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Managing wine quality

Volume 1: Viticulture and wine quality

Edited by Andrew G. Reynolds

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Managing wine quality

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Contents

Contributor contact details

(* = main contact)

Chapter 1

Professor Vicente Ferreira
Laboratory for Flavour Analysis and Oenology
Analytical Chemistry Department
Faculty of Sciences
University of Zaragoza
50009 Zaragoza
Spain

Email: vferre@unizar.es

Chapter 2

Dr Véronique Cheynier* and Dr Pascale Sarni-Manchado
INRA, UMR 1083 Sciences Pour l'Oenologie
2, Place Viala, Bât 28
34060 Montpellier Cedex
France

Email: cheynier@supagro.inra.fr

Chapter 3

Dr James A. Kennedy
The Australian Wine Research Institute
PO Box 197
Glen Osmond
SA 5064
Australia

Email: james.kennedy@awri.com.au

Chapter 4

Dr Bruce W. Zoecklein*
Professor and Head of Enology-Grape Chemistry Group
Department of Food Science and Technology
Virginia Tech
Food Science Building
Duckpond Drive
Blacksburg
VA 24061
USA

Email: bzoeckle@vt.edu

Dr K. C. Fugelsang
Professor of Enology
Department of Viticulture and Enology
California State University – Fresno
2360 E Barstow
Fresno
CA 93740
USA

Email: kennethf@csufresno.edu

Dr B. H. Gump
Professor of Analytical Chemistry and Enology
Department of Food and Beverage
Florida International University
North Miami
FL 33181
USA

Email: bgump@fiu.edu

Chapter 5

Dr Daniel Cozzolino* and Robert G. Dambergs
The Australian Wine Research Institute
Waite Road
Urrbrae
PO Box 197
Adelaide
SA 5064
Australia

Email:
Daniel.Cozzolino@awri.com.au;
bob.dambergs@awri.com.au

Chapter 6

Dr James P. Osborne
Department of Food Science and Technology
Oregon State University
100 Wiegand Hall
Corvallis
OR 97331
USA

Email:
James.Osborne@oregonstate.edu

Chapter 7

Dr Isabelle Lesschaeve*
Vineland Research and Innovation Centre
PO Box 4000
4890 Victoria Ave. N.
Vineland Station
Ontario
LOR 2E0
Canada

Email: isabelle.lesschaeve@vinelandresearch.com
or info@vinelandresearch.com

Dr Ann C. Noble
University of California
One Shields Ave
Davis
CA 95616
USA

Email: acnoble@ucdavis.edu

Chapter 8

Dr Ioannis S. Arvanitoyannis
(Associate Professor) School of Agricultural Sciences
University of Thessaly
Fytokon Str
Nea Ionia
Magnessias
Volos 38446
Hellas
Greece

Email: parmenion@uth.gr

Chapter 9

Professor Cornelis van Leeuwen
ENITA – Université de Bordeaux
Institut des Sciences de la Vigne et du Vin
1 Cours du Général de Gaulle
CS 40201
33175 Gradignan Cedex
France

Email: k-van-leeuwen@enitab.fr

Chapter 10

Dr Philip R. Young* and Professor Melané A. Vivier
Institute for Wine Biotechnology
Department of Viticulture and Oenology
Stellenbosch University
Matieland
ZA-7602
Stellenbosch
South Africa

Email: pryoung@sun.ac.za, mav@sun.ac.za

Chapter 11

Dr Andrew G. Reynolds
Brock University
St. Catharines
Ontario
LS2 3A1
Canada

Email: areynold@brocku.ca

Chapter 12

Dr Robert G. V. Bramley
CSIRO Sustainable Ecosystems
PMB 2
Glen Osmond
SA 5064
Australia

Email: rob.bramley@csiro.au

Chapter 13

Dr Eileen S. Scott*
School of Agriculture, Food and Wine
The University of Adelaide
PMB 1
Glen Osmond
SA 5064
Australia

Email: eileen.scott@adelaide.edu.au

Robert G. Dambergs
The Australian Wine Research Institute
c/o Tasmanian Institute of Agricultural Research
Private Bag 98, Hobart
Tasmania 7001
Australia

Email: bob.dambergs@awri.com.au

Belinda E. Stummer
CSIRO Entomology
PMB 2
Glen Osmond
SA 5064
Australia

Email: belinda.stummer@csiro.au

Chapter 14

Professor Paola Battilani*
Institute of Entomology and Plant Pathology
Faculty of Agriculture
Università Cattolica del Sacro Cuore
Via Emilia Parmense, 84
29100 Piacenza
Italy

Email: paola.battilani@unicatt.it

Professor Angela Silva
Institute of Oenology and Food Technology
Faculty of Agriculture
Università Cattolica del Sacro Cuore
Via Emilia Parmense, 84
29100 Piacenza
Italy

Email: angela.silva@unicatt.it

Chapter 15

Professor Dr Monika Christmann* and Maximilian Freund
Geisenheim Research Center
Department for Oenology and Wine Technology
Blaubachstr 19
D - 65366 Geisenheim
Germany

Email: m.christmann@fa-gm.de

Preface

A common adage is that 'wine is made in the vineyard'. Volume 1 of *Managing wine quality – Viticulture and wine quality* – emphasises this point with 15 fundamental chapters from leading experts around the world. We anticipate that this book, and its companion volume dealing with oenology and wine quality, will provide a valuable and useful resource for students, scholars and members of the wine industry. The two volumes might potentially form the basis for a senior level undergraduate or graduate level course in wine science. Volume 1 is divided into three sections dealing with: (I) Understanding grape and wine sensory attributes; (II) Measuring grape and wine properties; and (III) Viticulture technologies, grape composition and wine quality attributes. Topics range from the biochemistry of aroma (Chapter 1), taste and mouthfeel (Chapter 2) and wine colour (Chapter 3) to methods of analysis of grapes and wines, including conventional methods (Chapter 4) as well as new non-destructive techniques (Chapter 5), methods for the authenticity and traceability of wines by sophisticated analytical means (Chapter 8) and of course sensory analysis (Chapter 7). Viticulture is well represented also, with topics that include the definition of terroir (Chapter 9), precision viticulture (Chapter 12) and vineyard management practices influencing wine quality (Chapter 11). Related chapters include genetic and genomic approaches for the improvement of wine quality (Chapter 10) and a comprehensive look at grape processing up to the point of the beginning of fermentation (Chapter 15). Fundamental microbial aspects are also included in this volume, including fungal contaminants in the vineyard (Chapter 13), the control of ochratoxin A (Chapter 14) and microbial quality (Chapter 6). Volume 1 and its companion volume on oenology and wine quality represent the state of the art on winemaking science and technology from the vineyard to the glass. I hope that the reader finds *Managing wine quality* both edifying and enjoyable, and that it will be considered a valuable resource for years to come.

Andrew G. Reynolds
Editor

1

Volatile aroma compounds and wine sensory attributes

V. Ferreira, University of Zaragoza, Spain

Abstract: This chapter presents a revision of our knowledge and understanding of the role played by the different aroma chemicals in the positive aroma attributes of wine. In Section 1.1, some basic concepts concerning the characteristics of aroma chemicals, such as thresholds, odour activity values (OAVs) and the relationship between the intensity of odour and the concentration are presented. After this, a systematic approach to classifying the different aroma chemicals of wine is presented. One basic idea is that all wines share a common basic aromatic structure formed by ethanol and 27 different aroma compounds, most of them by-products of fermentation. The mixture of those products has the typical vinous aroma and exerts an aroma-buffering effect with the ability to suppress the effect of many odorants added to it, particularly those with fruity characteristics. The ability of the different odour chemicals to break such a buffer, and hence transmit a different aroma nuance to the wine, and the relationship between the transmitted aroma nuance and the aroma of the chemical are used to define the different roles played by aroma compounds on wine aroma. These roles can be as impact compounds, major contributors, net contributors, subtle aroma compounds, aroma enhancers and aroma depressors. The subjects can be individual aroma chemicals or well-defined mixtures of molecules sharing chemical and odour properties (aroma families). Different examples of the aroma chemistry behind some of the most relevant wine aroma nuances from simple or complex wines are also presented and discussed.

Key words: wine, aroma, flavour, odour activity value, odour-concentration relationship, aroma buffer, impact chemicals, aroma families, aroma enhancers, aroma suppressors.

1.1 Introduction: basic properties of aroma chemicals

All of us have experience with odours and with some odour chemicals and, for those involved with wine and other high-quality foods, the description of the odour and flavour attributes of a product is something familiar or even trivial. The words we use to describe the odour attributes of a product come from our personal experience and take the name of other products with which more or less everybody is familiar and whose aroma is clear, explicit and supposedly unambiguous.

However, most of us have received nearly no education about the way in which the chemical aroma molecules work to create an aroma. On the contrary, it is likely that we will have received some contradictory or even biased information about the role of chemicals in aroma. On the one hand, we know that some chemicals have clear and easily recognizable aromas that match nearly perfectly the aroma attributes of a given product (e.g. vanilla and vanillin, or corked wine and trichloroanisole – TCA), which suggests that single aroma molecules can drive the aroma attributes of a product. However, this happens only in a very limited number of cases, and we should be ready to accept the idea that, most often, the aroma nuances are the result of a complex equilibrium between different aroma chemicals. On the other hand, some complex products such as wine, coffee or cacao have in their composition more than 1000 volatile molecules (Maarse and Vischer, 1989), and therefore, one can be tempted to think that the final aroma perception is the result of the interactions of several hundred volatiles, which means that we have almost no chance to understand, model or predict the aroma of these products. In this case, fortunately, things are not that complicated and, even in those complex products, the numbers of chemicals really contributing to the different aroma nuances are limited (let's say some tens). It is also true that we are lacking well established tools to deal with the aroma of complex products and that we have to rely mainly on empirical evidence, but since the late 1990s there has been some progress that makes it possible to propose a first approximation of the roles played by the different aroma chemicals of wine in the perception of its different sensory attributes. This chapter deals with this question and will try to present more or less systematically a number of ideas, concepts and facts that will help in understanding the complex relationships between wine odour chemicals and wine odour nuances. Before dealing with those ideas, we will present some basic concepts about aroma chemicals.

1.1.1 Volatile compounds and aroma chemicals

An odour chemical is a chemical compound able to interact with our olfactory epithelium and elicit an odorous sensory response. As our olfactory epithelium is located in the nostrils in the upper interior part of our nose, only volatile molecules can reach this point. So far, all aroma compounds known at present are volatile compounds (Leffingwell, 2002). The opposite is also true, and nearly all volatile molecules (excluding permanent gases, water and a limited number of simple molecules) have some aroma. However, there is an extraordinary variability in the

range of concentrations at which a volatile compound is really able to impact the pituitary and elicit the sensory response. While some simple molecules, such as ethane, chlorotrifluoromethane or ethanol, will only be smelled when the concentration in the air is as high as 1 g/m^3 (van Gemert, 2003), there are some others, such as bis(2-furfuryl) disulphide or 1-methoxy-3-methyl-3-butanethiol (Guth and Grosch, 1991), which can be perceived at concentrations in the air as little as 0.1 ng/m^3: i.e. differences in sensitivities are up to 10 orders of magnitude. When a volatile compound is at concentrations well below (let's say 10-fold) its threshold, then its odour contribution can be considered null and the molecule can no longer be, strictly speaking, considered an aroma compound.

1.1.2 Thresholds, odour activity values (OAVs) and *I*/log*C*

Thresholds in air are related to the real ability of the odorant to impact the pituitary, but are not related to the potential relevance of the aroma compound in a given product. This is because some odorants are so strongly retained or dissolved in the matrix of the product that they are released to the headspace in contact with the product to a very limited extent. This is the reason why thresholds in water are more useful for predicting the potential importance of a given aroma compound in an aqueous product. In water, some hydrophilic compounds, such as vanillin, are so well dissolved and hence are poorly released to the air that, even if we are very sensitive to them (the threshold in air is very low), a large concentration is required to get a clear odour signal (the threshold in water is very high) (van Gemert, 2003).

Thresholds are simple values and collecting them is relatively easy. This is the reason why, together with the odour description of the chemical, these values are almost the only parameters defining the behaviour of a given odour chemical which are used and tabulated. Many researchers rely then on the quotient between the concentration of a compound in a given product and the threshold of that compound in a matrix similar to that of the product. This parameter is known odour activity value (OAV) or aroma value (Grosch, 1993). However, the threshold, and hence the OAV, is just a first and rough approximation to the potential sensory importance of a given aroma compound in a product. As a first approximation, we can say that being at a concentration above threshold (OAV > 1) is necessary, but it is not enough, particularly in complex products. We can also say that all aroma chemicals present in a product at a concentration one order of magnitude below the threshold (OAV < 0.1) are irrelevant to the aroma of that product.

The relationship between the concentration of an odorant (usually in log scale) and its odour intensity is a very important plot, known as psychophysical curve. Figure 1.1 shows this representation for ethyl 2-methylbutyrate in hydroalcoholic solution. The function is a typical sigmoid curve reaching a saturation point (Chastrette *et al.*, 1998).

As the figure shows, the concentration range can be divided roughly into three areas: the sub-threshold area, the supra-threshold area and the saturation area. The boundaries between these areas are not clearly delineated due to the individual differences in sensitivity; rather, these areas have quite blurred boundaries,

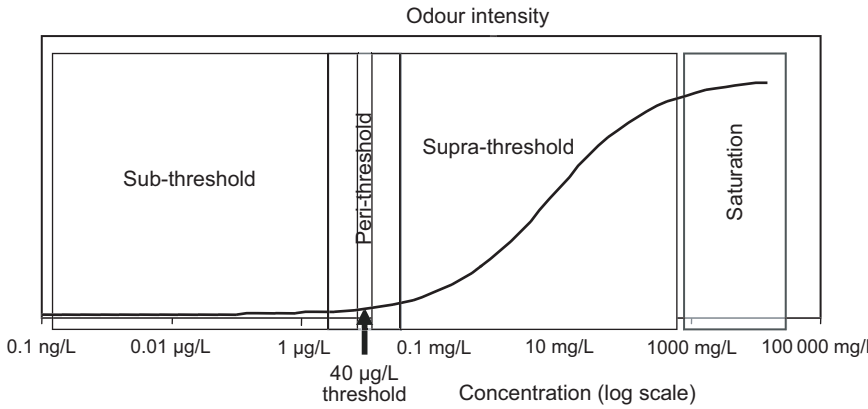

Fig. 1.1 Odour intensity versus *C* (log scale) for ethyl 2-methylbutyrate (Ferreira *et al.*, 2003a).

particularly in the area around the threshold which is known as the peri-threshold area. Compounds can differ not only in thresholds but also in the dynamic ranges of response, which have a significant effect on the final sensory effect of the compounds. This can be clearly seen in Fig. 1.2, which shows the *I*/log*C* plot of four different compounds (Ferreira *et al.*, 2003a). The thresholds of the compounds in the figure are widely distributed, and range from 0.1 ppt to 70 ppm, i.e., more than eight orders of magnitude. However, the plot also shows that the compounds have very different *I*/log*C* relationships. While the range between the 0 and the saturation area in 'normal' compounds, such as 4-methyl-4-mercaptopentanone or ethyl 2-methylbutyrate, is covered in four to five orders of magnitude of concentration, methyl benzoate reaches saturation just 1.5 orders to magnitude above threshold while β-damascenone requires 11 orders of magnitude to reach saturation. The practical impact of these facts is enormous and shows the serious limitations of the OAV concept for predicting the importance of a given compound in the aroma. For instance, it can be seen that the plots of β-damascenone and 4-methyl-4-mercaptopentanone intersect at a concentration of around 0.1 µg/L. This means that at this concentration both compounds have the same odour intensity; however, the OAV for β-damascenone is more than 1000 (OAV over-estimates its importance) while for 4-methyl-4-mercaptopentanone this parameter is just 20 (OAV under-estimates its importance).

There is an additional reason why OAVs can fail to predict the real relevance of a given aroma molecule in a complex mixture, and this reason is related to the 'miscibility' or 'differentiability' of the aroma under study in relationship with the aroma of the mixture (Livermore and Laing, 1998). Some odours can be very easily identified in a given mixture and, hence, a low OAV can have great impact (Escudero *et al.*, 2004). On the contrary, some other aromas meld nearly perfectly with the aroma of the mixture so that, even at high OAVs, the aroma is barely perceived.

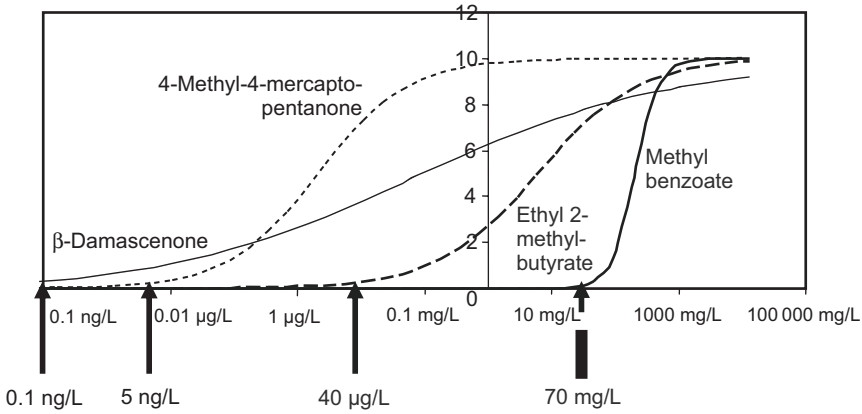

Fig. 1.2 Odour intensity versus *C* (log scale) for four different odorants (Ferreira *et al.*, 2003a).

1.1.3 Distribution of thresholds

A further reason why thresholds and OAVs are merely rough approximations to the importance of a given aroma in a product is that thresholds vary among individuals (Punter, 1983). Usually, the distribution of the thresholds for a single aroma chemical among the population follows a log-normal law and, in the most frequent case, the difference in sensitivity between the least sensitive individuals and the most sensitive is one order of magnitude. Again, there is a relatively large variability in this respect too. In extreme cases, the population is segregated in groups of sensitive and insensitive (or anosmic) individuals and, in this case, differences in sensitivity can be higher than four or five orders of magnitude (Amoore, 1977). This has been found to happen to some wine off-odours, which means that if the quality control in a company was carried out by a single individual and he/she happened to be anosmic to the compound, then the off-flavoured wine could be bottled and dispatched unnoticed (Coulter *et al.*, 2007). This is a good reason to rely on a sensory panel!

1.1.4 Odour, flavour and taste

Aroma compounds can reach the pituitary via the nose during normal olfaction (via orthonasal) or via the pharynx when the food is taken in the mouth and swallowed (via retronasal). In the first case, we smell the odour of the product; in the second case, we perceive the flavour of the product. There is no doubt that the smell of the product is almost entirely due to the signals elicited by the aroma molecules in the olfactory receptors (pure odour), although there are also trigeminal nerve endings in the olfactory mucosa able to interact with some aroma chemicals to produce trigeminal stimulation. In fact, some aroma chemicals produce a sensory perception that is a mixture of odour and trigeminal stimulation, such as acetic acid or menthol (Prescott, 1999a). The flavour is a complex perception that integrates

information from three different sensory systems: odour, taste and the chemosensory receptors, responsible for 'hot', 'cool', 'dry', 'irritant' or 'pungent' attributes (Prescott, 1999b). In the case of aroma molecules, the amount and proportion of molecules reaching the olfactory receptors when the food is taken into the mouth and during swallowing is not exactly the same as that when the product is smelled, because the conditions for the release of the aroma molecules from the product are fairly different in both cases (e.g. temperature, dilution with saliva, aggregation) (Linforth *et al.*, 2002). That complexity can make us think that flavour is totally different, while the truth is that most qualitative attributes of flavour are also produced by aroma molecules via the activation of the olfactory receptors.

1.2 Wine aroma 'organization'

1.2.1 The base of wine aroma

A normal table wine contains several hundreds of volatile compounds, but most of them are at concentrations well below the threshold, which means that they are not really relevant in the perception of the sensory attributes of the wine. The number of odour molecules really active in a normal wine lies between 20 and 40, and the total number of odour molecules that can be really active in the different kinds (without odour problems) of wines is around 70. What we need now is to find a series of rules to put some order into these relatively high numbers.

A first key to such issues consists of realizing that some aroma compounds are present in all wines, independent of their origin and kind. These groups of compounds are of course these volatile aroma compounds produced by fermentation at relatively well-defined proportions. These 27 compounds can be seen in Table 1.1. All these compounds are present at concentrations well above threshold in nearly all wines and they form a particular aroma mixture with the aroma we often define as vinous. It is slightly sweet, pungent, alcoholic and a little bit fruity (Escudero *et al.*, 2004). Another compound, β-damascenone, can also be included in this list because although it is not formed by yeast during fermentation, it can also be found in nearly all wines at concentrations above threshold which, as we saw in Fig. 1.2, is very low.

Of course not all wines have exactly the same composition in this base. The concentration of sugar in the must, the strain of yeast and the degree of anaerobiosis are well-known factors influencing its composition. For instance, this last factor means that whites and rosés are richer in fatty acids and their ethyl esters while they contain less alcohols and isoacids than reds (Ferreira *et al.*, 1996). Another less well-known factor is that the concentrations of fusel alcohols, fusel alcohol acetates, isoacids and their ethyl esters, all of them related to the yeast amino acid metabolism, are related to the varietal origin of the must (Ferreira *et al.*, 2000; Hernandez-Orte *et al.*, 2002). This is the reason why some wines, such as those made with Tempranillo, have a 'soft' and delicate aroma because of the low amount of fusel alcohols and isoacids they naturally contain. Finally, ageing is also an important factor and introduces important changes related to the different acid

Table 1.1 Aroma chemical compounds forming the base of wine aroma

Miscellaneous	Fusel alcohols	Organic acids	Isoacids	Organic acid ethyl esters	Fusel alcohol acetates	Ethyl esters of isoacids
Ethanol	Isobutanol	Acetic acid	Isobutyric acid	Ethyl acetate	Isobutyl acetate	Ethyl isobutyrate
Diacetyl	Isoamyl alcohol	Butyric acid	2-Methyl butyric acid	Ethyl butyrate	Isoamyl acetate	Ethyl 2-methyl butyrate
Acetaldehyde	Hexanol	Hexanoic acid	Isovaleric acid	Ethyl hexanoate	β-Phenylethyl acetate	Ethyl isovalerate
	β-Phenylethanol	Octanoic acid		Ethyl octanoate		
	Methionol	Decanoic acid		Ethyl decanoate		

Table 1.2 Effect of the omission in the mixture of wine major compounds (the aroma buffer) of one of the constituents (adapted from Ferreira *et al.*, 2002). Significance of discriminative tests (triangular test) comparing the original mixture with the mixture from which one aroma component has been removed. The qualitative description of the differences is also included

Compound	Significance	Qualitative effect
Ethyl isovalerate	NS	None
Ethyl 2-methylbutyrate	NS	None
Ethyl isobutyrate	NS	None
Ethyl butyrate	NS	None
Ethyl acetate	NS	None
Acetaldehyde	NS	None
Diacetyl	NS	None
β-Phenylethanol	0.05	Inappreciable
Butyric acid	0.05	Inappreciable
Isoamyl alcohol	0.05	Inappreciable
Ethyl octanoate	0.05	Inappreciable
Methionol	0.05	Inappreciable
Octanoic acid	0.05	Inappreciable
Hexanoic acid	0.05	Inappreciable
Ethyl hexanoate	0.05	Inappreciable
Isovaleric acid	0.05	Inappreciable
Isoamyl acetate	0.05	Slightly less fruity
β-Damascenone	0.05	Slight decrease in intensity

NS, 0.05: not significant or significant at $p < 0.05$, one-tailed test.

+ alcohol = ester equilibria. As the wine concentrations in acetic acid are low, fusel alcohol acetates are hydrolyzed and can be found at appreciable concentrations only in young wines. In contrast, as the wine concentrations in ethanol are relatively high, the concentrations of fatty acid ethyl esters are relatively constant and the concentrations of the ethyl esters of isoacids steadily increase during ageing, which causes a softening in the aroma of some red wines. These compositional differences have some remarkable sensory consequences but yet, it is the combined effect of all these compounds which exerts the most remarkable influence on wine aroma and follows a pattern common to all wines.

1.2.2 The aroma-buffer effect

The mixture formed by the aforementioned compounds forms what we call an aroma buffer. We call it a buffer because in some sense it resembles the buffer systems we usually use to fix pH. Those buffer systems have the ability to counteract the effect of small additions of acid or of alkali and, as such, the aroma buffer has both the ability to counteract the effect of the omission from the mixture of one of its components and the ability to counteract the addition to the mixture of many single odorants. Both effects can be seen in Tables 1.2 and 1.3. Table 1.2 (Ferreira *et al.*, 2002) shows the effects of omission from the mixture of one of the odorants. As can be seen, in most cases the omission from the mixture had no

Table 1.3 Sensory effects caused by the addition of a single aroma compound to a neutral wine from Maccabeo (adapted from Escudero *et al.*, 2004). Compounds represent different aroma families

Compound added (concentration added and relative increment in relation to wine content)	Effect	Observations
Hexanoic acid (6.2 mg/L; 2.5×)	Slight	Fruity; – candy
β-Phenylethanol (300 mg/L; 21×)	None	
Isoamyl acetate (5.5 mg/L; 2.2×)	Slight	+ Banana
Ethyl octanoate (6.0 mg/L; 8.6×)	None	
2,6-Dimethoxyphenol (2 mg/L; 4000×)	Slight	Flowery; – candy
Guaiacol (15 μg/L; 71×)	Slight	Pineapple; – candy; – flowery
Furaneol (800 μg/L; 27×)	None	
Sotolon (140 μg/L; 28×)	Clear	Fruity; – candy
β-Damascenone (4.5 μg/L; 1×)	None	

effect, or a just noticeable effect that the judges were not able to define. Only in the cases of isoamyl acetate and β-damascenone were there slight effects on the fruitiness of the mixture.

The effect of the addition of different aroma compounds to a neutral wine is presented in Table 1.3 (Escudero *et al.*, 2004). Results are again very surprising. It can be seen that the addition of huge amounts of some odorants has almost no effect, or even that the effect is not the perception in the mixture of the added odorant, but a decrease in some of the basic attributes of the mixture (except for isoamyl acetate). This buffering effect is something challenging for neurophysiologists and has such a strong influence on the way we should understand the hierarchical relationships between wine odorants that it can be used as a useful criterion to classify wine odorants.

1.2.3 Breaking the buffer

Fortunately, the aroma of many wines is very rich in aroma nuances that are quite different to the basic 'vinous' aroma of the aroma buffer. This clearly means that some aroma molecules succeed in some wines in breaking the buffer and produce a different sensory perception. As was mentioned earlier, OAVs are not a useful criterion to predict whether one aroma molecule is going to be clearly perceived in wine aroma, particularly in light of the previously explained fact that some odorants at very low OAVs cause large sensory impacts while others at such OAVs can be hardly detected. By observation, we have identified four different ways to break the buffer:

1. A single molecule at a concentration large enough, such as, for instance, isoamyl acetate in banana-smelling wines.
2. A group of molecules with close similarity in chemical and aromatic properties, such as, for instance, aliphatic γ-lactones in some red wines. Some of these associations can be so important, particularly if the group of chemicals is produced along the same chemical or biochemical pathway, that individual

compounds have no significance and it is much better to work directly with the group of chemicals. These groups of chemicals are considered families of chemical aroma compounds.

3. A large group of molecules with some similarity in a generic (non-specific) aroma descriptor (for instance sweet), such as for instance linalool, γ-lactones and ethyl cinnamates in some white wines. Those large groups of molecules are in most cases combinations of different families of chemical aroma compounds (Loscos *et al.*, 2007).
4. The association between an aroma enhancer and one or several aromatic molecules unable to break the buffer themselves. In this case, the aroma nuance can be that of the chemical enhanced or a new aromatic nuance mixture of those of the enhancer and the other aroma molecules.

1.2.4 Roles played by aroma chemicals

The exact role played by an aroma chemical in a complex aroma mixture can be assessed empirically by means of reconstitution, addition and omission sensory experiments (Grosch, 1993). In reconstitution experiments, a synthetic aroma mixture containing the aroma compounds present in the wine at the same concentrations is first prepared. Then, new mixtures similar in composition to the first one but in which one aroma chemical or a group of aromas have been omitted or added are further prepared and their sensory properties are assessed. These tedious procedures make it possible to observe the sensory effect linked to the presence of a compound in a chemical environment matching closely that of the wine.

How such sensory impact relates to the odour properties of the aroma chemical or group of aroma chemicals under study gives us the potential to define the different roles played by the different aroma chemicals or groups of them. For instance, in some wines in which isoamyl acetate is found at relatively high concentrations, it is possible to detect the typical banana nuance of his compound; i.e., in those wines this compound is transmitting to the wine its specific odour nuances. However, in some other wines in which this aroma compound is present at more modest concentrations, isoamyl acetate just adds a fruity or even a sweet–fruity (unspecific) odour nuance. In these latter cases, it is a generic descriptor (primary or secondary) of the acetate that is transmitted to the wine. These ideas can be easily understood with the help of Figs 1.3 and 1.4.

Figure 1.3 shows in schematic form the ability of isoamyl acetate to transmit its odour nuances to the wine as a function of concentration. As can be seen, below 200 μg/L this compound is just one of the many sweet–fruity compounds that wine contains, and removing it from the wine will have a null or very weak sensory effect. We can say that this compound at this concentration is a secondary or subtle contributor to the sweet–fruity note. Between 200 and 1400 μg/L, the importance of the compound in the wine grows to the point that it becomes a quite important contributor to the fruity note of the wine. If this compound is removed from the wine, then the intensity of the fruity note of the wine will decrease, but most surely we will not notice any qualitative change. At this point, this aroma molecule plays

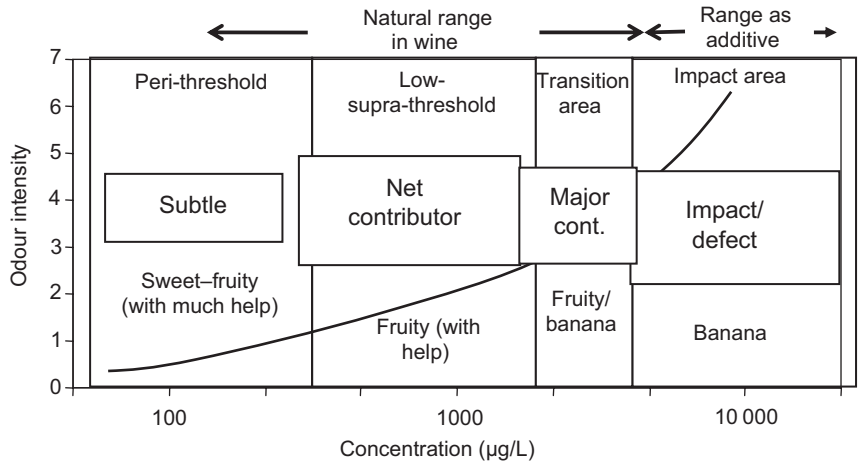

Fig. 1.3 Odour intensity versus *C* (log scale) for isoamyl acetate, including the contribution of this compound to wine aroma (Ferreira, unpublished).

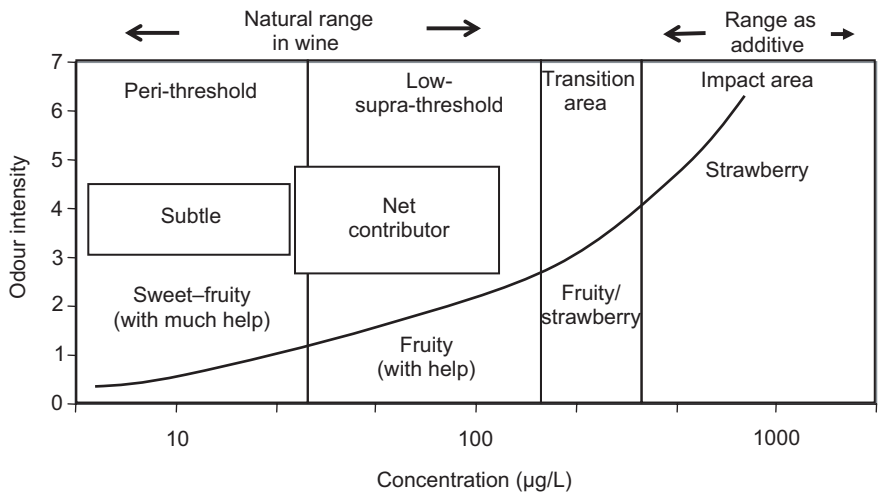

Fig. 1.4 Odour intensity versus *C* (log scale) for 2-methylbutyrate, including the contribution of this compound to wine aroma (Ferreira, unpublished).

the role of net contributor to the fruity note. Between 1400 and 2200 µg/L we come into the transition phase. At this point, the people more sensitive towards this chemical will perceive its banana note in the wine, while the less sensitive will not be able to identify the product. However, if the compound was removed from the wine, then an important sensory change implying quantitative and qualitative changes would be noted. At this point, this compound plays the role of major

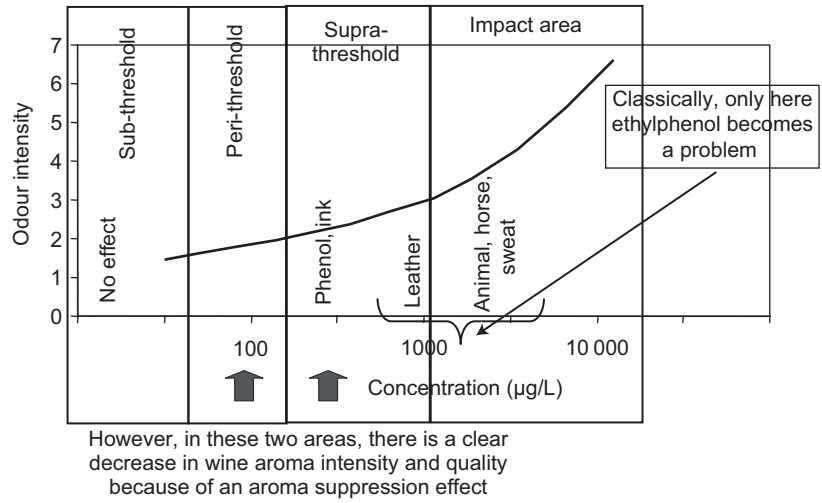

Fig. 1.5 Odour intensity versus *C* (log scale) for 4-ethylphenol, including the contribution of this compound to wine aroma (Ferreira, unpublished).

contributor. Above 2000 µg/L, nearly everybody will perceive the typical banana note of the compound in the aroma of the mixture and, if the sensory impact becomes too dominant, it will even become a defect. At this last concentration, removing the compound from the wine has a dramatic effect on its aroma properties with notable qualitative changes, which means that, at these concentrations, the compound acts as a genuine impact compound.

The second example is that of ethyl 2-methylbutyrate. This compound, as the plot in Fig. 1.4 shows, never reaches the concentration in wine at which it is used as an additive by the industry to produce a net impact. This means that, taken individually, this compound can play only the role of subtle or net contributor, but not that of impact compound. However, and as we will see later, this compound may act concurrently with the other members of its family, and the family in its entirety, can then play a role as net or major contributors to the fruit or red fruit aroma nuance of the wine.

A third different example is that of geraniol. Geraniol is a quite powerful aroma compound, but in many instances the sensory effect linked to its presence cannot be directly related to its specific or even generic sensory properties, but to the specific sensory properties of a different (but similar) molecule, such as linalool or *cis*-rose oxide, i.e., the addition of geraniol to a wine containing linalool will not bring about the increment of an odour nuance related to geraniol, but to linalool. This is a clear case of aroma enhancement, and the role played by geraniol is that of aroma enhancer.

A completely different situation is most often observed with some chemicals of relatively bad aroma, although some relatively good-smelling molecules can also follow a similar pattern. A typical example of this behaviour is that of 4-ethyl-

phenol, responsible for the undesirable sweat, animal, leather odour nuances of some red wines (Chatonnet *et al.*, 1992). The relationship between the sensory effect caused in wine by this chemical and its concentration can be observed in Fig. 1.5. At a concentration high enough (well above 1000 μg/L), the sensory effect of this molecule is the straightforward apparition of the off-odour note but, at smaller concentrations, certainly below the recognition thresholds, what the addition of this molecule to a fruity wine causes is the decrement of the fruity note (Aznar *et al.*, 2003). At those low concentrations, the molecule plays the role of aroma depressor.

All these different roles can be systematized in a list which will make it possible to classify the aroma compounds attending to the role they can play in a given wine. The classification is a new proposal based on observation, but it is based on well-established concepts of flavour chemistry (Belitz and Grosch, 1999).

1. **Genuine impact compound**. This role is played by individual compounds which, in a given wine, are at concentration high enough to transmit to that wine their specific aroma nuances, i.e., the aroma of the compound can be recognized in the wine.
2. **Major contributor**. This role is played by individual compounds or by families of aroma compounds that are present in the wine at a concentration high enough to transmit to the wine a primary generic descriptor (red fruit, citric, minty) of its aroma, but not the specific descriptor of the compound (i.e., the compound cannot be clearly recognized in the wine). The transmitted descriptor in the wine is nearly entirely due to the compound so that, if the compound or family of compounds are removed, then the sensory effect will be very intense qualitatively and quantitatively.
3. **Net contributors**. This role is played by individual compounds or by families of aroma compounds that are present in the wine at a concentration high enough to transmit to the wine a generic descriptor. Such a descriptor is also contributed by other compounds or families of compounds so that if the compound or family of compounds are removed, then a significant decrease in the intensity of the odour nuance will be noted, but a major change in the qualitative aroma profile will not be observed.
4. **Secondary or subtle contributors**. This role is played by those individual compounds or families of aroma compounds that are present in the wine at a concentration below that required to transmit individually to the wine one of its generic descriptors. However, such aromatic descriptor (usually very general, such as sweet or fruity) is noted because of the concerted action of many aroma molecules or families. Accordingly, if the compound or family of compounds is removed from the wine, the sensory effect will be very weak or even null.
5. **Aroma enhancer**. This role is played by those individual aroma molecules or families of aroma compounds which fail to transmit to the wine their specific or generic descriptors but nonetheless enhance the specific aroma of some other molecule or group of molecules present in the wine. In some cases, the

enhancement brings about a new aroma quality as a consequence of the mixture of the odours of aroma and enhancer, while in some others the effect of the enhancement is merely the increase of the aroma intensity. In any case, if the enhancer is removed, then a decrease in the intensity of an aroma nuance, not directly related to the aroma of the enhancer, will be noted.

6. **Aroma depressor**. This role is played by those individual aroma molecules or families of aroma compounds whose presence in the wine causes a decrease in the intensity of an odour note. If the depressor is removed from the wine, then an increase in the intensity of the depressed odour nuance is noted.

With these roles defined, it is now possible to present the aroma chemicals and families of aroma chemicals that are able to play some of the most significant roles.

1.3 Wine aroma molecules classified by their role

1.3.1 Impact compounds

According to our own research and from literature data, the following 16 compounds can act as impact compounds of some particular wines.

Varietal impact aroma compounds

Linalool. This was the first identified aroma component able to exert an impact on Muscat wines (Cordonnier and Bayonove, 1974; Ribéreau-Gayon *et al.*, 1975). Its contribution to the characteristic aroma of several wines made with grape cultivars from Galicia has been clearly demonstrated (Versini *et al.*, 1994; Campo *et al.*, 2005; Vilanova and Sieiro, 2006). Similarly, it also contributes to the flowery or even citrus notes of some other white cultivars (Arrhenius *et al.*, 1996; Lee and Noble, 2003; Campo *et al.*, 2005; Palomo *et al.*, 2006), always in combination with the other terpenols.

***cis*-Rose oxide**. This terpene of pleasant flowery character was first identified as a characteristic impact aroma compound of wines made with Gewürztraminer (Guth, 1997). Later, it was also found to be a key odorant in wines made with the varietal Devin (Petka *et al.*, 2006), and was also detected in the hydrolyzed fractions from precursors obtained from different neutral grape varieties (Ibarz *et al.*, 2006). As with linalool, it requires the presence of the other terpenols to be clearly perceived.

β-Damascenone. This compound is found in nearly all wines at concentrations around 1–4 μg/L. At these concentrations, the compound acts mainly as aroma enhancer, promoting the fruity aroma of wine esters (Escudero *et al.*, 2007; Pineau *et al.*, 2007). However, this compound can be present at much higher concentrations in wines made from sun dried grapes (Campo *et al.*, 2008) or overripe grapes (Pons *et al.*, 2008). It is a key odour compound in Pedro Ximénez wines (Campo *et al.*, 2008).

4-Mercapto-4-methylpentan-2-one. This compound has a characteristic scent of box tree (*Buxus* spp.), which can be perceived in some wines made with Sauvignon blanc (Darriet *et al.*, 1991, 1993, 1995) or Scheurebe (Guth, 1997). At lower concentrations, the compound is not strictly speaking an impact compound, but a major contributor to the fresh fruity notes (Escudero *et al.*, 2004).

3-Mercaptohexan-1-ol. This compound has a smell reminiscent of green mango and box tree with some rubbery notes. Its odour is very complex and changes with concentration and with the aroma environment in which it is found. It was first identified in wines from Sauvignon blanc, Cabernet-Sauvignon and Merlot (Bouchilloux *et al.*, 1998) but afterwards it was found in many others (Tominaga *et al.*, 2000). It is an impact compound of some rosé wines (Murat *et al.*, 2001; Ferreira *et al.*, 2002), of white wines made with Petit Arvine (Fretz *et al.*, 2005) and Sauternes wines (Bailly *et al.*, 2006; Sarrazin *et al.*, 2007; Campo *et al.*, 2008).

3-Mercaptohexyl acetate. This compound was first found in wines from Sauvignon blanc (Tominaga *et al.*, 1996), but it can also be found in many other wine types (Tominaga *et al.*, 2000; Lopez *et al.*, 2003; Culleré *et al.*, 2004; Gomez-Miguez *et al.*, 2007). It has been recently shown that it is the impact aroma compound of the wines made with the Spanish variety Verdejo, imparting the characteristic tropical fruit aroma nuance to the wine (Campo *et al.*, 2005).

Rotundone. This compound is a sesquiterpene responsible for the spicy notes of Shiraz wines and also of black and white pepper, and was recently reported in Australian wines (Wood *et al.*, 2008). It elutes out of the chromatographic phases very late, which precluded its identification when the aroma composition of pepper was first addressed. Its odour threshold in water was found to be around 25 ng/L, although 25% of tasters were insensitive to this compound. Concentrations in Shiraz grapes are highly variable, ranging from less than 10 to more than 600 ng/L (Wood *et al.*, 2008).

Fermentative impact compounds

Diacetyl. This compound is another odorant with a complex role in wine aroma. It was one of the first identified wine aroma molecules (Fornachon and Lloyd, 1965), and it has often been blamed as the cause of a defect when it is present at high concentrations (Clarke and Bakker, 2004). Its sensory effect is extremely dependent on the type of wine (Martineau *et al.*, 1995a; Bartowsky *et al.*, 2002), and its concentration is also time dependent and related to the concentration of sulphur dioxide in the wine (Nielsen and Richelieu, 1999). Diacetyl is responsible for the buttery note appreciated in some Chardonnay wines (Martineau *et al.*, 1995b; Bartowsky *et al.*, 2002), and its role in the sweet notes of some Port wines has also been suggested (Rogerson *et al.*, 2001). Several authors agree on its ambiguous character (Lonvaud-Funel, 1999; Bartowsky *et al.*, 2004).

Isoamyl acetate. This is the only ester capable of imparting its characteristic aroma nuance to wines, sometimes too overtly. In wines made with Pinotage or

Tempranillo varieties it is a characteristic aroma compound (van Wyck *et al.*, 1979; Ferreira *et al.*, 2000).

Age-related impact aroma compounds

(E)-Whiskylactone. This is an impact compound in wines aged in oak wood (Boidron *et al.*, 1988). Above a given concentration it can produce an excessive and unpleasant woody characteristic (Pollnitz *et al.*, 2000).

Sotolon (3-hydroxy-4,5-dimethyl-2(5H)-furanone). This is also an impact compound in wines made with botrytized grapes (Masuda *et al.*, 1984), or wines from biological ageing (Martin *et al.*, 1990, 1992; Moreno *et al.*, 2005), natural sweet wines (Cutzach *et al.*, 1998, 1999), Pedro Ximénez (Campo *et al.*, 2008), Oporto (Ferreira *et al.*, 2003b) or Madeira (Camara *et al.*, 2004). Its concentration, in general, increases with oxidation (Escudero *et al.*, 2000a).

Furfurylthiol (FFT, or 2-furanmethanethiol). This strong coffee-smelling compound is formed by reaction between furfural from the oak cask and sulphydric acid formed during the fermentation (Blanchard *et al.*, 2001), and is able to transmit its aroma to some types of wine. There is not a lot of analytical data on the occurrence of FFT because of difficulties in its determination, but it has been found at relatively high concentrations in aged wines from Champagne (Tominaga *et al.*, 2003a) and in some other wines (Tominaga *et al.*, 2006; Mateo-Vivaracho, 2009).

Benzylmercaptan (or benzenemethanethiol). This is a compound with a powerful toasty aroma and, together with FFT, it can impart smoky and empyreumatic nuances to some aged wines, such as Champagne or Chardonnay sur lie (Tominaga *et al.*, 2003a,b) but also to normal aged dry wines (Mateo-Vivaracho, 2009).

Dimethyl sulphide (DMS). This compound was identified some time ago in aged wines (Marais, 1979) and apparently plays an ambiguous role in wine aroma. Quite often it is related to a defect (sulphury odour) (Park *et al.*, 1994; Ferreira *et al.*, 2003c), but some other authors have demonstrated that it exerts a powerful enhancing effect on the fruity note of some highly appreciated red wines (Segurel *et al.*, 2004; Escudero *et al.*, 2007).

Methional (3-(methylthio)propanal). This compound also plays an ambiguous role. In young white wines it causes unpleasant odours (Escudero *et al.*, 2000b), but in complex wines, such as some Chardonnays or some great red wines, is a net contributor to some appreciated odour nuances (Ferreira *et al.*, 2005).

Phenylacetaldehyde. This is also a compound with an ambiguous role. Its smell of honey is very pleasant but gives to the wine oxidation notes that are considered to be defective and that depress fruitiness (Aznar *et al.*, 2003; Ferreira *et al.*, 2003d). However, this compound can act as impact compound in Sauternes or

Pedro Ximénez wines, in which it is found at very high concentrations (Sarrazin *et al.*, 2007; Campo *et al.*, 2008).

All the aforementioned aromas, at lesser concentrations do not play a role of impact compound, but that of major, net or even subtle contributor to some aroma nuance related to one of its more or less general or specific aroma descriptors.

1.3.2 Homogeneous aroma families

A particular case of additive (or eventually synergistic) action is that of all of the groups of compounds which share aromatic characteristics and also share common formation pathways (Jarauta *et al.*, 2006), a case relatively frequent in fermented natural products. In this case, it is possible to define families of odorants. The exact role of these families has been less studied because of the difficulties in defining thresholds and sensory properties for groups of compounds. However, the concept has been latent in the aroma groupings made by some authors (Moyano *et al.*, 2002; Aznar *et al.*, 2003) and has been the subject of some research in our laboratory (Jarauta, 2004; Culleré, 2005; Jarauta *et al.*, 2006; Culleré *et al.*, 2007; Loscos *et al.*, 2007). In this group different families can be identified.

1. Ethyl esters of fatty acids, responsible for fruity notes (apple-like, ester-like) of some white wines (Ferreira *et al.*, 1995).
2. Aliphatic γ-lactones which contribute to the peachy aroma of some reds (Ferreira *et al.*, 2004; Jarauta, 2004) but can also be contributors to the sweet nuances of many other wines (Loscos *et al.*, 2007).
3. Volatile phenols such as guaiacol, eugenol, 2,6-dimethoxyphenol, iso-eugenol and allyl-2,6-dimethoxyphenol, responsible for phenolic and toasted notes of wines (Escudero *et al.*, 2007).
4. Vanillas (vanillin, methyl vanillate, ethyl vanillate and acetovanillone) that can contribute to sweet flowery notes in many wines (Loscos *et al.*, 2007).
5. Burnt-sugar compounds (furaneol, homofuraneol, maltol) that can contribute to the general fruitiness of red wines (Jarauta, 2004; Ferreira *et al.*, 2005).
6. Fusel alcohol acetates that can contribute to the flowery and/or fruity notes of white wines (Campo *et al.*, 2005).
7. Aliphatic aldehydes with 8, 9 and 10 carbon atoms, that can contribute to the citric notes of some wines (Culleré, 2005).
8. Branched aldehydes 2-methylpropanal, 2-methylbutanal and 3-methylbutanal, that contribute to the characteristic odours of aged red wines (Culleré, 2005).
9. Ethyl esters of branched or cyclic fatty acids (ethyl 2-, 3- and 4-methylpentanoates and ethyl cyclohexanoate) (Campo *et al.*, 2006a,b), some of which have been recently identified. The aroma of these compounds could act additively with that of the other wine ethyl esters of branched acids (ethyl isobutyrate, ethyl isovalerate and ethyl 3-methylbutyrate) and contribute to the fruity notes of red wines, as has been recently suggested (Ferreira *et al.*, 2009).
10. Ethyl cinnamate and ethyl dihydrocinnamate that can contribute to the sweet

and floral notes of some wines, particularly Chardonnays (Loscos *et al.*, 2007, 2009).

The previous classifications involve 16 potential impact compounds and 31 other aroma compounds grouped in 10 different aroma families. This makes a palette of 26 different odours which, by combination with the base of the aroma, can explain most wine aroma nuances. Not all the combinations have been completely described and studied up to this date, but it is possible to interpret some relevant wine aroma nuances.

1.4 Interpretation of some wine aroma nuances

The key message coming out of this chapter about the aroma of wine is how the basic aroma buffer produced by ethanol and the other major fermentation volatiles is broken. The way in which this happens (through more or less impact compounds, or families, or through large numbers of subtle compounds) determines the complexity and aroma characteristics of wines.

1.4.1 Wines whose aromatic perception is driven mainly by a single odour chemical

In general, wines whose aromatic perception is driven by a single odour chemical are simple and have a clear, simple and distinctive aroma nuance caused by a chemical acting as a genuine impact aroma compound. Its degree of complexity, of course, will depend on the concentration of such a chemical, and on the presence of other aroma compounds which can modify or add some more aroma nuances.

The most typical and well-known example of these kinds of wines is Muscat. Other examples are some rosé wines whose aroma characteristics are due to the presence of high concentrations of 3-mercaptohexan-1-ol (Murat *et al.*, 2001; Ferreira *et al.*, 2002). Others are Sauvignon blanc wines, whose aroma characteristics are due mainly to 4-mercapto-4-methylpentan-2-one (Darriet *et al.*, 1995), or wines made from Verdejo, whose aroma characteristics are due to 3-mercaptohexyl acetate (Campo *et al.*, 2005). Other particular examples of this type of wine are some Cabernet Sauvignon or Cabernet Franc red wines made in some parts of New Zealand or France which show a very intense cassis aroma, nearly entirely due to the presence of high concentrations of 3-mercaptohexyl acetate. Of course, if the wines are rich in some other compounds, such as ethyl esters of fatty acids, linalool or isoamyl acetate, the final perception will be more complex, and surely more appreciated. In the case of Sauvignon blanc wines, some producers find value in the presence of methoxypyrazines, which no doubt, adds some complexity (even if this is controversial). Another case of simple wines that, nowadays, are not very appreciated is that of white wines with large amounts of isoamyl acetate, displaying a strong banana aroma. Finally, there are some white wines with a simple fruity aroma which is mainly due to the presence of high concentrations of ethyl esters of fatty acids.

1.4.2 Wines not having any genuine impact aroma compound

Wines that do not have any genuine impact aroma compounds include some very interesting wines showing complex aromas which cannot be attributed to a single chemical identity. In the case of whites made with Maccabeo or Chardonnay, for instance, their aroma nuances are related to the simultaneous presence of many relevant aroma families present at quite modest concentrations. For instance, the flowery notes of some of them can be related to the simultaneous presence of small amounts of linalool, γ-lactones, vanillins, ethyl cinnamates and norisoprenoids (Loscos *et al.*, 2007). Their fruity notes are the result of a complex interaction between those compounds and ethyl esters of fatty acids, fusel alcohol acetates and small amounts of some cysteine-related mercaptans, such as 4-mercapto-4-methylpentan-2-one or 3-mercaptohexyl acetate and eventually also to some aliphatic aldehydes (Escudero *et al.*, 2004; Loscos *et al.*, 2007). In these cases, obviously, the quality vectors of wine are extremely complex and multivariate.

1.4.3 Complex wines containing several potential impact compounds

Typical examples of complex wines containing several potential impact compounds are some Chardonnays fermented in barrel or aged *sur lies*. In this case, the concentrations of some fermentation compounds are lower, and several powerful odorants appear. These are whiskylactones, of course, but also diacetyl, methional and furfurylthiol. The aroma is still complex, since it retains a large part of the compounds previously cited, but now the typical woody notes, together with the creamy–buttery nuance given by diacetyl and eventually a cauliflower undertone given by methional and a toasty-coffee-like note given by furfurylthiol, can be easily detected.

Other examples of these types of wines are Sherry-like or Sauterne-like wines. In Sherry, acetaldehyde, diacetyl and several isoaldehydes (isobutyraldehyde, isovaleraldehyde, 2-methylbutyraldehyde) act as a family of impact compounds (Culleré *et al.*, 2007), but they also contain sotolon at high concentrations, which gives them their characteristic nutty flavour (Campo *et al.*, 2008). In the case of Sauternes, wines contain relatively high concentrations of 4-mercapto-4-methylpentan-2-one, 3-mercaptohexan-1-ol, phenylacetaldehyde and sotolon (Campo *et al.*, 2008).

1.4.4 Most complex wines: the case of big reds

Red wines are, by nature, much more complex since, among many other factors, they contain quite large amounts of volatile phenols which exert a suppression effect on fruity notes (Atanasova *et al.*, 2004). This phenomenon is still more intense when the wines have been aged in oak casks, increasing the concentrations of volatile phenols and adding whiskylactones. In this chemical environment, the perception of the different notes, particularly fruity notes, is extremely complex. In addition, great red wines do not have explicit or specific odour nuances, but a large palette of many subtle odours. It is not surprising, therefore, that in red wines, leaving aside whiskylactones, most often we do not find genuine impact compounds,

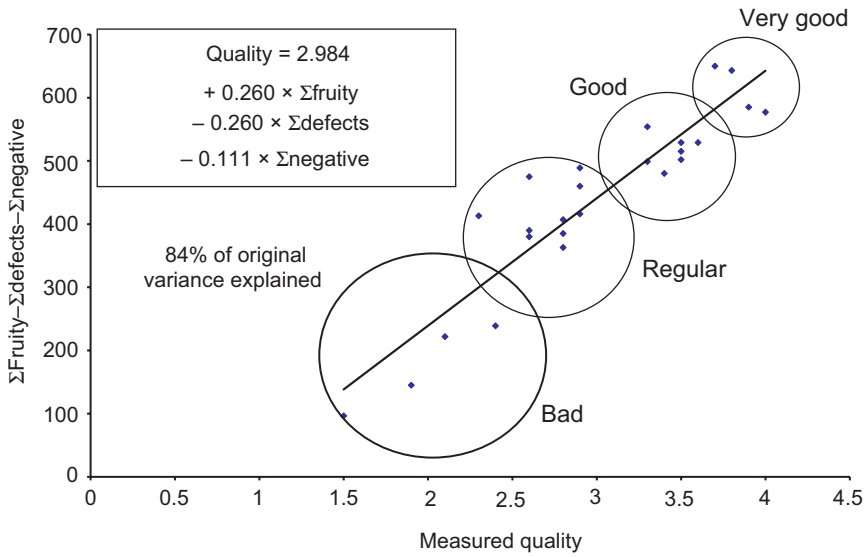

Fig. 1.6 Relationship between the measured sensory quality of high quality Spanish red wines with the quality predicted with a three-parameter model. ΣFruity is the vector comprising all the fruity wine odorants. ΣDefects is the vector comprising 4-ethylphenol, TCA, *o*-cresol and 3,5-dimethyl-2-methoxypyrazine. ΣNegative is a vector comprising 13 aroma chemical compounds with unpleasant aroma (Ferreira *et al.*, 2009).

but relatively large groups of compounds which contribute to the different odour nuances. Up to this date, we have identified several major contributors to the fruity notes of red wines:

1. The concerted action of ethyl esters, including here several recently discovered branched ethyl esters, with norisoprenoids (β-damascenone and β-ionone) and with the enhancing effect of DMS, that can impart berry fruit notes to the wine (Escudero *et al.*, 2007).
2. The concerted action of five γ-lactones (γ-octa-, nona-, deca-, undeca- and dodecalactones) that can be responsible for the peach notes of some reds, particularly from certain areas of Spain and Portugal (Jarauta *et al.*, 2006).
3. The concerted action of furaneol, homofuraneol, maltol, sotolon, norisoprenoids and methional that can be responsible for some cherry and chocolate notes of some reds (Ferreira *et al.*, 2005).

It is very interesting to note the hierarchy of the sensory notes and hence of the aroma chemicals in wine. In a recent study relating the odorant composition, measured by gas chromatography-olfactometry, with wine quality, this parameter was found to be related to three major vectors formed by the summation of groups of odorants (Ferreira *et al.*, 2009), as is shown in Fig. 1.6. The vector with major and more negative influence was formed by the summation of ethyl phenols, TCA and 3,5-dimethyl-2-methoxypyrazine, while most wines were of course not corked (no one was classified as such) nor too rich in ethylphenols. Therefore, this result

not only means that a wine containing a high concentration of ethylphenols or TCA is a bad wine, but that the general quality of a commercial red wine is inversely related to the content it has in these compounds, i.e., all these compounds at concentrations far below the recognition threshold are exerting a strong depressing effect on the wine fruitiness as previously suggested (Aznar *et al.*, 2003).

1.5 Conclusions and future trends

The complexity of wine aroma is in accordance with its chemical complexity. As happens in complex perfumes, and far from the artificially flavoured products, wine aroma is the result of complex interactions between many odour chemicals. Only in some particular and simple cases is it possible to find genuine impact compounds able to transmit to the product their primary sensory descriptors. In the most complex and most valuable products, however, the sensory notes are created by the concerted action of many molecules, many of which, surprisingly, are at concentrations near threshold. As the most important aroma compounds are known and as today there are analytical techniques available for their determination, it is only a matter of time before the different aroma compounds of the most valuable products will be unscrambled and fully understood and their vectors of quality defined. This potential is going to open a number of opportunities in the study of the precursors of the different wine aroma nuances. It is expected that in a first step, the chemical aroma quality vectors for different types of wine or for different aroma sensory attributes will be defined, and that further research will make it possible to determine which ones are the precursors and what is the potential of a given grape or of a recently fermented must to produce a wine of the desired quality. Particular attention will be paid to those aroma chemicals or families of aroma chemicals really able to create a sensory impact. Similarly, the discovery that many 'bad' aroma chemicals have negative consequences for wine quality at concentrations well below those usually considered risky will promote in the industry the need for more efficient and better directed quality control tools. An exciting world of knowledge with much better wines as recompense is ahead for those daring to deal with aroma chemicals in a systematic and comprehensive way.

1.6 Acknowledgements

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2

Wine taste and mouthfeel

V. Cheynier and P. Sarni-Manchado, INRA, UMR 1083, France

Abstract: Taste and mouthfeel are essential elements of wine quality and major drivers for expert evaluation and consumer liking. Major taste qualities in wine are sweetness, sourness and bitterness, contributed by sugars, organic acids and ethanol, respectively, while mouthfeel encompasses a number of inter-related tactile sensations. The present chapter reviews current knowledge on the compounds and mechanisms responsible for wine taste and mouthfeel properties, with special emphasis on astringency, and presents physico-chemical and sensory analysis approaches involved in this research. It then includes a discussion on the impact of some viticulture and wine-making practices on wine composition and associated taste and mouthfeel properties.

Key words: taste, mouthfeel, astringency, bitterness, sourness, tannins, interactions, acids, alcohol, glycerol, polysaccharides, proteins, saliva.

2.1 Introduction

Sourness, astringency and bitterness are major sensory attributes of red wines while white wines are usually not astringent but can be perceived as sour or bitter. Sourness is due to organic acids and related to pH. Bitterness and astringency are primarily elicited by phenolic compounds but can be enhanced by ethanol and acids, respectively. Sweetness is mostly imparted by sugars, although glycerol contribution has been reported. Other common sensory descriptors in wine tasting include hotness, associated with ethanol, and viscosity, which has been attributed to ethanol, glycerol, sugar or polysaccharides. Thus, taste and mouthfeel are essential elements of wine quality and major drivers for expert evaluation and consumer liking. Mouthfeel encompasses a number of inter-related sensations,

among which astringency, contributed by tannins, is especially important for red wine quality. It is commonly accepted that astringency is a tactile sensation (Breslin *et al.*, 1993; Green, 1993; Clifford, 1997; Noble, 1998), resulting from interactions of tannins with salivary proteins and glycoproteins (Bate-Smith, 1954), and/or from subsequent adsorption of excess tannins onto the mouth epithelium (Guinard *et al.*, 1986b; Green, 1993; Clifford, 1997). However, its physico-chemical bases are still poorly understood. Major advances have been made in the understanding of astringency perception since the 1990s. The structure of grape tannins and of some of their reaction products in wine has been unravelled. The availability of well-characterised tannin fractions has made it possible to study the relationships between structure of grape and wine tannins, their interaction properties and their sensory perception. Similar progress has been made on structural determination of wine polysaccharides, and their influence on the astringency of tannins has been determined.

The present chapter reviews current knowledge on the compounds and mechanisms responsible for wine taste and mouthfeel properties, with special emphasis on astringency. It also presents new developments in sensory analysis and physico-chemistry approaches involved in this research. Finally, it includes a discussion on the impact of some viticulture and wine-making practices on wine composition and associated taste and mouthfeel properties.

2.2 Components contributing to taste and mouthfeel properties

2.2.1 Definitions

In theory, the term taste should be restricted to five particular qualities: sweetness, sourness, bitterness, saltiness and umami. These sensations are mediated by specialised neuroepithelial cells (taste receptor cells), clustered into onion-shaped organs (taste buds), which specifically detect the dissolved substances that come in contact with them (Kinnamon and Margolskee, 1996). Nevertheless, this term, as well as the term flavour, is often used more broadly to designate taste together with other sensations: on the one hand, retro-nasal perception of aroma, involving interactions of volatile compounds with olfactory receptors situated in the nose cavity, on the other hand, tactile sensations felt in the mouth such as heat or astringency, which correspond to mouthfeel or texture. Unlike perception of taste or smell, that of mouthfeel attributes is experienced through the sense of touch and the related signals transmitted to the brain by the trigeminal nerve.

Texture, employed for describing fabric long before being used for foods and beverages, has been defined as 'the attribute of a substance resulting from a combination of physical properties and perceived by the senses of touch, sight, and hearing' (Jowitt, 1974), following from the earlier definition of Szczesniak (Szczesniak, 1963). Mouthfeel is specifically the tactile properties perceived once the food or beverage is placed in the mouth (Guinard and Mazzucchelli, 1996). In the context of wines, texture, like mouthfeel, has been defined as 'the dimension of

tasting that draws together attributes such as smoothness or astringency that produce tactile rather than flavour sensations on the surface of the mouth' (Robinson, 2006). In addition, a narrower sense of texture, restricted to creamy and syrup attributes, has been proposed within a mouthfeel wheel developed for structuring the vocabulary associated with these complex characteristics in red wine (Gawel *et al.*, 2000). To avoid such confusions, it thus seems preferable to use the term mouthfeel rather than texture, although oral sensations can be influenced by visual perception of viscosity or colour.

As aroma compounds are treated in Chapter 1, this chapter will focus on perceptions in the mouth, using the terms taste and mouthfeel, respectively, to designate perceptions that are tasted through interactions with taste receptors and those that are felt by tactile sensations.

2.2.2 Compounds contributing to wine taste and mouthfeel properties

Wine contains a wide array of compounds, that vary in quantity and composition depending on the grape and wine-making process. Some of these constituents are well known to impart taste or mouthfeel properties. Moreover, many compounds, including some that do not have a direct effect, modify wine perception, due to interactions processes within the wine matrix and in the mouth.

Wine is primarily a hydro-alcoholic solution acidified to pH values in the range 3–4 by organic acids. Major organic acids in grapes are tartaric acid and malic acid, but the latter is often converted to lactic acid through malo-lactic fermentation in wines. Wines also contain large amounts of glycerol (5–10 $g.L^{-1}$), which represents their third constituent after water and ethanol (Flanzy, 1998). The compounds most cited with respect to taste and mouthfeel in wine are certainly phenolic compounds, which contribute bitterness and astringency (Noble, 1990, 1998). These compounds are extremely diverse in nature, from very simple molecules, to large complex polymers. Their structures and physico-chemical properties, as well as their impact on taste and mouthfeel, will be discussed in detail below. Other macromolecules present in wine, namely proteins and polysaccharides, are known to interfere with polyphenols and thus modify their perception.

Proteins are relatively minor in wine, with concentrations up to 300 $mg.L^{-1}$ in un-fined wines (Waters *et al.*, 2005) but usually below 100 $mg.L^{-1}$ (Fukui and Yokotsuka, 2003; Jones *et al.*, 2008a). Wine proteins originate mostly from grape, but yeast proteins have also been detected by immunodetection (Dambrouck *et al.*, 2003) and by mass spectrometry (Yokotsuka *et al.*, 1991; Kwon, 2004). Major protein fractions responsible for heat-induced haze in white wine have been identified as thaumatin-like proteins and chitinases (Waters *et al.*, 1996). Wine also contains glycoproteins including, in particular, yeast mannoproteins that act as haze-protective factors (Waters *et al.*, 2005). These will be presented below along with polysaccharides as they are heavily glycosylated.

Polysaccharides (Table 2.1), released from the cell walls of both grapes and yeast in the course of wine-making, are found in wine in concentrations ranging from 0.2 to 1 $g.L^{-1}$ (Doco *et al.*, 1999). Pectic polysaccharides originating from

Table 2.1 Average polysaccharide concentrations in wines

	Red wines	White wines
Mannoproteins[b]	100–150 mg.L^{-1}	100–150 mg.L^{-1}
Pectic polysaccharides		
AG and AGP[c,d]	100–200 mg.L^{-1}	50–150 mg.L^{-1}
RGII[a]	50–250 mg.L^{-1}	10–50 mg.L^{-1}

[a]Doco *et al.*, 1999.
[b]Vidal *et al.*, 2003.
[c]Brillouet *et al.*, 1990.
[d]Pellerin *et al.*, 1995.

grapes are arabinans, arabinogalactans (AG) and rhamnogalacturonans while yeast polysaccharides are mannans and mannoproteins that are excreted during fermentation or released after autolysis. Arabinogalactans and arabinogalactan proteins (AGP) are the major polysaccharides in wines (Brillouet *et al.*, 1990; Pellerin *et al.*, 1995). Rhamnogalacturonan II (RGII), containing rare sugars, is also very abundant in wines as it resists enzymatic degradation (Doco and Brillouet, 1993). Its concentration increases during skin contact and is thus much higher in red wines than in white wines. Other grape polysaccharides include rhamnogalacturonan I, but its concentration is rather low in wine (< 20 mg.L^{-1}) (Pellerin *et al.*, 1995). Small amounts of arabinans have also been reported (Pellerin *et al.*, 1995). They are quantified together with arabinogalactans as polysaccharides rich in arabinose and galactose (PRAG).

2.3 Physico-chemical bases for astringency perception

Astringency, coming from the latin *ad* (to) and *stringere* (bind), is defined as 'the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins'(ASTM, 1989). The nature of physiological processes underlying this perception has been a matter of debate, some authors suggesting that it is a primary taste like bitterness or sourness (Schiffman *et al.*, 1992; Critchley and Rolls, 1996). Nevertheless, it is widely accepted to be triggered by a tactile sensation, based on the following arguments. Astringency perception is perceived even in regions of the mouth epithelium devoid of primary taste sensory receptors, although interaction of astringent compounds with taste pathways cannot be precluded (Breslin *et al.*, 1993). Moreover, it does not decrease in successive exposures, as observed with sensations involving taste or smell receptors, but rather increases, especially as the delay between ingestions is shortened (Guinard *et al.*, 1986b).

Although acids also elicit astringency, in wine, this sensation is primarily associated with tannins (Noble, 1998). Tannin astringency is considered to be related to their ability to precipitate salivary proteins. A two-stage mechanism, involving adsorption of tannins on mouth epithelium as the salivary proteins

become depleted, has also been proposed (Guinard *et al.*, 1986b). However, interactions of tannins with proteins do not necessarily lead to precipitation. Formation of soluble or colloidal complexes may induce sufficient conformational changes to alter the lubricating properties of salivary proteins and generate the in-mouth friction that triggers astringency perception (Bate-Smith, 1973). Rheological measurement of saliva visco-elastic properties has shown significant reduction of saliva elasticity upon mixing with citric acid or epigallocatechin gallate. This suggests that astringency arises from disruption of the salivary film lubricating the mouth surface following salivary protein aggregation, although this may not be the only mechanism involved (Rossetti *et al.*, 2008). Complexation and precipitation depend on the structure and conformation of both the tannins and the proteins but also on the medium composition. Information on the mechanisms involved in interaction, precipitation and adsorption processes is essential for understanding the physico-chemical bases of astringency perception.

2.3.1 Saliva composition

Saliva ensures the lubrication of mouth tissues and facilitates mastication and ingestion. It is involved in maintenance of tooth mineralisation and control of the mouth microflora and participates in predigestion as it contains enzymes. Human saliva, secreted by three major types of glands (parotid, submandibular and sublingual), along with minor glands, contains over 99% water and about 0.5% w/v of solids, mainly minerals and proteins. Salivary protein concentration commonly ranges from 1 to 3.5 $mg.mL^{-1}$ (Bennick, 1982), but much higher values (up to 8.2 $mg.mL^{-1}$) have been reported for some individuals (Clifford, 1997). Proline-rich proteins (PRP) represent about 70% of the proteins in parotid saliva (Kauffman and Keller, 1979) and are also produced by submandibular glands. They are present in the saliva of primates and herbivorous animals but almost absent in that of carnivores. Moreover, they are induced in some rodents upon feeding tannin-rich diets. Such diets can be lethal to hamsters in which they are not induced (Mehansho *et al.*, 1987a). Differences in salivary protein contents in children and adults, except in populations on low-tannin diets (Clifford, 1997), also suggest induction of salivary proteins in response to dietary tannin exposure in humans.

PRP are divided into acidic, basic, and glycosylated types which, despite their sequence similarities, have different functions (Bennick, 1982). The primary structure of PRP shows repeated sequences, with high proportions of proline, glycine and glutamine (in basic PRP) or glutamic acid (in acidic PRP) residues. Proline represents alone 40% and, together with glycine and glutamine, 80% of amino acid residues in basic PRP. Because of this particular primary sequence, PRP do not show a folded three-dimensional structure and thus belong to a particular family of proteins called natively unfolded or instrinsically unstructured proteins (Tompa, 2002, 2003; Uversky, 2002). The functions of these proteins, related to increased binding specificity or resistance to changes in environmental conditions, are imparted by their flexible open structure and further enhanced by repetitive segment sequences (Tompa, 2003). The only known role of basic PRP is

to bind dietary tannins (Lu and Bennick, 1998) and thus prevent the deleterious health effects of these compounds (Mehansho *et al.*, 1983, 1987b). Histatins, that are peptides with high levels of histidine, arginine and lysine in their amino acid sequence, also found in saliva, have been shown to precipitate tannins even more efficiently than PRP (Yan and Bennick, 1995). Glycosylated PRP, which accounts for 17% of salivary proteins and 25% of PRP in humans (Bennick, 1982), may participate in mouth lubrication (Hatton *et al.*, 1985). Mucins, another class of glycoproteins in saliva, are major components of the visco-elastic mucous barrier coating the mouth epithelium, believed to contribute to saliva viscosity (Strous and Dekker, 1992). However, mucins do not mimic the rheological properties shown by whole human saliva (Rossetti *et al.*, 2008).

2.3.2 Grape tannins

Tannins have been defined as water-soluble phenolic compounds originating for plants with molecular weights in the range 500–4000 (Haslam, 1979), the higher molecular weight compounds being considered insoluble and consequently not astringent (Lea and Arnold, 1978). However, the isolation from grape (Souquet *et al.*, 1996) and apple (Guyot *et al.*, 1997, 2001) of higher molecular weight tannins that are both soluble in 12% alcohol and astringent (Vidal *et al.*, 2003) has led to this upper limit being revised.

Tannins (Fig. 2.1) can be divided into several groups: hydrolysable tannins, which are polyesters of sugars and gallic or ellagic acids, condensed tannins, also called proanthocyanidins, which are oligomers and polymers of flavan-3-ols, and complex tannins in which both types are covalently bound. Proanthocyanidins are the only tannins found in grape. They make up a large group of compounds that differ by the nature of their constitutive units, their number (degree of polymerisation) and the position of linkages between them, as illustrated in Fig. 2.1. When heated under acidic conditions, proanthocyanidins release red anthocyanidin pigments, and several groups of proanthocyanidins can be distinguished, based on the nature of the anthocyanidin released. Thus, polymers of (epi)catechin, that release cyanidin, are called procyanidins while polymers of (epi)gallocatechin, that release delphinidin, are called prodelphinidins.

The presence of both procyanidins and prodelphinidins in grape extracts was established by acid degradation and ^{13}C nuclear magnetic resonance (NMR) analysis back in 1980 (Czochanska *et al.*, 1980). Reversed-phase high-performance liquid chromatography (HPLC) analysis has enabled the detection of monomers, dimers and trimers in grape extracts, but its resolution becomes very poor as the molecular weight increases, due to the larger number of isomers. Polymer characterisation can be achieved after acid catalysed cleavage in the presence of a nucleophilic agent (toluene α-thiol (thiolysis) or phloroglucinol (phloroglucinolysis)). In these methods, the upper units (initially substituted in C4) are released as carbocations that react with the nucleophile to yield an adduct, while the terminal unit (initially substituted in C8 or C6) is released as such. HPLC analysis of the reaction products (lower units and adducts from upper units) gives

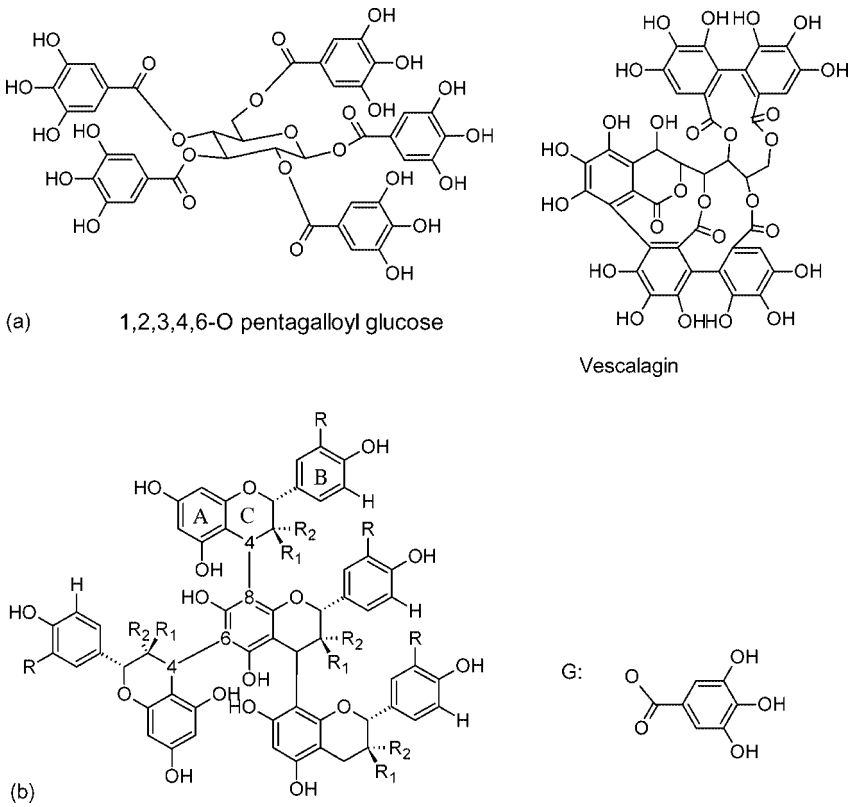


Fig. 2.1 Examples of hydrolysable (a) and condensed tannins (b).

access to the nature and proportions of each constitutive unit, the total proanthocyanidin concentration (calculated as the sum of all units) and the mean degree of polymerisation (mDP, calculated as the ratio of terminal units to total units).

Grape seed tannins are partly galloylated procyanidins consisting of catechin, epicatechin and epicatechin 3-gallate units (Prieur *et al.*, 1994), while proanthocyanidins from skins (Souquet *et al.*, 1996), stems (Souquet *et al.*, 2000) and pulp (Mané *et al.*, 2007; Verries *et al.*, 2008) are much less galloylated and also contain epigallocatechin units. In addition, the mDP of seed proanthocyanidins is much

Table 2.2 Average tannin composition of grape berries

	% Galloylated procyanidins	% Prodelphinidins	Content	mDP
Grape berry				
Stems	++	+	+	9
Skins	–/+	+++	+++	30–51
Pulp	++	++	Trace	18–46
Seed	+++	–	+++	5–11

lower than that of skin proanthocyanidins, that calculated for pulp proanthocyanidins being intermediate. The tannin content is higher in seeds than in skins and very low in pulp (Souquet *et al.*, 1996), but the contribution of skins to the berry content may be predominant in some varieties (Souquet *et al.*, 2006; Mané *et al.*, 2007) (Table 2.2).

2.3.3 Wine tannins

Wine tannin composition depends on that of the grape from which the wine is made but also on tannin extraction from the various berry compartments and on their subsequent reactions in wine. Besides, wines aged in barrel or with some added oenological tannins may also contain ellagitannins originating from oak (e.g. vescalagin and castalagin; Fig. 2.1). Moreover, tannins are rather unstable compounds, which undergo various types of reactions, leading to further complexity of wine composition. The role of these reactions in colour and astringency changes occurring during wine ageing has been known for a long time. Various reaction mechanisms have been postulated in pioneer works (Hathway and Seakins, 1957; Haslam, 1966; Somers, 1971; Ribéreau-Gayon, 1982). Major progress has been recently achieved. Studies carried out in parallel in wines and in model solutions have enabled structural determination of numerous products, confirming the occurrence in wine of some of the known reactions and leading to the discovery of others so far unsuspected.

2.3.4 Chemical reactivity of tannins

Reactions of proanthocyanidins involve three major processes, based on the chemical reactivity of the compounds (Fulcrand *et al.*, 2006). Flavan-3-ols have a nucleophilic character (i.e. show an electron excess and are able to react with electrophiles, showing an electron deficiency) in the C6 and C8 positions of their A-ring. They are also prone to oxidation, especially on their *o*-diphenolic B-ring, oxidised to electrophilic *o*-quinones. In addition, the interflavanic bonds of proanthocyanidins are susceptible to acid-catalysed cleavage. This reaction, spontaneous at wine pH, yields a strongly electrophilic intermediate. Based on these reactivities, flavan-3-ols undergo electrophilic substitution reactions in which they act either as nucleophiles (through their A-ring) or as electrophiles (through their B-ring after oxidation or C-ring after acid-catalysed cleavage of interflavanic linkages).

Oxidation reactions

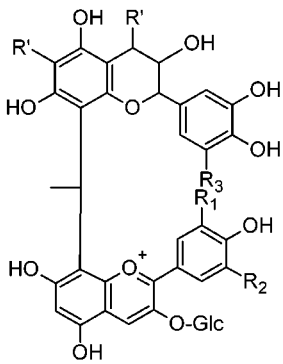
Phenolics oxidise through enzymatic and non-enzymatic reactions. Enzymatic reactions catalysed by grape polyphenoloxidase (PPO) take place very early in the winemaking process, when the grape cells are damaged. With the exception of monomers, flavan-3-ols are not substrates for grape PPO, but they can be involved in enzymatic oxidation through reactions with quinones generated from other PPO substrates, such as hydroxycinnamic acids. Thus, (epi)catechin, epicatechin 3-gal-

late (Cheynier *et al.*, 1988) and procyanidins (Cheynier and Ricardo Da Silva, 1991) can be oxidised to the corresponding quinones by caffeoyltartaric acid quinone, in a coupled oxidation reaction. Moreover, flavan-3-ols, acting as nucleophiles, can react with the quinones arising from enzymatic or coupled oxidation. In particular, catechin dimers, in which the B-ring of one unit is linked with the A-ring of the other, can be formed by reaction between catechin and its quinone (Guyot *et al.*, 1996b). These molecules (dehydrodicatechins) are isomers of procyanidin dimers and can also be regarded as tannins. After fermentation, chemical reactions become prevalent. Chemical oxidation of catechin yields dehydrodicatechins, like enzymatic oxidation (Young *et al.*, 1987; Guyot *et al.*, 1996b). When other phenolics are present, co-dimers can be formed. In particular, addition of anthocyanins onto catechin quinones has been observed in model solutions (Duenas *et al.*, 2006).

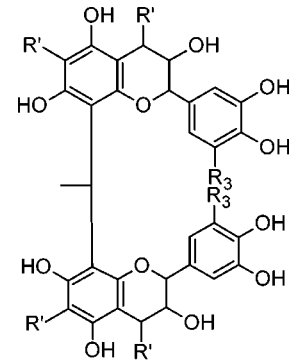
Autoxidation of flavan-3-ols is very slow in acidic media like wine but can be accelerated by metal ion catalysts. However, in the presence of iron (Oszmianski *et al.*, 1996) or copper (Clark and Scollary, 2002; Es-Safi *et al.*, 2003), other types of pigments, identified to xanthylium cations (Es-Safi *et al.*, 1999b), were formed predominantly. These pigments result from condensation of flavan-3-ols with glyoxalic acid, which is an oxidation product of tartaric acid. Its formation, like oxidation of ethanol to acetaldehyde (Wildenradt and Singleton, 1974), requires the presence of phenolic compounds and catalysts such as metal ions. Both aldehydes, as well as their reaction products, can be considered as markers of oxidation in wine (Fulcrand *et al.*, 2006).

Condensation with aldehydes

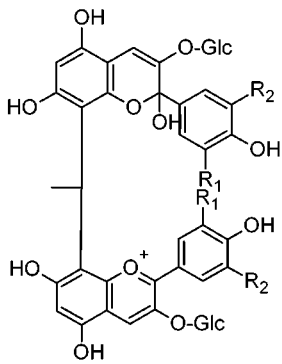
Anthocyanins and flavan-3-ols react with acetaldehyde to form purple pigments in which one anthocyanin and one flavan-3-ol are linked in their C8 positions through a methylmethine bond, often (and improperly) called ethyl bond (Timberlake and Bridle, 1976). In this condensation reaction, both flavonoids act as nucleophiles, the electrophilic species being the aldehyde, under its protonated form. Oligomers containing variable proportions of flavan-3-ol and anthocyanin units (Es-Safi *et al.*, 1999a) (Fig. 2.2 (1)), only flavan-3-ols (Fig. 2.2 (2)) (Fulcrand *et al.*, 1996), and only anthocyanins (Fig. 2.2 (3)) (Atanasova *et al.*, 2002b) were formed by the same mechanism. This indicates that flavan-3-ols and anthocyanins compete in this reaction and that C6 and C8 are reactive in both types of units. The methylmethine linkages, like the interflavanic bonds, are sensitive to acid-catalysed cleavage. This releases vinylflavanol derivatives that can react with anthocyanins to yield the orange flavanylpyranoanthocyanins (Fig. 2.2 (4)) (Cheynier *et al.*, 1999; Mateus *et al.*, 2002). Blue pigments in which the flavanols are linked to pyranoanthocyanins through an additional vinyl bridge have also been detected (Mateus *et al.*, 2003). Glyoxylic acid (Fulcrand *et al.*, 1997), furfural and 5-hydroxymethylfurfural that are degradation products of sugars (Es-Safi *et al.*, 2000b) and other aldehydes present in fortifed wines (Pissara *et al.*, 2003) react in the same way as acetaldehyde, but the resulting oligomers further proceed to xanthylium pigments (Es-Safi *et al.*, 1999c, 2000a). However, to our knowledge, none of these products has been detected in wine.



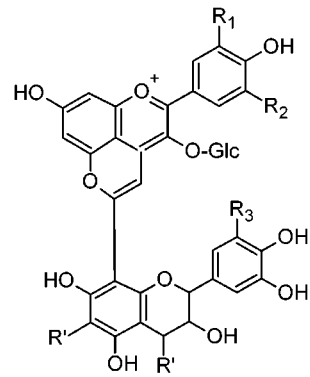
Flavan 3-ol-ethyl-anthocyanin (1)



Flavan 3-ol-ethyl-flavan 3-ol (2)



Anthocyanin-ethyl-anthocyanin (3)



Flavanylpyranoanthocyanin (4)

Anthocyanin units:

R_1 = OH R_2 = H: cyanidin

R_1 = OH R_2 = OCH_3: petunidin

R_1 = R_2 = OH: delphinidin

R_1 = R_2 = OCH_3: malvidin

R_1 = OCH_3 R_2 = H: petunidin

Flavan 3-ol units:

R_3 = H or OH

R' = H or flavan 3-ol

Fig. 2.2 Derived tannins resulting from reactions with acetaldehyde.

Direct reactions of flavan-3-ols with anthocyanins.
Two groups of direct anthocyanin/flavan-3-ol reactions have been demonstrated in wine (Fig. 2.3). In the first one (Fig. 2.3a), an anthocyanin adds onto the carbocation generated by acid-catalysed cleavage of a proanthocyanidin interflavanic linkage, to yield a flavanol–anthocyanin (F–A) adduct (Salas *et al.*, 2003). This reaction consists in replacing part of the tannin chain by an anthocyanin monomer, so that the resulting species are usually shorter than their tannin precursor. In the second reaction (Fig. 2.3b), addition of a flavan-3-ol onto an anthocyanin yields a flavene anthocyanin-flavan-3-ol (A–F) intermediate that can oxidise to the corresponding flavylium pigment (Somers, 1971) or convert to colourless A-type species (Bishop and Nagel, 1984; Remy-Tanneau *et al.*, 2003).

Few papers are available on the reactions of ellagitannins in wine, but they are also oxidisable, due to the presence of numerous *o*-triphenolic rings in their structure. Besides, vescalagin, when incubated in mildly acidic conditions, generates an electrophilic intermediate that undergoes nucleophilic substitution with flavan-3-ols and anthocyanins to yield complex tannin structures (Quideau *et al.*, 2003).

All these molecules can be considered as tannins; those containing anthocyanin units are often referred to as polymeric pigments or pigmented tannins.

2.3.5 Tannin–protein interactions

Tannin–protein association, as well as self-association of polyphenols, involves first formation of soluble complexes that can aggregate further and precipitate out. This second step is strongly dependent on the polyphenol structure and on other components present in the medium. Tannin–protein interactions have been extensively studied, using a variety of techniques and experimental models, in relation with haze, astringency and anti-nutritional effects. Earlier works have measured proteins and/or tannins remaining in solution after precipitation. To avoid problems related to interferences of tannins with the reagents classically used in protein assay, particular proteins (coloured such as methylene blue or haemoglobin (Porter and Woodruffe, 1984; Okuda *et al.*, 1985), or presenting an enzymatic activity) have been proposed. However, these measurements may not reflect the intensity of astringency as tannin–protein interactions show some specificity (Hagerman, 1989). Moreover, they do not take into account the formation of soluble complexes which may also play a part in astringency perception. Other investigations have been focussed on the mechanisms involved in interaction processes, using thermodynamic methods such as equilibrium dialysis or microcalorimetry (McManus *et al.*, 1985; Artz *et al.*, 1987; Pascal *et al.*, 2007; Poncet-Legrand *et al.*, 2007b). Information on the structure of soluble complexes has been provided by spectrometric methods such as NMR (Luck *et al.*, 1994; Murray *et al.*, 1994; Hatano and Hemingway, 1996; Baxter *et al.*, 1997), sometimes associated with molecular modelling (Vergé *et al.*, 2002a; Simon *et al.*, 2003), circular dichroism (Simon *et al.*, 2003; Pascal *et al.*, 2006) and mass spectrometry (Sarni-Manchado and Cheynier, 2002; Vergé *et al.*, 2002b; Simon *et al.*, 2003; Chen and Hagerman,

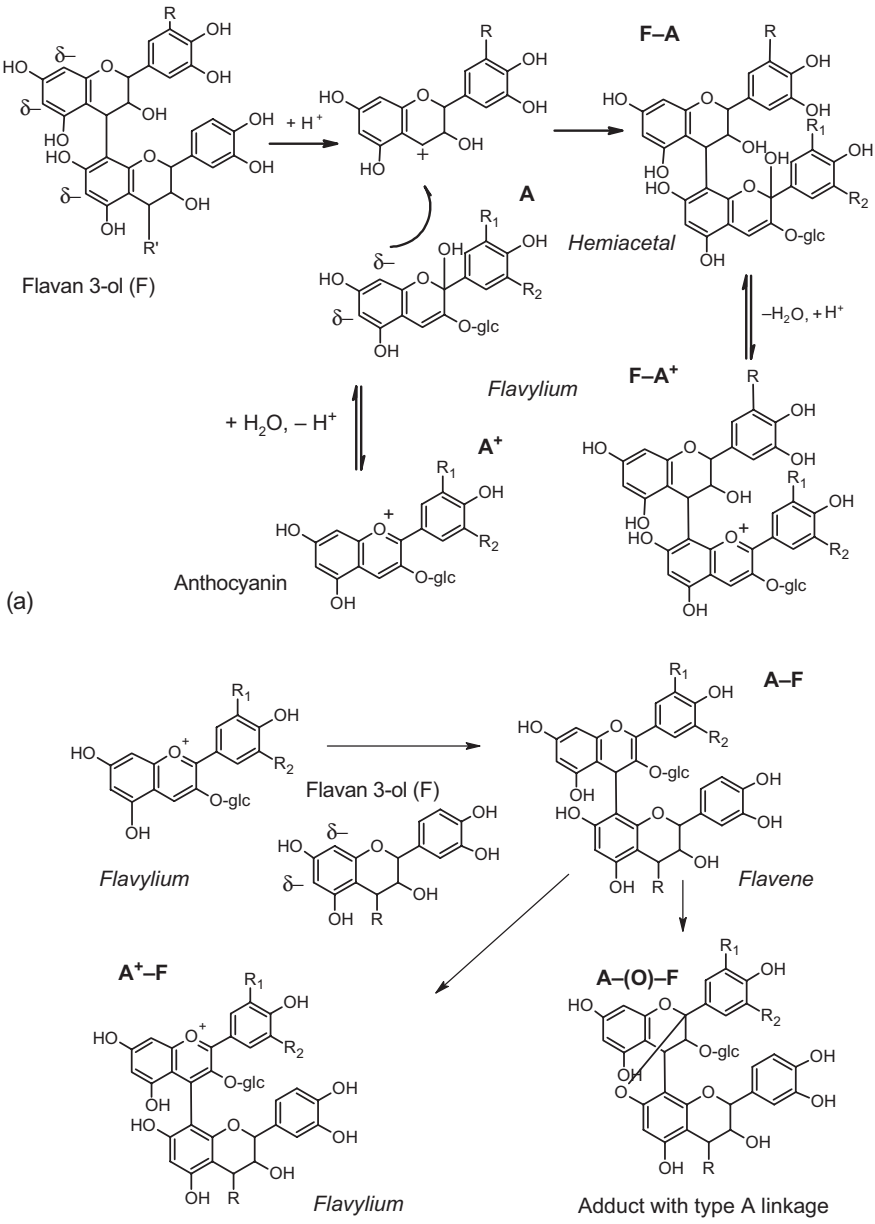


Fig. 2.3 Direct reactions of tannins with anthocyanins: (a) formation of F–A; (b) formation of A–F (R_1, R_2, R, R' as in Fig. 2.2).

2004), while colloidal behaviour of the complexes has been studied by dynamic light scattering (Pascal *et al.*, 2006; Poncet-Legrand *et al.*, 2006).

Influence of tannin structure

Tannin affinity towards proteins depends primarily on the number of *o*-diphenolic rings that are the major interaction sites (Haslam, 1974; McManus *et al.*, 1985; Haslam and Lilley, 1988). The presence of several such groups in the molecule enables formation of bridges between proteins (McManus *et al.*, 1985) and with other polyphenols (Baxter *et al.*, 1997). Procyanidins and galloylated flavan-3-ol monomers aggregate through auto-association (Poncet-Legrand *et al.*, 2003) and through interaction with poly-L proline (Poncet-Legrand *et al.*, 2006) while non-galloylated monomers do not. Aggregation of procyanidins increases with their concentration and with their chain length (Riou *et al.*, 2002). Association constants (10^4–10^5 M^{-1}) determined by isothermal titration microcalorimetry indicated relatively high affinity of galloylated flavan-3-ol monomers for poly-L-proline (Poncet-Legrand *et al.*, 2007b). In the case of procyanidin oligomers, a cooperative mechanism, in which already bound procyanidins increase the following binding, has been established.

Other approaches similarly demonstrated that the formation of soluble (Baxter *et al.*, 1997; Sarni-Manchado and Cheynier, 2002) and of insoluble (Kennedy *et al.*, 1984; Okuda *et al.*, 1985; Kumar and Horigome, 1986; Ricardo da Silva *et al.*, 1991; Maury *et al.*, 2001) complexes in the presence of various peptides or proteins increases with tannin DP and with galloylation. C4–C6 linked dimers were also more precipitated than their C4–C8 isomers (Ezaki-Furuichi *et al.*, 1987; Ricardo da Silva *et al.*, 1991), indicating a role of molecular conformation in interaction properties. The conformational mobility of the phenolic molecules appears essential for protein binding as shown by the much lower affinity for bovine serum albumin (BSA) of rather rigid vescalagin and castalagin, compared to flexible penta-*O*-galloyl-D-glucose (Beart *et al.*, 1985). Higher protein affinity has been reported for prodelphinidins than for procyanidins (Hagerman, 1989). In agreement with this finding, epigallocatechin gallate interacted more strongly than epicatechin gallate with immobilised salivary proteins (Bacon and Rhodes, 1998). The same trend was found when studying their complexation with the human salivary PRP IB5 by mass spectrometry (Canon *et al.*, 2008), but the reverse was found when calculating their binding constants with poly L-proline (Poncet-Legrand *et al.*, 2007b).

Very little is known about protein interactions of derived tannins present in wine. Catechin dimers generated by enzymatic oxidation of catechin and procyanidin B3 similarly inhibited β-glucosidase activity (Guyot *et al.*, 1996a). Oxidation of epigallocatechin gallate, generating polymeric species, also resulted in enhanced protein interactions as evidenced by changes in casein adsorption properties at the air/liquid interface (Sausse *et al.*, 2003). Methylmethine-linked catechin oligomers (Fig. 2.2) formed colloidal particles that further aggregated and precipitated out (Saucier *et al.*, 1997). Under the same conditions, procyanidins also aggregated, but no precipitation occurred (Saucier *et al.*, 1996). This may be

related to differences in molecular weight, as the mDP was estimated at 9 for methylmethine-linked oligomers versus 5 for the procyanidin fraction, and to the higher hydrophobicity of the acetaldehyde condensation products. Replacement of flavan-3-ol units by anthocyanins in the structure of wine-derived tannins has been suggested to result in decreased astringency, but its impact on interactions with protein has not yet been investigated.

Influence of protein structure

All proteins co-precipitate with tannins, but some specificity has been reported. For instance, the PRP of herbivorous mammals show particular affinity for the tannins ordinarily found in their diet (Hagerman and Robbins, 1993). Proline-rich sequences, encountered in PRP but also in proteins most commonly used as fining agents (e.g. gelatin, casein), are particularly prone to interact with tannins. This is attributed to their open and flexible sequence. A NMR study performed on a short PRP sequence has shown that prolines in proline clusters are the major residues involved in interactions with pentagalloylglucose (Murray *et al.*, 1994).

Interaction involves stacking of the planar proline cycle with the phenolic ring, stabilised by hydrogen bondings with the peptide bonds adjacent to the proline and with other amino acids like glycine. Involvement of both enthalpy-driven (attributed to hydrogen bonding) and entropy-driven (associated with hydrophobic effect and conformational changes) phenomena in interactions of flavan-3-ols with poly-L-proline was confirmed by isothermal titration calorimetry (Poncet-Legrand *et al.*, 2007b). The former is prevalent in the case of flavanol monomers and the latter in that of polymers. Other studies performed with procyanidin dimers established the prevalence of hydrogen bonding (Hatano and Hemingway, 1996; Hagerman *et al.*, 1998; Simon *et al.*, 2003). Interaction did not alter the secondary structure of a proline-rich peptide, but its conformational dynamics was reduced (Simon *et al.*, 2003). This is probably related to the short peptide length, as studies carried out with β-casein (Jobstl *et al.*, 2004) and with the entire salivary PRP sequence (Pascal *et al.*, 2006) demonstrated that interaction occurs in successive stages, involving first binding of the ligands together with folding of the protein, followed by formation of relatively small aggregates, and further aggregation leading to precipitation. Cooperative binding implicating polyphenol/polyphenol and polyphenol/protein interactions has been reported for a proline-rich peptide (Baxter *et al.*, 1997). The affinity increases with the peptide chain length (Charlton *et al.*, 2002), and the stability of tannin–protein complexes increases strongly with the number of repeated amino-acid sequences (Charlton *et al.*, 1996). This suggests that longer peptides can wrap around the polyphenol and interact with it simultaneously through several sites. An interesting consequence is that only PRP–tannin complexes made with full length PRP sequences resist degradation under conditions such as encountered in digestion (Lu and Bennick, 1998).

Glycosylation of the proteins favours their association with tannins but enhances the solubility of the resulting complex (Asquith *et al.*, 1987; Sarni-Manchado *et al.*, 2008). Consequently, precipitation of mucins (Jones and Nangan, 1977) and

of glycosylated salivary PRP (Sarni-Manchado *et al.*, 1999, 2008) requires large concentrations of tannins.

Other factors influencing interaction

Self-association of tannins, as well as their interactions with proteins and with other macromolecules, depends on the medium composition. Aggregation of tannins increases with ionic strength and decreases with ethanol concentration, confirming the role of hydrophobic interactions in the process (Poncet-Legrand *et al.*, 2003). Tannin–protein interactions depend on the stoichiometry and on the concentration of both species. Precipitation of the complexes can be reversible or irreversible. Increasing the amount of proteins may induce redistribution of tannins between the protein molecules and dissolution of the precipitates (Haslam *et al.*, 1992b). This seems specific to proteins with open conformation (Luck *et al.*, 1994) but has not been observed with salivary PRP (Haslam *et al.*, 1992b). It also depends on the polyphenol: redissolution of complexes has been observed with procyanidin dimers and trimers but not with higher molecular weight fractions (Prieur-Delorme, 1994). Procyanidin aggregation is strongly modified by the presence of some polysaccharides (Riou *et al.*, 2002). RGII monomer had no effect while RGII dimer promoted aggregation. Arabinogalactan proteins and mannoproteins isolated from wine did not prevent procyanidin aggregation but strongly inhibited particle growth. The stabilising effect of mannoproteins decreased as their molecular weight increased and with increased ionic strength, suggesting a steric stabilisation mechanism (Poncet-Legrand *et al.*, 2007a). Polysaccharides also limited precipitation of tannin protein complexes, due to formation of soluble ternary protein–polysaccharide–tannin complexes (Haslam *et al.*, 1992a; Luck *et al.*, 1994; Cheynier *et al.*, 2006).

Other variables such as solvent characteristics and temperature affect complexation and precipitation. Increasing the temperature weakens the affinity between tannins and PRP but favours larger particles (Charlton *et al.*, 2002). It enhances BSA-induced precipitation of pentagalloylglucose, but not of a procyanidin dimer, supporting different interaction mechanisms for the two tannins (Hagerman *et al.*, 1998). Increasing pH between 3.8 and 6.0 did not affect affinities but increased aggregation, suggesting that smaller particles are stabilised by charge repulsion between them and that precipitation occurs through phase separation (Charlton *et al.*, 2002).

Ionic strength, pH and ethanol affect the solubility of proteins and of tannin–protein complexes. Aggregates formed between a human salivary PRP and epigallocatechin gallate were more stable when the ionic strength was increased with sodium chloride or sodium tartrate (Pascal *et al.*, 2006). In 12% ethanol, partial aggregation of the PRP decreased its ability to interact with epigallocatechin gallate. Precipitation of BSA by a procyanidin dimer was not modified in the presence of 25% methanol whereas precipitation by pentagalloylglucose was decreased (Hagerman *et al.*, 1998). Again, this indicates that different mechanisms, driven by hydrogen bonding and hydrophobic coating of the tannin on the surface of the protein, respectively, are involved in the precipitation process.

2.3.6 Physico-chemical methods to predict wine astringency

Several assays have been proposed to estimate tannin composition and/or predict astringency in wine. Their general principle consists in measuring turbidity or precipitation induced by addition of phenolic compounds or extracts to protein, glycoprotein or polysaccharide solutions, assuming that this correlates with the astringency intensity.

Bate-Smith was first to propose haemanalysis, based on haemoglobin precipitation (Bate-Smith, 1973). However, this method suffered from various interferences. An alternative assay, based on precipitation with BSA, dissolution of the precipitate in alkaline medium and total phenol measurement by a colour reaction using ferric chloride, was developed for quantitative determination of tannins (Hagerman and Butler, 1978). A gelatin precipitation method, in which BSA is replaced with gelatin and proanthocyanidins are assayed specifically by spectrophotometry after heating in acidic ethanol (Bate-Smith, 1954), was proposed for red wine (Glories, 1984). The gelatin index, expressed in % precipitated tannins, is calculated from the difference between values measured before and after gelatin precipitation.

Further refinements of these methods have been described more recently. The methodology developed by Hagerman and Butler (Hagerman and Butler, 1978) has been adapted for routine measurement of tannins in grapes and wine (Harbertson *et al.*, 2002) and later transformed to a high-throughput assay by using a microplate reader for spectrophotometric measurements (Heredia *et al.*, 2006). Combining precipitation, that is expected to selectively precipitate higher molecular weight phenolics, with sulphite bleaching, that has been proposed to distinguish polymeric pigments (resistant to bleaching) from grape anthocyanins (non-resistant) (Somers, 1971), gives access to so-called large (LPP) and small polymeric pigments (SPP). However, it is worth pointing out that some polymeric pigments, such as flavanol anthocyanin adducts, behave like their anthocyanin precursors with respect to hydration and sulphite bleaching (Salas *et al.*, 2004). Tannin concentrations of very diluted and very concentrated samples in the BSA assay are under-estimated and recommendations were made to avoid this bias (Jensen *et al.*, 2008). Variants using absorbance at 280 nm to measure total phenolics in the wine before and after precipitation with ovalbumin (Llaudy *et al.*, 2004) or with methylcellulose, an insoluble polysaccharide (Sarneckis *et al.*, 2006), have been developed. The latter has been adapted to high-throughput format and combined with spectrophotometric colour measurements that give an insight into the pigment composition (Mercurio *et al.*, 2007). Results obtained with methylcellulose and BSA precipitation assays were highly correlated ($r^2 = 0.96$ and 0.80, for grapes and wines, respectively), but the values determined with the methylcellulose assay were about three-fold higher (Mercurio and Smith, 2008). Tannin concentrations measured by ovalbumin precipitation were much lower than those estimated with the gelatin index, but the ovalbumin method was faster, more reproducible and correlated better with the astringency scores given by sensory panels (Llaudy *et al.*, 2004). The methylcellulose and BSA precipitation assays also showed strong correlations with perceived wine astringency ($r^2 = 0.83$ and 0.90, respectively).

It is not clear whether precipitation is actually requested for the elicitation of astringency. Nephelometry has been proposed as a sensitive technique to measure tannin–protein interactions for predicting haze formation (Siebert *et al.*, 1996) or astringency (Horne *et al.*, 2002). Recently, an Astringency Mucin Index based on nephelometric measurement of tannin interactions with bovine salivary mucin, chosen as a model of salivary protein, has been developed as a predictive test (Monteleone *et al.*, 2004) and further up-scaled for routine use by using the ability of haze particles to screen the fluorescence emitted by formazin solutions (Fia *et al.*, 2008). Nephelometric turbidity unit (NTU) readings increased with increasing concentrations of tannins, but different curves were obtained with tannic acid (a mixture of galloylglucose derivatives) and grape seed proanthocyanidins. Although turbidity measurements are known to suffer from various drawbacks, and in particular strongly over-estimate large particles, linear predictive models were obtained for each tannin extract, by relating mean astringency ratings determined by a sensory panel and mean NTU values, on a range of tannin concentrations.

2.4 Sensory analysis of wine taste and mouthfeel properties

Sensory analysis is the only way to assess taste and mouthfeel properties, although studies carried out *in vitro* have enabled much progress in the understanding of the physico-chemical and physiological processes underlying them. Relevant and reliable sensory information can be generated by objective methods, based on rigorous protocols and statistical data treatment, which gradually replace empirical techniques traditionally used in sensory evaluation.

2.4.1 Sensory analysis methods

Sensory methods can be divided in two groups: discriminant methods and descriptive methods (Piggott *et al.*, 1998). The former aim at establishing differences between samples and can be used in particular for the determination of detection thresholds, by comparing a range of increasing concentrations. They do not require specific training of the panel, and are relatively easy to perform, but they do not distinguish between the various sensations exhibited by a given compound or sample. Thus, recognition thresholds, focussing on a particular attribute, are sometimes preferred. Determination of detection or recognition thresholds in serial HPLC fractions can be used for screening for taste active compounds in an extract, in the so-called taste dilution analysis (TDA) (Frank *et al.*, 2001). The half-tongue test, in which the diluted fraction and pure water are applied in random order on one side of the subject's tongue and the subject asked on which side he or she perceives the expected sensation (Scharbert *et al.*, 2004), is a particular version of this TDA method.

Descriptive sensory analysis aims to identify and quantify the intensity of particular characteristics and provide an exhaustive picture of the sensory properties associated with a given set of samples. It is performed following established

procedures (ISO, 1994), first requiring selection and training of the panel to ensure that panellists have a good discriminatory ability and sensitivity, and that they are reproducible in their assessment. The next steps involve generation of descriptors on the set of samples to be analysed and training of the panel for consistent use of the generated terms, before carrying out sensory analysis itself, that consists in scoring the intensity of each attribute for each sample. Comparison of data obtained independently by two sensory panels in two different countries on the same series of wines has shown very high reproducibility (Preys *et al.*, 2006). However, this method does not take into account the dynamics of sensory perception, which is particularly important for taste and mouthfeel properties such as bitterness or astringency, that last for a relatively long time even after expectorating or swallowing the sip (Lee and Lawless, 1991).

To fully characterise such sensations, time–intensity (TI) procedures, that consist in continuously recording the perceived intensity of a single characteristic as it changes over time, have been developed (Pangborn *et al.*, 1983; Lee and Pangborn, 1986; Lee and Lawless, 1991). These methods have proven particularly useful in the assessment of astringency and bitterness in wines (Guinard *et al.*, 1986b; Noble, 1995). The data are produced in the form of a TI curve from which up to 11 significant parameters (Cliff and Noble, 1990), including maximum intensity, time to maximum, total duration and total area under the curve, are then extracted. More recently, methods considering the whole curve (Dijksterhuis and Eilers, 1997) and combining multivariate analysis with statistical modelling have also been proposed for analysing TI data (François *et al.*, 2007). Specific recommendations have been made for training the sensory panel for application of TI procedures (Peyvieux and Dijksterhuis, 2001).

Because of the time-dependence of individual tastes, the dominant perception of a food or beverage changes during the tasting. To describe this phenomenon, a new method called temporal dominance of sensations (TDS) has recently been proposed (Pineau *et al.*, 2009). This consists in identifying and rating sensations perceived as dominant until another one takes over. Comparison of the sensory profiles provided by TDS and by descriptive sensory analysis showed that TDS differentiated products on more attributes but could not detect some sensations, that were never dominant (Meillon *et al.*, 2008).

Regardless of the sensory method used, specific care should be taken to avoid carryover effects when assessing astringency. The duration of astringency perception upon repeated ingestions of wine increased significantly (Guinard *et al.*, 1986b). The intensity was also increased when successive sips were taken at 20-second intervals (Noble, 1995) but not after 40-second intervals (Guinard *et al.*, 1986b). To avoid this bias, very strict protocols involving rinses and minimum delays between samples are requested. Various compounds known to interact with tannins have been tested for their efficacy in reducing carryover effects in the evaluation of red wine astringency by TI (Colonna *et al.*, 2004; Ross *et al.*, 2007). Pectin and carboxymethylcellulose at 1 $g.L^{-1}$ were superior to water, polyvinylpyrrolidone (4 $g.L^{-1}$), ovalbumine (4 $g.L^{-1}$), or gelatin (6 $g.L^{-1}$) in decreasing astringency. Unsalted crackers were found more effective than pectin at 1 $g.L^{-1}$

(Ross *et al.*, 2007) but less than pectin at 5 $g.L^{-1}$ (Colonna *et al.*, 2004). For the palate cleansing to be most effective, it had to be followed by an extensive water rinse (Colonna *et al.*, 2004). The pectin rinse was also tested in a descriptive analysis protocol in which the intensities of bitterness, acidity and astringency in red wine samples were assessed simultaneously. The discrimination capacity and homogeneity of the panel in the evaluation of all three attributes were significantly improved with the use of the pectin rinse (Müller *et al.*, 2007).

Finally, careful training of the panel is required as bitterness, astringency and acidity may be confused with each other (Lee and Lawless, 1991). This is usually achieved using quinine sulphate or caffeine (for bitterness) and alum, tannic acid or gallic acid (for astringency). However, the last two have both bitter and astringent properties (Robichaud and Noble, 1990; Kielhorn, 1999) while alum does not reflect the exact sensation elicited by phenolic compounds. Finally, experienced wine tasters often argue that astringency can be further subdivided into several sensations. This has been taken into account in the development of the wine mouthfeel wheel (Gawel *et al.*, 2000), and an approach based on the feeling of tactile sensations elicited by substances such as chalk, silk, velvet and sandpapers with different grains has been used to train the jury to distinguish and rate these attributes.

2.4.2 Impact of ethanol, glycerol and acids on wine taste and mouthfeel

Ethanol is itself bitter (Mattes, 1994) and enhances the bitterness elicited by other substances. Increasing ethanol by 3% significantly increases bitterness of wines containing different levels of catechin (Fischer and Noble, 1994) and of wine-like model solutions containing grape seed tannins (Vidal *et al.*, 2004a) but also of some model white wines containing no phenolic compounds (Jones *et al.*, 2008a). Ethanol also exhibits pungency, with burning and tingling sensations (Cliff and Heymann, 1992). The pungency of ethanol was most diffuse in nature, with some burning and tingling sensations. It had the shortest perceived onset and overall duration.

Increased ethanol concentrations resulted in an increase of hotness perception in Riesling wines (Gawel *et al.*, 2007b) and in model wines (Jones *et al.*, 2008a). Ethanol is also suggested to contribute to the viscosity of wine. Indeed, viscosity perception increased with the ethanol concentration, but there were no differences in white wines ranging from 7% to 14% ethanol (Pickering *et al.*, 1998), or in model white wines (Jones *et al.*, 2008a). An increase of viscosity perception was, however, reported in another study (Gawel *et al.*, 2007b). Finally, higher alcohol concentrations enhanced mouthfeel characteristics such as palate dryness and roughness and contributed an unpleasant metallic character, but only in the absence of polysaccharides (Jones *et al.*, 2008a).

Studies on the effect of glycerol on taste and mouthfeel have led to contradictory results. Difference tests established that glycerol increased the sweetness of dry white wine but not its viscosity at concentrations normally found in wine (Noble and Bursick, 1984). In ice wine, glycerol enhanced perceived viscosity but

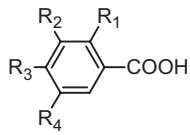
less than ethanol or sugar (Nurgel *et al.*, 2004). In another study (Gawel *et al.*, 2007b), the effect of glycerol on perceived viscosity was inconsistent, but the wines containing 10 $g.L^{-1}$ glycerol were rated higher in viscosity than those containing 5 $g.L^{-1}$. A significant effect of glycerol on viscosity perception was also demonstrated in model white wine, along with a reduction of hotness perception, but only when polysaccharides were not present (Jones *et al.*, 2008a). In the last two studies, ethanol, glycerol or polysaccharides did not impact sweetness.

Organic acids, that are present in rather large concentrations in wine, are both sour and astringent (Thomas and Lawless, 1995). In model solutions, the sourness elicited by organic acids appears primarily determined by pH (Kallithraka *et al.*, 1997b; Norris *et al.*, 1984; Sowalsky and Noble, 1998). However, at a given pH value, it also increases as the normality is increased and depends on the acid (Sowalsky and Noble, 1998). Lactic acid, which has only one carboxylic group, appears more sour than malic and tartaric acids, and triprotic citric acid less sour than the other three. In wine, sourness perception is also dependent on pH (Fischer and Noble, 1994), and wines acidified with malic or lactic acid are not different (Kallithraka *et al.*, 1997b). Astringency perception due to organic acids increases as the pH decreases (Lawless *et al.*, 1996) but does not depend on the concentration or nature of the acid (Sowalsky and Noble, 1998).

2.4.3 Taste and mouthfeel properties of wine phenolics

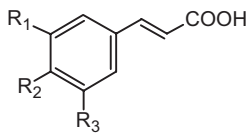
Phenolic compounds (Fig. 2.4), and especially flavan-3-ols, are major contributors to the bitterness and astringency of wines (Noble, 1990). Caffeic acid derivatives are often reported as bitter. However, they are not perceived in wines, and their rather low perception thresholds in water (Okamura and Watanabe, 1981) or hydro-alcoholic solutions (Dadic and Belleau, 1973) have been ascribed to their acidic character (Nagel *et al.*, 1987; Verette *et al.*, 1988). Benzoic acid derivatives show bitterness, sourness, sweetness, astringency and pungency (Peleg and Noble, 1995). Gallic acid appears more bitter than astringent, especially at higher concentration (Robichaud and Noble, 1990). Flavonols may also contribute bitterness, but they are present in rather low concentrations in wine. When evaluating fractions obtained by sequential solvent extraction of a red wine, most of the bitter compounds were found in the ethyl acetate fraction (representing 4.6% of the dry matter) whereas astringency, sweetness and sourness were contributed mostly by the water-soluble compounds (> 95% of the dry matter) (Hufnagel and Hofmann, 2008a). HPLC fractionation of these extracts and taste dilution sensory analysis of the resulting fractions revealed that phenolic acids, along with procyanidin oligomers, were the key astringent components in the ethyl acetate fraction. Flavonol glycosides were perceived as velvety astringent, as reported earlier in tea (Scharbert *et al.*, 2004) or cocoa (Stark and Hofmann, 2006), while the ethyl esters of phenolic acids contributed both astringency and bitterness. Taste reconstruction and omission experiments confirmed that red wine bitterness was imparted by ethyl esters of phenolic acids and lower molecular weight flavan-3-ols at sub-threshold concentration, whilst astringency was due to water-soluble higher

Hydroxybenzoic acids



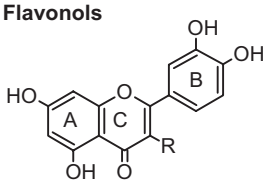
$R_1 = R_2 = R_4 = H$, $R_3 = OH$	*p*-Hydroxybenzoic acid
$R_1 = R_4 = H$, $R_2 = R_3 = OH$	Protocatechuic acid
$R_1 = H$, $R_2 = R_3 = R_4 = OH$	Gallic acid
$R_1 = H$, $R_2 = R_4 = OCH_3$, $R_3 = OH$	Syringic acid

Hydroxycinnamic acids



$R_1 = R_2 = R_3 = H$	Cinnamic acid (non-phenolic)
$R_1 = R_3 = H$, $R_2 = OH$	*p*-Coumaric acid
$R_1 = R_2 = OH$, $R_3 = H$	Caffeic acid

Flavonols



R = OH Quercetin
R = O-glc Quercetin 3-*O*-glucoside

Fig. 2.4 Structures of some major phenolic compounds of wine.

molecular weight phenolics and enhanced by organic acids (Hufnagel and Hofmann, 2008b).

Anthocyanins have been reported to contribute to astringency in model solution studies (Brossaud *et al.*, 2001; Vidal *et al.*, 2004c). However, after further purification, grape anthocyanins tasted similar to the model wine (Vidal *et al.*, 2004b), suggesting that astringency properties reported in earlier studies were due to contamination, possibly by flavonols. Anthocyanins were also strongly associated with the chalk-like character in Syrah wines, but this does not necessarily reflect a direct causative effect (Gawel *et al.*, 2007a).

Flavan-3-ols are bitter and astringent, the maximum intensity and duration of both perceptions increasing as a function of concentration (Robichaud and Noble, 1990). Catechin and epicatechin are more bitter than astringent (Lea and Arnold, 1978; Robichaud and Noble, 1990) and epicatechin is both more bitter (Thorngate and Noble, 1995) and more astringent (Kallithraka *et al.*, 1997a) than its isomer, possibly due to differences in C-ring configuration. Bitterness decreases and astringency increases as the tannin chain length increases (Lea and Arnold, 1978; Robichaud and Noble, 1990). Systematic investigation of monomers, dimers and

trimers have confirmed this finding and shown that the C4–C6 dimers are more bitter than their C4–C8 isomers (Gacon *et al.*, 1996). It is commonly admitted that larger molecular weight proanthocyanidins (beyond DP8 or so) are poorly soluble and thus do not exhibit astringency (Goldstein and Swain, 1965; Lea, 1990). However, recent studies have established that larger polymers are both soluble in wine (Maury *et al.*, 2001) and highly astringent (Vidal *et al.*, 2003). The presence of galloyl groups, that increases tannin affinity for proteins, further enhanced astringency intensity.

Experiments aiming at correlating phenolic composition with sensory data have been performed on series of commercial red wines. The astringency of 35 wines from British Columbia was linearly predicted from total phenolics and co-pigmented anthocyanins (Cliff *et al.*, 2002), that of 40 Dornfelder and Beaujolais wines from phenolic composition data determined by HPLC analysis (Preys *et al.*, 2006). In the latter study, astringency appeared strongly related to the concentration of tannins, and to their average degrees of polymerisation and of galloylation. Phenolic acids were not correlated to sourness but seemed associated with astringency while flavonol aglycones were associated with bitterness. However, these associations require further investigation as they may have been fortuitous.

Changes in sensory properties over wine ageing are commonly ascribed to polymerisation of tannins or formation of polymeric pigments. However, experimental evidence is still missing. Astringency increases rather than decreases when tannin chain length increases (Vidal *et al.*, 2003) and, although formation of ethyl-linked flavan-3-ol oligomers has been postulated to take part in the astringency decrease observed during ripening of persimmon (Tamura *et al.*, 1999), these compounds tasted as astringent as proanthocyanidins. In contrast, fractions containing water-soluble tannin–anthocyanin adducts did not contribute to mouthfeel properties (Vidal *et al.*, 2004b). This suggests that conversion of tannins to tannin–anthocyanin adducts rather than polymerisation of tannins is responsible for the loss of astringency in red wine ageing. Wines fermented with red grape skins and seeds have been reported to be more astringent than those fermented with skins and seeds from white grape while addition of anthocyanins increased the 'fine grain' perception (Oberholster *et al.*, 2009). This suggests that anthocyanins, when present together with tannins, are converted to astringent compounds or that they increase extraction of tannins into the wine, as reported earlier (Singleton and Trousdale, 1992). However, the observed differences may also have been related to differences in tannin composition between the white (Chardonnay) and red (Shiraz) grapes used in this study.

2.4.4 Influence of interactions on wine taste and mouthfeel properties

Taste and mouthfeel properties are also strongly modulated by the presence of other wine components, such as alcohol, acids, glycerol and macromolecules. Lowering the pH of solutions containing catechin (Fischer and Noble, 1994), tannic acid (Guinard *et al.*, 1986a) or grape seed tannins (Kallithraka *et al.*, 1997b) and of wines (Guinard *et al.*, 1986a; Kallithraka *et al.*, 1997b) increased astringency

perception, possibly due to effects on the conformation and charge state of salivary glycoproteins (Gawel, 1998). Changes in pH did not affect the viscosity of mucins (Veerman *et al.*, 1989) but modified that of glycosylated PRP (Nordbo *et al.*, 1984). These contradictory results may be explained by an effect of ionic strength (Gawel, 1998). However, the role of mucins in astringency perception may be questioned as it has been recently shown that they do not form the strong elastic interface observed with whole saliva (Rossetti *et al.*, 2008). Increasing tartaric acid content while maintaining constant pH had no effect on tannin perception (Fontoin *et al.*, 2008).

Ethanol content did not modify astringency but significantly increased the bitterness of flavanols (Delcour *et al.*, 1984; Fischer and Noble, 1994; Vidal *et al.*, 2004a; Fontoin *et al.*, 2008). Mutual suppression of bitterness and sweetness is well documented (Lawless, 1979; Calvino *et al.*, 1990; Bartoshuk, 1993) and daily experienced, for instance when sweetening coffee. Addition of sugar also suppressed perceived acidity of a white wine, but the reverse was not observed (Jones *et al.*, 2008b), in agreement with earlier results (Bartoshuk, 1975).

Addition of sweeteners reduced mouth dryness induced by tannic acid (Lyman and Green, 1990) and astringency of alum (Breslin *et al.*, 1993) or red wine (Ishikawa and Noble, 1995). This has been attributed to an increase of viscosity due to the sweetener itself or to simulation of saliva secretion. Aspartame, a non-viscous artificial sweetener, did not affect astringency but was also less efficient than sucrose in simulating saliva secretion (Lyman and Green, 1990; Smith *et al.*, 1996). In contrast, viscous carboxymethylcellulose decreased astringency perception (Noble, 1998). Nevertheless, polysaccharides also interact with tannins to yield soluble complexes (Riou *et al.*, 2002) and may modify their interaction with salivary proteins. Increased solubilisation of pectins has been proposed to explain the loss of astringency during persimmon ripening (Taira *et al.*, 1998) and rhamnogalacturonan II decreased tannin astringency in model solution (Vidal *et al.*, 2004a). Similarly, the effect of gelatin fining on wine astringency may be imparted not only to precipitation of tannins, but also to their incorporation in soluble tannin–gelatin complexes (Maury *et al.*, 2001). Incorporation of tea polyphenols in milk casein micelles (Luck *et al.*, 1994) or complexation of tannins with caffeine to form tea cream are also reported to result in lower astringency (Haslam *et al.*, 1992b; Powell *et al.*, 1992).

While simple models examining two factor interactions provide useful information, they may not be able to predict sensory properties of complex media such as wine (Vidal *et al.*, 2004a; Jones *et al.*, 2008b). When simultaneously assessing the effect of volatiles, ethanol, glycerol, polysaccharides and proteins on sensory properties of a model white wine, a number of second and higher order interactions have been found. Thus, the trends were for proteins to reduce viscosity and for polysaccharides to increase it. Proteins also increased metallic character at high ethanol or high glycerol levels. In a red-wine model containing ethanol, tannins, anthocyanins and two polysaccharide fractions, secondary interactions were shown between ethanol and polysaccharides and between the two polysaccharide fractions (Vidal *et al.*, 2004a).

2.4.5 Individual variations

Variance analysis of sensory data obtained on red wines to which tannic acid (2 $g.L^{-1}$), quinine sulphate (40 $mg.L^{-1}$) or both were added showed significant impact of the panellists on taste and mouthfeel attributes (Ross and Weller, 2008), as shown earlier for bitterness and astringency (Arnold and Noble, 1978). Individual perception depends on physiological factors, which can be genetically determined or result from food habits, and on psychological and cultural factors. A classical example of taste genetic dependence is the response to 6-n-propylthiouracil (PROP), perceived as tasteless by some individuals and as extremely bitter by others, who have a higher number of taste buds and more taste pores in each bud (Bartoshuk, 1993). Sensitivity to other bitter substances such as naringin, a flavonoid responsible for citrus bitterness, is related to the PROP status (Drewnowski *et al.*, 1997), but this does not seem to be the case for flavan-3-ols (Noble, 1998). Nevertheless, PROP non-tasters gave lower intensity ratings for bitterness, astringency and acidity in red wine compared to tasters (Pickering *et al.*, 2004).

Astringency perception is also highly heterogeneous. Various studies have tried to relate individual differences in astringency perception with saliva characteristics. It has been reported that astringent solutions are perceived more intensely by persons with low salivary flow rate (Fischer *et al.*, 1994; Ishikawa and Noble, 1995), but this was not confirmed in another study (Guinard *et al.*, 1998). The salivary protein profiles show individual variations (Clifford, 1997). Intra- and inter-individual variations suggest some adaptation of saliva composition to dietary exposure to tannins, as shown for some animal species (Mehansho *et al.*, 1987b; Hagerman and Robbins, 1993). No correlation was found between salivary protein content before wine tasting and astringency perception (Kallithraka *et al.*, 2001), confirming earlier results (Guinard *et al.*, 1998). In contrast, the amount of proteins in saliva after wine tasting was negatively correlated with the maximum astringency intensity and the time needed to reach it, suggesting that these parameters are related to the amount of protein lost through precipitation (Kallithraka *et al.*, 2001). The concentration of individual salivary proteins may also be important for astringency, as shown by negative and positive correlations between astringency perception and the relative areas of some particular protein peaks in saliva HPLC profiles. High proportions of salivary PRP may be associated with high astringency perception, while high proportions of other proteins, including non-PRP and hydrophilic PRP (presumably glycosylated) may result in reduced astringency perception. In a recent study, individual saliva exhibiting different protein profiles were discriminated on their ability to precipitate tannins (Sarni-Manchado *et al.*, 2008). A high level of glycosylated proteins was associated with lower precipitation rates.

Individual responses to taste or mouthfeel stimuli depend on the person's experience and expectations and on the particular context (Leland, 1997). Rejection of bitter substances is considered to be an evolutionary process preventing consumption of potentially toxic foods. Association between liking of bitterness and daring behaviour has been suggested (Mattes, 1994), but this may be specific of molecules that also exert a stimulating effect, such as caffeine. Sensitivity to

caffeine and possibly other bitter substances decreases with exposure frequency (Mattes, 1994). Bitterness liking is also higher for frequent consumers, but it does not increase with regular consumption, unlike liking of sweetness or saltiness. A study of the perception and liking for a naturally polyphenol-rich, astringent pear juice in 10-year-old children during six weeks of exposure showed large differences in liking evolution patterns following consumption of the astringent juice, but they were not explained by differences in perceived astringency or in oral parameters such as saliva pH and flow rate (Nicklaus *et al.*, 2006).

Furthermore, interactions between sensory stimuli may take place at the cognitive level (Keast and Breslin, 2003). Taste and mouthfeel perception is strongly influenced by other sensory characteristics and particularly colour (Clydesdale, 1993). Thus bitter substances have a higher detection threshold in coloured and especially red solutions which are also perceived as sweeter (Maga and Lorentz, 1973). This is probably due to mental association related to the concomitant increase of sweetness and of red colour as fruits ripen. Interferences between colour and taste result from learning processes (Clydesdale, 1993). Addition of red colouring to a white wine changes its taste perception, but more significantly for a professional jury than for an inexperienced panel (Sauvageot and Struillou, 1997). A shift from 'soft' tannins to 'green' tannins was observed when the same wines were tasted by a panel of wine experts in black versus white glasses, due to mental association between intense red colour and good maturity of the grapes (Cheynier *et al.*, 2006). It is thus recommended that taste evaluation is performed in black glasses independently of colour evaluation.

Similar interaction processes have been reported with aroma characteristics. For instance, almond flavour increases perception of sweetness and decreases that of saltiness (Frank *et al.*, 1993) and fruit flavour decreases astringency in soy fortified yogurt (Drake *et al.*, 2001). These cognitive associations are highly variable, depending on the particular experience of each panel member and they cannot always be eliminated by training (Noble, 1996; Simons and Noble, 2003).

2.5 Viticulture and oenology practices to optimise wine taste and mouthfeel

Most of the components responsible for taste and mouthfeel properties can be modified to some degree through appropriate selection of grape material, and by grape growing and wine-making practices. Particular targets are selection of the harvest date to ensure optimum maturity of the grapes, control of extraction steps in red wine-making and treatments of the final wines to prevent or correct eventual defects. Traditional goals in viticulture and oenology have been to increase the level of alcohol in wines, but the trend is currently reversed and new research developments aim at reducing it. Possible strategies include selection of varieties with lower sugar content at maturity, development of vine growing practices enabling limitation of sugar accumulation, selection of yeasts and control of fermentation kinetics to reduce the yield of sugar to alcohol conversion, and

physical processes removing sugar or alcohol. Wine acidity varies greatly according to climatic conditions and sometimes requires correction.

2.5.1 Impact of genetic factors and vine-growing practices on grape composition

The phenolic composition of grapes is primarily determined by genetics, and chemotaxonomic classification of grapes based on quantitative and qualitative profiles of anthocyanins (Roggero *et al.*, 1988; Mazza and Miniati, 1993), flavonols and dihydroflavonols (Mattivi *et al.*, 2006; Masa *et al.*, 2007) or hydroxycinnamic esters (Boursiquot *et al.*, 1986; Cheynier *et al.*, 1989b) has been proposed. Information on flavan-3-ol composition is still limited, but varietal differences have been reported (Souquet *et al.*, 1999, 2006).

Development stage and growth conditions are also important determinants of grape composition. Ripening of the berries is accompanied by sugar accumulation, a loss of acidity and an increase of the sugar to acid ratio, classically used to determine commercial maturity and harvest date. Lack of acidity in the grapes results from excessive maturity or excessive soil potassium levels. Hydroxycinnamic acids (Romeyer *et al.*, 1983) and flavan-3-ols (Kennedy *et al.*, 2001; Downey *et al.*, 2003; Fournand *et al.*, 2006; Verries *et al.*, 2008) accumulate very early during berry development. Their concentration decreases during ripening (Romeyer *et al.*, 1983; Kennedy *et al.*, 2001; Downey *et al.*, 2003), probably due to dilution as a result of berry growth, as proanthocyanidin content remains almost constant when expressed on a per berry basis (Fournand *et al.*, 2006). No change (Fournand *et al.*, 2006; Verries *et al.*, 2008), an increase (Kennedy *et al.*, 2001) and a decrease (Downey *et al.*, 2003) of proanthocyanidin mDP have been reported over the same period.

Proanthocyanidin extraction rates decrease as the grape ripens, suggesting that they adsorb on the plant cell wall (Downey *et al.*, 2003) or are converted *in vivo* to other molecular species (Kennedy *et al.*, 2001), for instance by enzymatic oxidation, as shown for *Arabidopsis* seeds (Pourcel *et al.*, 2007). Grape composition is further modulated by environmental factors such as sun exposure, temperature or water stress and by viticultural practices (Jackson and Lombard, 1993). Titratable acidity declines as sunlight exposure increases (Bergqvist *et al.*, 2001). Variable response of anthocyanins to light and temperature has been observed (Bergqvist *et al.*, 2001; Kennedy *et al.*, 2002; Spayd *et al.*, 2002). Anthocyanin content normally increases with sun exposure but excessive exposure should be avoided for maximum berry colour in warm regions. Spraying with ethanol (El-Kereamy *et al.*, 2002) or with 2-chloroethylphosponic acid (El-Kereamy *et al.*, 2003), an ethylene-releasing compound, triggers expression of genes related to anthocyanin biosynthesis and accumulation of anthocyanins in grape berries. Flavonols are particularly affected by sun exposure, in agreement with their role as UV protectants, but not by temperature (Price *et al.*, 1995; Spayd *et al.*, 2002; Downey *et al.*, 2004). Seed proanthocyanidin composition is slightly influenced by exposure (Downey *et al.*, 2004) and vine water status (Kennedy *et al.*, 2002; Ojeda, 2002). Increased

contents were found in berries from zones with low vine vigour (Cortell *et al.*, 2005).

2.5.2 Extraction of phenolics and other macromolecules in winemaking

Wine composition depends primarily on the selective extraction of grape components in the winemaking process. Compounds present in pulp cells, such as sugars or organic acids, are readily extracted into the must at pressing, whilst extraction of skin and seed constituents, including anthocyanins, flavonols and most proanthocyanidins, requires maceration. Thus, hydroxycinnamic acids are the major phenolic compounds of white musts and wines made by direct pressing. Increasing the delay before harvest and pressing, as well as pomace contact before fermentation (Cheynier *et al.*, 1989a; Ricardo da Silva *et al.*, 1993) and thorough pressing (Yokotstuka, 1990; Somers and Pocock, 1991), increased the concentration of flavanol monomers and oligomers in wine. To our knowledge, there is no report of proanthocyanidin polymers in white wines, and we have been unable to detect them in Champagne wines (Mané, 2007), in spite of their presence in the grape berry pulp (Mané *et al.*, 2007). This may be due to their adsorption on plant cell walls, as shown in cider making (Renard *et al.*, 2001), or to their oxidation during pressing. As previously stated (Section 2.3.3), proanthocyanidins can be oxidised by quinones generated from hydroxycinnamic acids (Cheynier and Ricardo Da Silva, 1991). This resulted in much lower concentrations of proanthocyanidins in wines produced using hyperoxidation at the must stage (Cheynier *et al.*, 1989a; Ricardo da Silva *et al.*, 1993).

In red winemaking, extraction of anthocyanins and of proanthocyanidins increases with alcohol, sulphur dioxide and temperature. Anthocyanin concentration peaks relatively early and then declines as they are converted to other compounds, both phases occurring faster at higher temperature (Morel-Salmi *et al.*, 2006), while extraction of proanthocyanidins increases with the duration of maceration. Skin (and pulp) tannins can be distinguished from seed tannins by the nature of their constitutive units. Thus, epigallocatechin units (absent in seed tannins) and epicatechin gallate units (present in much larger amounts in seeds) can serve as markers of proanthocyanidins originating from skin or pulp and from seed, respectively (Cheynier *et al.*, 1997).

Monitoring of tannin composition in fermenting red musts showed that tannins from skins and pulp are extracted very early, along with the anthocyanin pigments, while extraction of galloylated procyanidins from seeds continues until pressing (Cheynier *et al.*, 1997; Morel-Salmi *et al.*, 2006). Extraction of phenolic compounds from seeds appeared more influenced by the concentration of ethanol than their extraction from skins (Canals *et al.*, 2005), due to differences in solubility of proanthocyanidins from both origins or to the particular structures of each plant tissue.

Several wine-making techniques aiming at enhancing extraction of phenolic compounds have been developed (Sacchi *et al.*, 2005). Cold soak at 10–15 °C before fermentation is sometimes performed to improve wine colour, with variable

success. This has led to increased levels of anthocyanins (Reynolds *et al.*, 2001) and of both anthocyanins and proanthocyanidins originating from skins (Cheynier *et al.*, 2006) in Syrah wines but not in Pinot noir (Feuillat, 1996; Heatherbell *et al.*, 1996), Pinotage and Sangiovese (Marais, 2003). Treatments intended to break the cell membranes are commonly used. They include treatments using pectolytic enzymes, that degrade the berry cell walls, and physical treatments such as freezing of the must and flash release, which consists of heating the grapes in a closed tank and then placing them under vacuum to fragilise cell walls and cool the must. Must freezing with dry ice increased the concentrations of both anthocyanins and tannins (Couasnon, 1999). Flash release accelerated extraction of all phenolic compounds and resulted in higher tannin to anthocyanin ratios and higher levels of derived pigments in the wines (Morel-Salmi *et al.*, 2006). Flash release and heating also accelerated extraction of grape polysaccharides (Doco *et al.*, 2007). However, wines obtained by pressing immediately after flash release contained lower amounts of polyphenols and of grape polysaccharides than those made with pomace contact, indicating that extraction continued during maceration.

Pectolytic enzyme treatment resulted in increased extraction of RGII, and partial degradation of pectins rich in arabinose and galactose (Doco *et al.*, 2007; Ducasse *et al.*, 2010). Either increase (Ducruet *et al.*, 1997; Bakker *et al.*, 1999; Revilla and Gonzalez-SanJose, 2003; Kelebek *et al.*, 2007) or decrease (Wightman *et al.*, 1997; Bautista-Ortin *et al.*, 2005) of anthocyanins and/or colour following enzyme treatments have been reported. Tannin extraction was also enhanced in some cases (Ducasse *et al.*, 2010) but not in others (Souquet *et al.*, 2008). Run-off, which consists of removing part of the juice, thus increasing the solid to juice ratio, at the beginning of maceration, increased anthocyanin concentration and colour intensity of Grenache and Syrah wines but did not modify their tannin content (Souquet *et al.*, 2008).

2.5.3 Oenological practices

Ethanol content can be altered by increasing or decreasing sugar concentration or by removing alcohol using membrane techniques. Reverse osmosis is allowed to concentrate musts and increase sugar concentrations as an alternative to chaptalisation, and can be used to produce low-alcohol wines (Bui *et al.*, 1986). Excessive acidity can be reduced by malo-lactic fermentation or, in some vine-growing regions, by addition of potassium tartrate, potassium bicarbonate or calcium carbonate (Schaeffer, 1998). Insufficient acidity, resulting in poorer stability and quality, is also observed, especially in hot climates, or due to excessive potassium fertilisation (Morris *et al.*, 1983). Corrections can be achieved by adding tartaric acid, but this is rather difficult to control, or by ion exchange systems using resins or membrane technology. Selective removal of potassium enabling controlled acidification of wine can be obtained by an electromembrane process with bipolar membranes (Escudier and Moutounet, 1998). A red wine acidified to pH 3.5 using this technique was perceived as more sour and less bitter than the control at pH 3.9, but no difference was detected in astringency perception (Müller *et al.*, 2010, submitted).

Progressive loss of astringency observed during wine ageing is attributed to conversion of astringent tannins to less astringent derivatives (Singleton and Noble, 1976; Haslam, 1980). Oxidative polymerisation of flavanols and their condensation with acetaldehyde yield polymeric species that are equally prone to interact with proteins (Guyot *et al.*, 1996a) and as astringent (Vidal *et al.*, 2004b) as tannins of similar molecular weight. However, as explained earlier, reactions of tannins in wine also generate lower molecular weight species. Replacement of flavanol units by anthocyanins in such reactions seems particularly efficient in reducing astringency, as no astringency was reported for tannin–anthocyanin copolymers (Vidal *et al.*, 2004b). The sensory properties of other anthocyanin derivatives such as flavanol-ethyl-anthocyanins and flavanyl-pyranoanthocyanins remains to be determined. The relative proportions of the various products formed are primarily determined by the tannin to anthocyanin ratio, which depends on grape composition and extraction, by pH, as some of the reaction involves acid-catalysed cleavage or proton addition steps, and by oxidation (Fulcrand *et al.*, 2004, 2006). Thus, practices such as micro-oxygenation enhance the conversion of anthocyanins and tannins, first to ethyl-linked derivatives, and then to pyranoanthocyanin derivatives (Atanasova *et al.*, 2002a). The relationships between these reactions and reported changes in wine mouthfeel properties require further investigation.

Addition of fining proteins such as gelatins or caseins to precipitate tannins out is another common practice to improve mouthfeel properties, along with colloidal stability. Higher molecular weight tannins, which are also the most astringent, are selectively precipitated by gelatins (Maury *et al.*, 2001) and by other proteins tested as alternative fining agents (Maury *et al.*, 2003). However, tannin concentration in wines was only slightly lower after fining, as the amount removed was very small.

The loss of astringency induced by the treatment has been attributed to removal of highly polymerised tannins, which are also highly astringent. However, this may also be partly due to involvement of tannins in soluble complexes with gelatin, impeding their interactions with salivary proteins, as some of the added protein remained in the fined wine. Tannin precipitation rate was higher in reconstituted tannin solution than in wine. Addition of wine polysaccharides to the tannin solution restored the wine precipitation level, meaning that polysaccharides prevented precipitation of tannin–protein complexes, due to competition between both macromolecules or to formation of ternary complexes (Cheynier *et al.*, 2006). RGII, one of the main wine polysaccharides, reduced the astringency exhibited by tannins (Vidal *et al.*, 2004a).

Other polysaccharides such as gum arabic or yeast mannoproteins are used as protective colloids, preventing haze and precipitation phenomena in wine. These polysaccharides have been shown to inhibit tannin aggregation (Riou *et al.*, 2002; Poncet-Legrand *et al.*, 2007a) but had no effect on astringency (Vidal *et al.*, 2004a). Finally, addition of tannins is also commonly used to modify wine quality. This will be discussed in volume 2 of *Managing wine quality.*

2.6 Future research trends

Ongoing research in this area is mostly aimed at gaining a better understanding of mechanisms underlying mouthfeel and especially astringency perception. The structure of wine tannins is still incompletely understood. Current analytical methods give access either to a few individual compounds or to rather crude values (total phenols, total proanthocyanidins or average composition of proanthocyanidins) that are insufficient to characterise extremely heterogenous mixtures of polymers such as wine tannins. When comparing two proanthocyanidin fractions with identical average compositions and mDP but different molecular weight distributions, the fraction containing larger oligomers tasted significantly more astringent, demonstrating that average values cannot predict astringency. One of the major challenges is to develop specific methods for the characterisation of this heterogeneity and of the distribution of molecular species (different molecular weights, units or sequences, branched or linear, etc.). Further research will aim at understanding existing relationships between the structure and the properties of both individual compounds and increasingly complex mixtures that may behave differently from the sum of individual component behaviours. The final objective will be to link mouthfeel properties as measured by sensory analysis with the physico-chemical processes taking place within the wine matrix and with the saliva components. Particular effort is focused on developing high throughput methods enabling chemical characterisation, often referred to as fingerprinting, of large numbers of samples and/or prediction of astringency properties. The outcomes of this research will be extremely helpful for the development of suitable technologies and decision-making tools to control the final quality of the wines.

Last but not least, the physiology of mouthfeel perception is a very active research area, and many questions regarding individual differences in saliva composition, induction mechanisms of saliva or salivary protein secretion in human, the respective role of genetic and cultural factors, and their impact on individual perception differences remain to be answered.

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3

Wine colour

J. A. Kennedy, The Australian Wine Research Institute, Australia

Abstract: This chapter summarizes the importance of colour in wines, starting with a rationalization of why wine colour is important from a production as well as a consumer standpoint. It then introduces the compounds responsible for wine colour, their reactivity, and ways in which they can be managed during wine production.

Key words: red wine, white wine, chemistry, management.

3.1 Introduction: contribution of colour to sensory properties

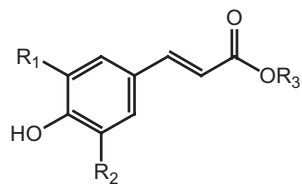
When one considers the sensory properties of wine, its colour is generally the first attribute evaluated. Along with other visual features such as clarity, fluidity and effervescence, a wine's colour can provide information on the integrity of the wine. For colour, its depth or intensity can provide an indication of its extraction and structure. The tint or hue of a wine can indicate such things as its age or degree of oxidation. For many winemakers, wine colour provides the first cue for wine evaluation. As Emile Peynaud, the esteemed wine scientist, wrote, 'A wine's color is its face in which age and character can be read.' (Peynaud, 1987, p.32). This generally-accepted relationship is also supported by a number of scientific investigations which have looked at the influence of colour on wine preference (Somers and Evans, 1974; Jackson *et al.*, 1978; Somers *et al.*, 1983), and these studies show that there is a relationship between the judged quality of wine and colour. It is therefore important that winemakers understand the relationship between production practices and wine colour in order to manage wine composition effectively. To

understand wine colour, one needs to understand the chemical composition of wine from a colour standpoint, the reactivity of these compounds, and how production practices can be used to manage perception.

3.2 Chemistry of wine colour

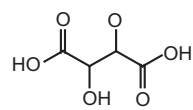
3.2.1 Chemistry of white wine colour

When white wine is produced, the skins and seed are generally separated from the juice prior to fermentation with very little if any contact with the juice. If skin contact is desirable, it is generally for the purpose of extracting aroma compounds. The lack of desired skin contact time is due to the strong relationship between wine yellowness and skin/seed contact (Cheynier *et al.*, 1989a; Ricardo-da-Silva *et al.*, 1993; Fernandez-Zurbano *et al.*, 1998; Gómez-Míguez *et al.*, 2007; Li and Wang, 2008). The colour of white wine is due to the content of the juice (primarily phenolics) in combination with the conditions that these components are exposed to during production. The principal phenolic compounds in white wine are the hydroxycinnamic acids, with small concentrations of flavan-3-ols likely (Fig. 3.1). In grapes, hydroxycinnamic acids are found esterified with tartaric acid (Ribéreau-



Hydroxycinnamic acid	R_1	R_2
p-coumaric	H	H
Caffeic	OH	H
Ferulic	OCH_3	H
Sinapic	OCH_3	OCH_3

R_3 esterification



Tartaric acid

Fig. 3.1 Hydroxycinnamic acids found in *Vitis vinifera* (reprinted with permission from Kennedy *et al.* 2006 *AJEV* **57**:3, p. 240).

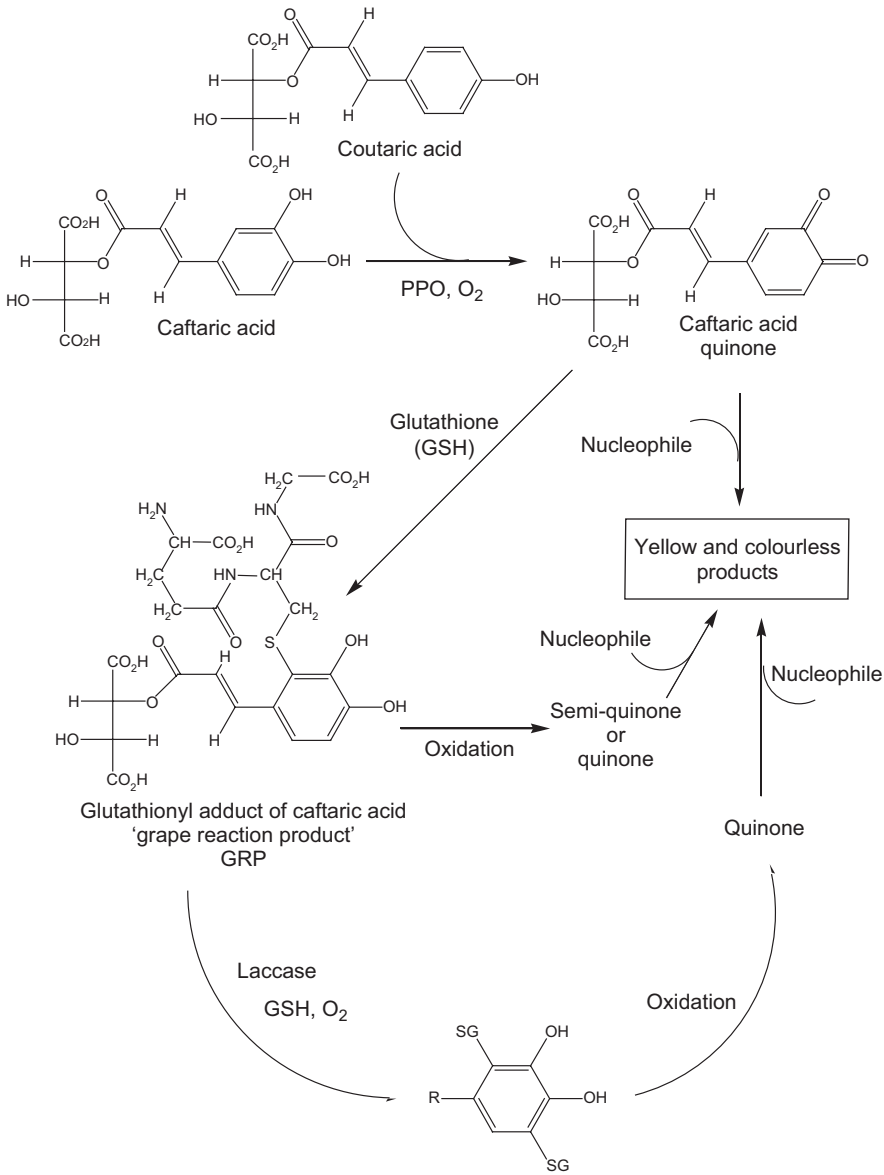


Fig. 3.2 Generalized schematic explaining the formation of coloured compounds in white wine.

Gayon, 1965), with the hydroxycinnamic acid predominantly in the *trans* configuration (Singleton *et al.*, 1978; Vrhovšek, 1998). In general, the most abundant hydroxycinnamic acid in grapes is *trans*-caftaric acid (caffeic acid combined with tartaric acid), followed by *p*-coutaric (*p*-coumaric and tartaric acids) and fertaric (ferulic and tartaric acids) (Ong and Nagel, 1978; Nagel *et al.*, 1979b; Romeyer *et al.*, 1983).

To provide the familiar yellow colour of most white wine, some oxidation of the phenolic compounds occurs unless special precautions are undertaken. The process of hydroxycinnamic acid oxidation in white musts and wine is shown in Fig. 3.2, and is best described by following the wine production process. Initially, when white juice is separated from the solid parts of the grape berry, exposure of the juice to oxygen results in enzyme-mediated oxidation (grape polyphenoloxidase, PPO, EC 1.10.3.1) of the phenolics present in the juice. The quinones formed initially react with reduced glutathione present in the grape berry and form a colourless glutathione adduct, often referred to as grape reaction product (Singleton *et al.*, 1985; Cheynier *et al.*, 1986). Higher pH musts are generally considered to be more susceptible to PPO-mediated oxidation (Guyot *et al.*, 1995)

Upon continued formation of hydroxycinnamic acid quinones, the concentration of reduced glutathione is exhausted and the formed quinones are available for coupled oxidation with other hydroxycinnamic acids, and if present flavan-3-ols. Since flavan-3-ols are primarily derived from the solid parts of the grape, longer skin contact time and harder press fractions will contain higher concentrations of these compounds. Because flavan-3-ols are more susceptible to oxidation and condensation reactions than hydroxycinnamic acids (Cheynier *et al.*, 1989b), musts with elevated flavan-3-ol concentrations are more susceptible to oxidation. Coupled oxidation reactions eventually lead to the formation of oxidized yellow pigments. This oxidation process proceeds after the fermentation process although the oxidation is no longer mediated by PPO and is likely to involve non-enzymatic, metal catalyzed radical reaction pathways (Oszmianski *et al.*, 1996; Danilewicz, 2003; Waterhouse and Laurie, 2006).

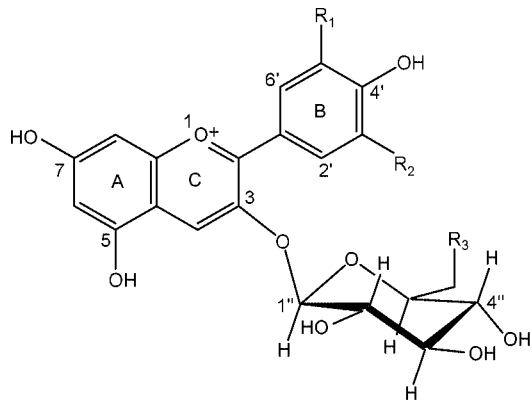
Based upon the above reaction sequence for white wine oxidation, managing white wine colour involves controlling the various portions of the reaction cascade. To limit oxidation in must for example, PPO can be deactivated using sulphur dioxide and/or oxygen which can be controlled by the addition of ascorbic acid (Rigaud *et al.*, 1991). Additional examples for managing white wine colour will be discussed below, but all can generally be interpreted using the mechanisms described above.

3.2.2 Chemistry of red wine colour

Young red wine

The compounds initially responsible for the colour of red wine are grape-derived anthocyanins, which in *Vitis vinifera* consist of an anthocyanidin moiety glycosylated with glucose (Ribéreau-Gayon, 1964; Fig. 3.3). The anthocyanidin-3-*O*-monoglucosides can also be acylated at C-6 of the glucose (Fong *et al.*, 1971). For essentially all grape varieties of commercial significance, the anthocyanins are restricted to the skin tissue.

The composition of anthocyanins in the grape varies considerably with variety (Wenzel *et al.*, 1987; Francis, 1989; Mazza, 1995). In addition to variations in the anthocyanidin portion of the molecule, anthocyanins also vary in the proportion



Anthocyanidin moiety	R_1	R_2
Cyanidin	OH	H
Peonidin	OCH_3	H
Delphinidin	OH	OH
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

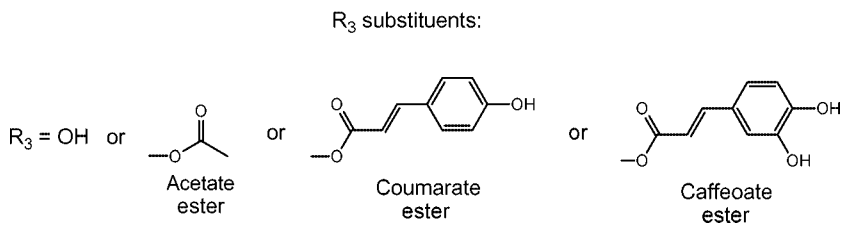


Fig. 3.3 Anthocyanins found in *Vitis vinifera.*

and extent of acylation. Generally speaking and for commercially-significant varieties, malvidin-3-*O*-glucoside is the most abundant anthocyanin. Acylation can vary from zero (e.g. cv. Pinot noir) to substantial amounts for other varieties (e.g. cv. Tinta Cao).

Anthocyanins have various equilibrium forms (Brouillard and Delaporte, 1977;

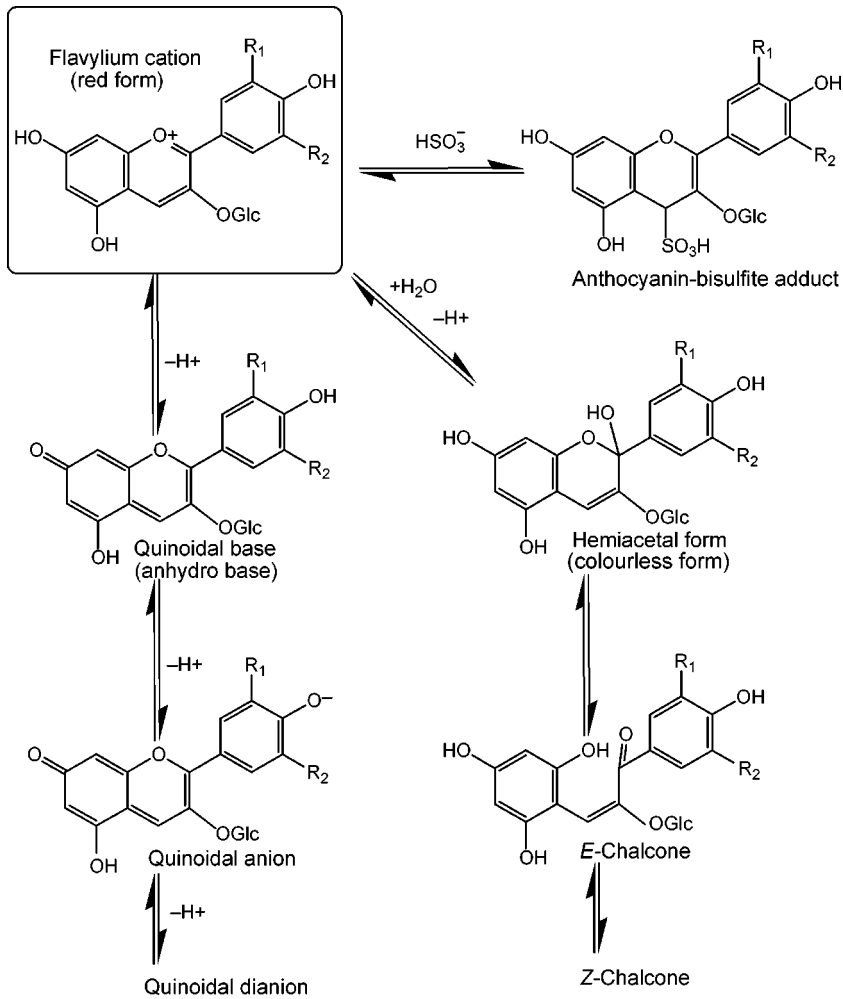


Fig. 3.4 Anthocyanin equilibrium forms observed in red wine (reprinted with permission from Kennedy *et al.* 2006 *AJEV* **57**:3, p. 242).

Pina, 1998; Asenstorfer *et al.*, 2003a; Fig. 3.4), and at wine pH (pH 3–4), two equilibrium forms dominate, the flavylium form and the hemiacetal form. The flavylium form is the desirable form from a winemaker's standpoint because it is the observed form (i.e., the red form). The hemiacetal form, in contrast, is colourless. The hemiacetal form comprises the major portion of the equilibrium forms present, with the flavylium form making up the second most abundant (Cheminat and Brouillard, 1986). The other equilibrium forms are present in minor quantities. As the pH of red wine declines, there is an increase in the proportion of the red or flavylium form of the anthocyanin; and hence, at an equivalent anthocyanin concentration, wines will appear redder at a lower pH.

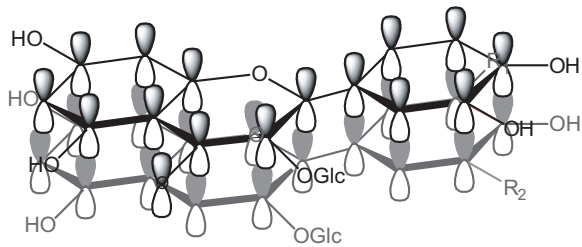


Fig. 3.5 Copigmentation between an anthocyanin (grey) and a cofactor (black, flavonol) with non-covalent interaction between π orbitals shown.

In addition to the effect of pH, the phenomenon of copigmentation affects the anthocyanin equilibrium in wine (Boulton, 2001; Liao *et al.*, 1992; Mazza and Brouillard, 1990; Mistry *et al.*, 1991). In wine, copigmentation is characterized by intermolecular non-covalent interactions between pigment π orbitals and those of a cofactor, resulting in the stabilization of a specific equilibrium form (Fig. 3.5). In the case of red wine colour, it is desirable to stabilize the red-coloured flavylium form. Hydroxycinnamic acids, flavonols and flavan-3-ol monomers are examples of cofactors found in wine, and it is expected that at higher cofactor concentrations, an increase in red colour as a result of copigmentation should occur. Flavonols are known to be particularly strong cofactors and flavan-3-ol monomers are comparatively weak (Mistry *et al.*, 1991). Other factors that could influence copigmentation include wine pH, ethanol concentration, ionic strength and wine temperature. It is generally thought that copigmentation declines proportionally as wine ages (due to a reduction in the concentration of anthocyanins), although substantial copigmentation remains after two years of age (Darias-Martín *et al.*, 2007).

Aged red wine

The above discussion generally characterizes the colour of new red wine. Managing the colour of new red wine generally comes down to managing the extent to which anthocyanins are extracted during red wine production and the chemistry (e.g. pH, copigmentation) of the anthocyanins once extracted.

Once a red wine is pressed, the concentration of anthocyanins in the wine is maximal. During ageing, the concentration of anthocyanins declines fairly quickly, with an observed reduction in red and an increase in yellow colour within the first two years (Nagel and Wulf, 1979a; Boido *et al.*, 2006; Monagas *et al.*, 2006). From a production standpoint, a major goal of colour management is stabilizing the red colour and reducing the rate at which yellowness increases. To understand the transformations that occur in red wine, it is important to understand the reactivity of anthocyanins since it is generally accepted that the red colour in older red wines is largely due to the presence of anthocyanins that have become modified.

From above, there are two predominant anthocyanin species in red wine, the flavylium form and the hemiacetal forms. From a reactivity standpoint, these equilibrium forms differ in their reactivity, with the flavylium form being electrophilic and the hemiacetal form nucleophilic (Fig. 3.6). It is expected then

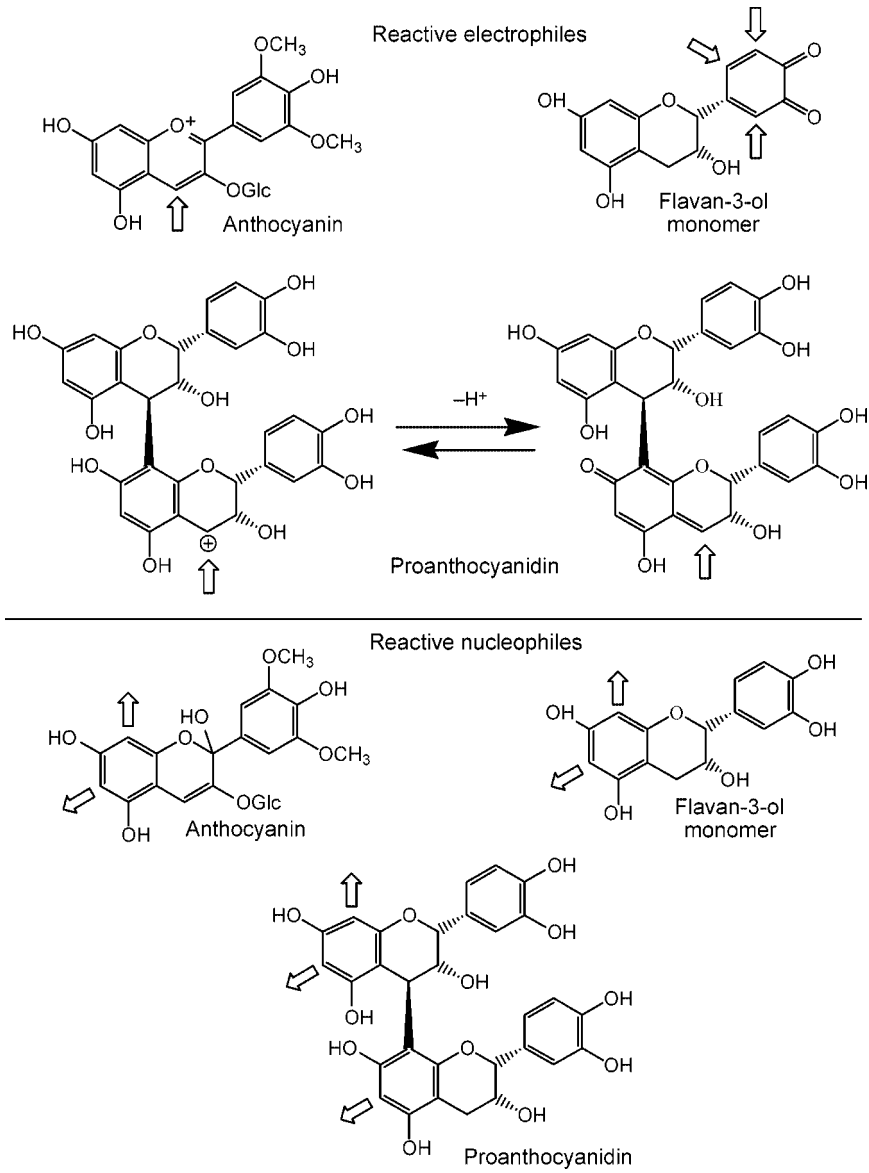


Fig. 3.6 Electrophilic and nucleophilic reactive centres for anthocyanins and other common phenolics found in red wine with the reactive centres designated with arrows (reprinted with permission from Kennedy *et al.* 2006 *AJEV* **57**:3, p. 243).

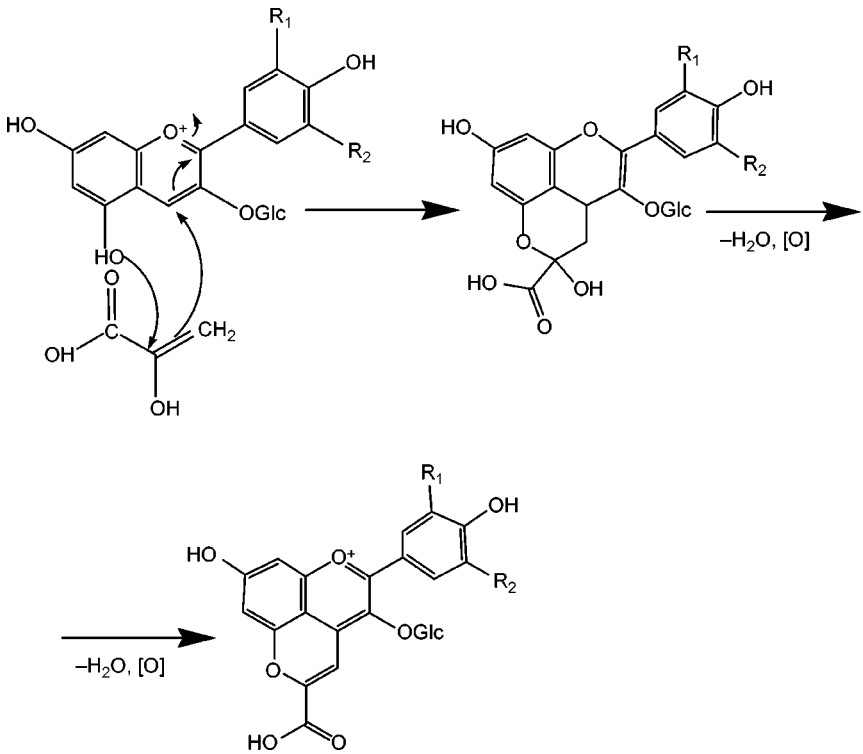


Fig. 3.7 Reaction of an anthocyanin with pyruvic acid to produce vitisin A.

that the anthocyanins will combine with compounds that have contrasting reactivity. The bleaching of anthocyanins with bisulphite anion is one example of this type of reaction (Timberlake and Bridle, 1967; Berké *et al.*, 1998; Fig. 3.4). Additional examples of species that would be expected to combine with anthocyanins are shown in Fig. 3.6. Historically, it has been speculated that products combining anthocyanins with these compounds would be a component of stable red wine colour (Jurd, 1969; Somers, 1971; Ribéreau-Gayon, 1973; Berg and Akiyoshi, 1975; Haslam, 1980; Ribéreau-Gayon *et al.*, 1983). Although until recently, these products were only speculated.

As wine colour transitions from grape-based anthocyanin to modified anthocyanin, its appearance changes from blue–red to brick-red. One example of a modified anthocyanin that partly explains this colour change is the formation adduct between the yeast metabolite pyruvic acid and anthocyanins (malvidin-3-*O*-glucoside) to form vitisin A (Bakker and Timberlake, 1997; Fulcrand *et al.*, 1998; Fig. 3.7). Vitisin A forms early after fermentation has completed, when pyruvic acid is maximal in concentration (Asenstorfer *et al.*, 2003b). The formed vitisin A has an absorption maxima at about 509 nm which would appear like the familiar brick-red colour of an older wine as opposed to a new wine which is dominated by absorption at 520 nm. In addition, the equilibrium proportion of the

flavylium form of vitisin A increases relative to the initial anthocyanin. Although it is likely that vitisin A is a minor contributor to aged red wine colour (Schwarz *et al.*, 2003a), it is a contributor nonetheless.

As wine ages, it is exposed to oxygen and the effects of oxidation. A product of wine oxidation is acetaldehyde, which is an oxidation product of ethanol oxidation (Wildenradt and Singleton, 1974). It has been observed for some time that acetaldehyde formation leads to a modification of red wine colour (Trillat, 1908; Singleton *et al.*, 1964; Timberlake and Bridle, 1976a). One of the major findings in red wine colour chemistry has been the identification of the acetaldehyde adduct of malvidin-3-*O*-glucoside in wine (Bakker and Timberlake, 1997). The formation of the vitisin-type pyranoanthocyanins in wine can lead to various modified anthocyanin products (Mateus *et al.*, 2004; Rentzsch *et al.*, 2007; Fig. 3.8). Given the recent progress in mass spectrometry (Hayasaka *et al.*, 2005), there is little doubt that additional compounds will be identified that are relatively low in molecular weight and which will contribute to the classic brick-red colour of aged red wine.

Additional chemistry which is likely to lead to the formation of compounds which give red wine its brick-red colour is the formation of xanthylium-type compounds (Es-Safi and Cheynier, 2004). These compounds have been known for some time (Jurd and Somers, 1970; Hrazdina and Borzell, 1971), but advances in their chemistry have recently occurred. The products formed are yellow in colour although products that appear purple–red have also been observed. The formation of yellow compounds in wine coupled with the red colour of anthocyanins would generate a wine colour that is consistent with aged wines.

Many of the compounds formed in red wine with age that have been described above have a low molecular weight. It is generally recognized, however, that of the observed colour in aged red wines, the dominant form is polymeric (Schwarz *et al.*, 2003a). Some time ago, structures were proposed for these pigmented polymers and, because of the prevalence of proanthocyanidins, these were speculated to involve anthocyanins and proanthocyanidins (Jurd, 1969; Somers, 1971; Ribéreau-Gayon, 1973; Berg and Akiyoshi, 1975; Haslam, 1980; Ribéreau-Gayon *et al.*, 1983). These structures have in large part, been confirmed (Dallas *et al.*, 1996; Remy *et al.*, 2000; Salas *et al.*, 2003, 2004; Fig. 3.9), and this chemistry has been extended into higher molecular weight material (Hayasaka and Kennedy, 2003). It is interesting to note that pigmented polymers begin formation early in a wine's life (Eglinton *et al.*, 2004).

The chemistry of red wine colour has advanced tremendously and the identification of anthocyanins incorporated into polymeric phenols indicates that the colour of wine is of interest for other sensory reasons, namely astringency. Given that astringency is an important attribute of red wine, modification of proanthocyanidins with anthocyanins is likely to be important for other reasons beyond simple colour stabilization. Current evidence suggests that the mechanism for astringency perception is driven by hydrophobic and hydrogen bonding interactions between salivary proteins and proanthocyanidins leading to the precipitation of the lubricating proteins (Haslam, 1998). A reduction in the

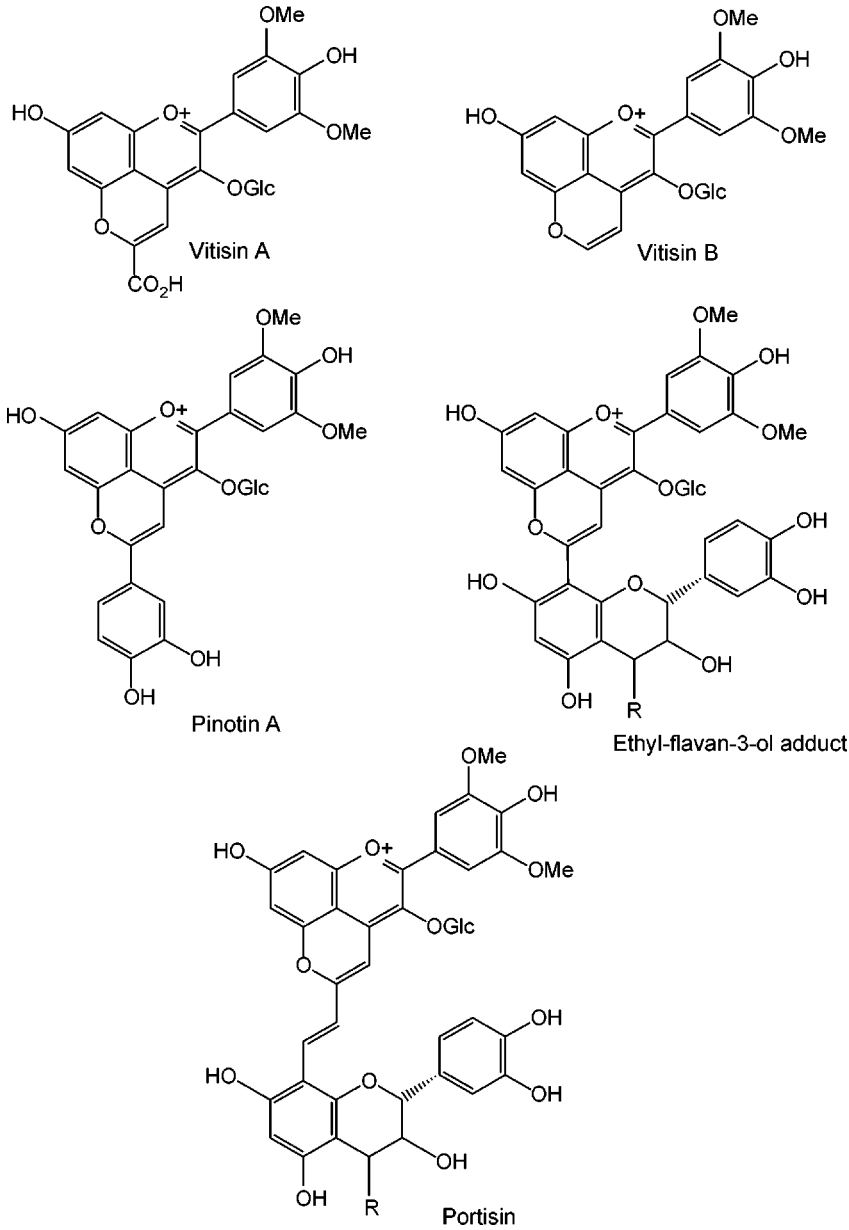


Fig. 3.8 Examples of malvidin-3-*O*-glucoside-based pyranoanthocyanins that have been identified in red wine.

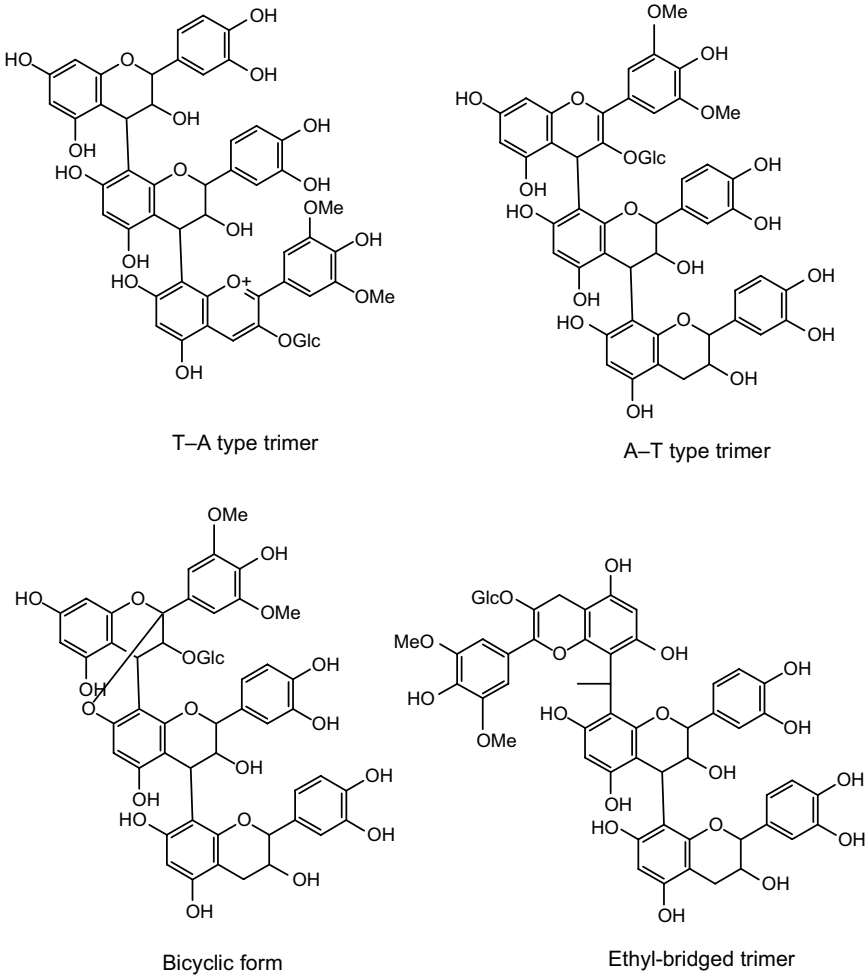


Fig. 3.9 Examples of malvidin-3-*O*-glucoside proanthocyanidin derivatives.

hydrophobicity of proanthocyanidins by modification with anthocyanins would likely reduce the interaction between salivary proteins and proanthocyanidins and therefore reduce their astringency. Therefore, while our understanding of red wine colour has become clearer, it is likely that sensory studies will confirm that red wine colour has importance for reasons beyond appearance.

3.2.3 Analysis of wine colour

Objective wine colour analysis can be divided into two categories, analysis for production and analysis for research purposes. The main distinction between these approaches is often the underlying cost of technology used and the information that is provided.

For production analysis, wine colour is generally accomplished using a UV/Vis spectrophotometer. Several methods have been developed based upon measuring the absorbance of wine (generally some combination of 420, 520 and 620 nm; Sudraud, 1958; Somers and Evans, 1978; Glories, 1984). For white wines, the degree of yellowness can be evaluated by measuring wine absorbance at 400–420 nm. For red wines, an increase in yellow (measured at 420 nm) relative to red (measured at 520 nm) is related to the perception of the 'brick-red' colour in wine, and absorbance at 520 nm is related to the depth of red colour.

Another tool that is useful in a winery setting is the use of tristimulus measurements to provide information on wine colour (Monagas *et al.*, 2006; Price, 2008). Tristimulus measurements are considered to be an improvement over basic absorbance measurements because they provide additional information on the human perception of colour (perception is based upon lighting and the observer, in addition to the sample).

Predictive visible and mid-infrared spectroscopy of grape homogenates and wine directly is gaining in use because of its simplicity and rapidity (Edelmann *et al.*, 2001; Cozzolino *et al.*, 2003, 2006; Skogerson *et al.*, 2007). This technique relies on correlation spectroscopy and therefore 'training' the instrument with well-characterized samples is a critical first step to routine and reliable use.

For young red wine, some may want to understand the extent to which anthocyanins are copigmented (Levengood and Boulton, 2004). Also for red wines, it is important to understand the development of stable colour. Methods that have utility for stable red wine colour assessment include methods developed by Somers that rely on bleaching anthocyanins with SO_2 (Somers and Evans, 1978). By doing this, and given that older red wines have an increased proportion of red colour after SO_2 bleaching (Peng *et al.*, 2002), an indication of stable colour development can be determined.

An additional method that can be used for measuring modified anthocyanins is to measure red wine visible absorbance before and after protein precipitation (Harbertson *et al.*, 2003). With this method, it is generally accepted that anthocyanins that are covalently associated with tannins are precipitable with protein and are colour stable. Measuring this form of colour may also provide information on the sensory properties of red wine tannins (Vidal *et al.*, 2004a,b).

For wine production, the general goal of wine colour measurement is to optimize perceived colour or provide correlative information on secondary wine attributes (e.g., oxidation, microbial spoilage). In research, the objectives of colour monitoring are often related more to understanding the chemistry of colouring matter *per se* in order to improve our ability to manage colour (and other attributes) in a production setting (Jensen *et al.*, 2008). In order to obtain this information, more specific analytical approaches are generally undertaken.

Chromatography is probably the most effective initial tool that researchers use to understand colour in wine. This approach has been used for some time (Ribéreau-Gayon, 1964). Today, high performance liquid chromatography (HPLC) is the most commonly used tool for researchers interested in wine colour. It can be used to monitor compounds important to both white and red wine (Ong and Nagel,

1978; Wulf and Nagel, 1978). The development of HPLC followed other chromatography methods which were initially used to understand the pigments in grapes and wine (Vaccari and Pifferi, 1978).

Today, HPLC is used to provide information including among other things: specific pigments in grapes (Wenzel *et al.*, 1987), wine (Bakker and Timberlake, 1985), how management practices can be used to modify them (Dallas and Laureano, 1994a), and the varietal integrity of red wine (Etiévant *et al.*, 1988; Otteneder *et al.*, 2004). Studies have found that results determined by HPLC correlate with results obtained with a UV/Vis spectrophotometer and other less specific methods for analysis (Vrhovšek *et al.*, 2001).

One of the initial steps often taken when analyzing wine colour is to subject the wine sample to an initial solid phase extraction of the sample in order to simplify and/or concentrate the sample (Kraemer-Schafhalter *et al.*, 1998; Oszmianski *et al.*, 1988; Cozzolino *et al.*, 2003, 2006). Although concentration is generally not an issue with colouring material, the sample matrix can potentially interfere with isolation and/or analysis.

Achieving a separation of compounds is most commonly achieved by using reversed-phase HPLC (Nagel *et al.*, 1979b; Lamuela-Raventos and Waterhouse, 1994: Berente *et al.*, 2000; Castellari *et al.*, 2002; Revilla *et al.*, 2001). This approach is effective for separating low molecular weight material (Woodring *et al.*, 1990). Higher molecular weight material (stable red wine colour for example) can be separated by using other chromatography approaches (Shoji *et al.*, 1999; Kennedy and Waterhouse, 2000; He *et al.*, 2006; Versari *et al.*, 2007). In addition, other methods are available for separation (Degenhardt *et al.*, 2000; Asenstorfer *et al.*, 2001; Sáenz-López *et al.*, 2004).

Following separation, compounds are detected using several techniques. Because the compounds of interest have chromophores that absorb light in the visible region, UV/Vis spectrophotometry in the visible region is often the detection method of choice. An important exception to this is that cofactors that are important in copigmentation phenomena often do not absorb light in the visible region and yet are important to the visible colour of red wine. In this case, monitoring in the ultraviolet region is also important.

A detection method that has found increased utility is mass spectrometry. Mass spectrometry has the advantage of providing mass structural information and therefore is more useful in the identification of specific compounds. It has utility in the identification of white wine phenolics (Chamkha *et al.*, 2003) as well as those found in red wines (Hayasaka and Asenstorfer, 2002; Salas *et al.*, 2003, 2004, 2005; Schwarz *et al.*, 2003b; Mateus *et al.*, 2004). Mass spectrometry has also been applied to large molecular mass pigments, and has been useful in providing preliminary structural information (Hayasaka and Kennedy, 2003).

Wine colour analysis has become a routine feature in many wineries, and its utility has been widely accepted. Analytical methods are fairly well developed for production use and, for the future, those that provide rapid, real-time information will find increased application.

3.3 Vineyard influences on wine colour

3.3.1 Development in grapes

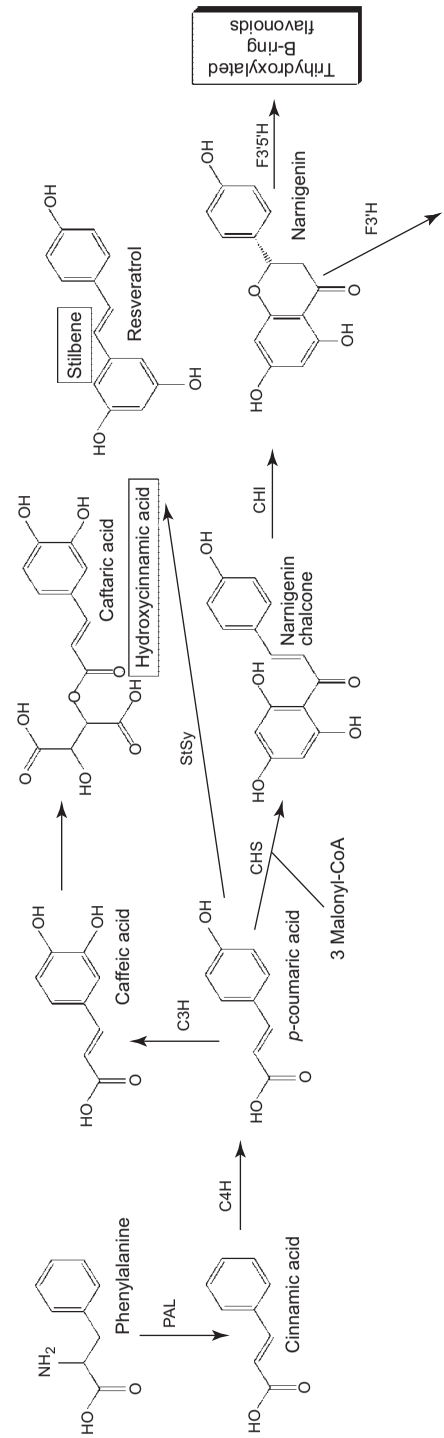
To effectively manage the colour of wine, it is important to understand the role that grape production plays. The compounds that are responsible for wine colour are in large part grape-derived, so it is essential to understand when they are biosynthesized, where they are localized in the grape berry and how production practices influence their concentration and extraction in the grape.

An important distinction must be made when discussing the role of grape production practices on wine colour. A considerable body of research is directed towards the understanding of how environmental factors influence grape berry metabolism, but it is important to understand that changes at the physiological level may not have relevance from a winemaking standpoint. An example of this is that water deficits applied to a vineyard prior to *véraison* are often used to improve red wine colour concentration, but the explanation for the colour increase in wine can sometimes be explained by a decrease in berry weight and hence an increase in the concentration of colouring material on a weight basis. Determining causality is an important part of research because it can lead to the development of new management options. In the case of an increase in red wine colour observed in irrigation studies, a reduction in berry size can also be achieved by modifying pruning practice (Holt *et al.*, 2008) or by draining off a portion of the juice prior to fermentation to mimic a reduction in berry size.

The phenolic compounds important to wine colour are biosynthetically derived from the amino acid phenylalanine (Fig. 3.10). The compounds important in white wine colour, the hydroxycinnamic acids and flavan-3-ols, are produced in the grape berry early in development, before *véraison* (Romeyer *et al.*, 1983; Kennedy *et al.*, 2000, 2001; Downey *et al.*, 2003). During fruit ripening, these compounds are likely modified, but these reactions are not well understood. In red grape varieties, anthocyanin production begins at *véraison* and continues throughout fruit ripening. Late in the growing season, when the grape berry becomes physiologically compromised, there is a reduction in the per berry concentration of anthocyanins (Kennedy *et al.*, 2002). The concentration on a weight basis, however, may increase due to berry desiccation.

The compounds responsible for white wine colour are distributed in the pulp and skin of the grape berry (Ong and Nagel, 1978) but, given that most white wines are produced without contact with the solid parts of the grape, the colour is essentially pulp-derived. As mentioned earlier, the major hydroxycinnamic acid found in the grape is caftaric acid (Ong and Nagel, 1978; Nagel *et al.*, 1979b; Herrick and Nagel, 1985).

For red wine, the anthocyanins for all but a few cultivars, are restricted to the skin tissue, and are generally dominated by malvidin-3-glucoside (Wenzel *et al.*, 1987). For those varieties that also have pulp-derived anthocyanins (teinturier varieties), the anthocyanins in the pulp are generally dominated by cyanidin-3-glucoside. The anthocyanins in skin tissue are considered to be vacuolar, unlike the tannins which may also be associated with the plant cell wall (Amrani-Joutei *et al.*,



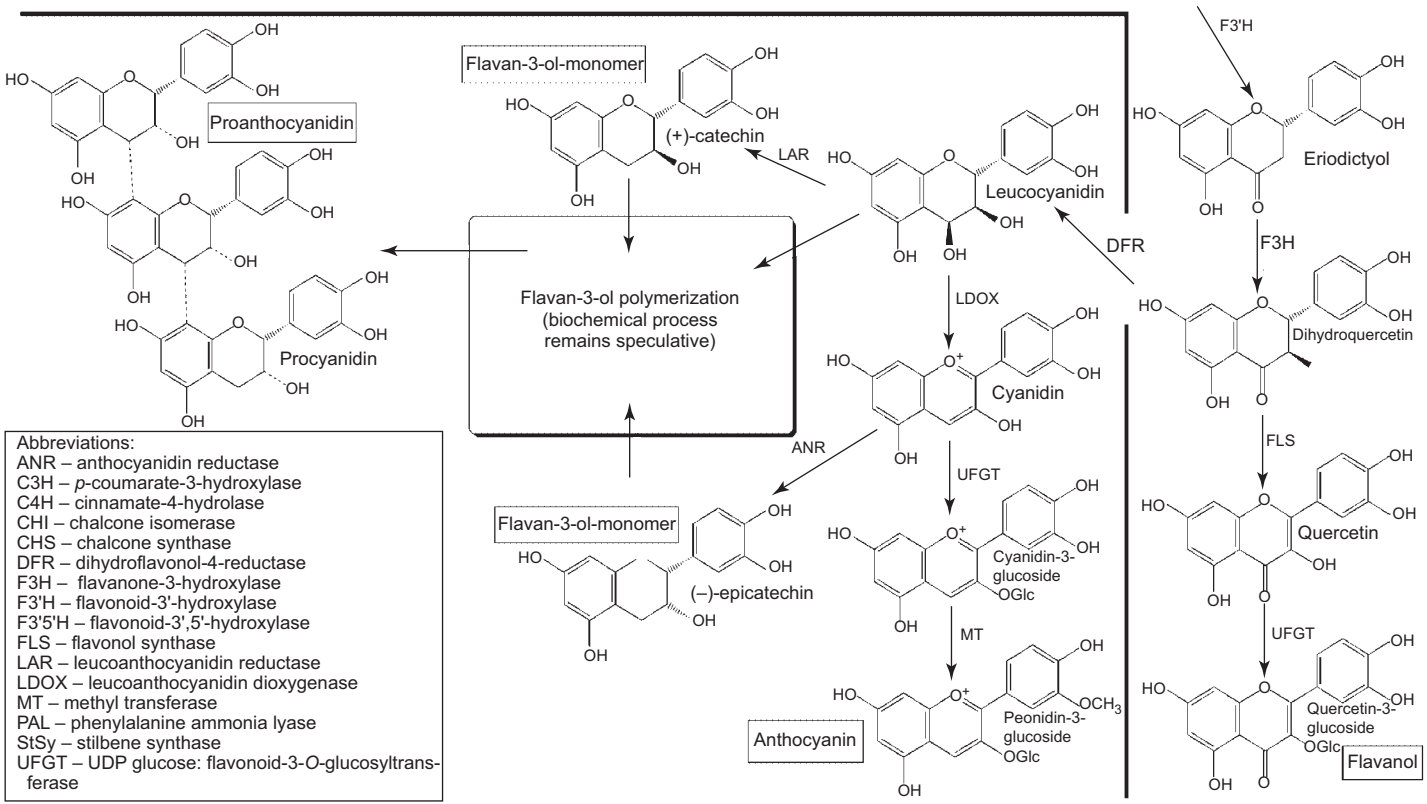


Fig. 3.10 Simplified biosynthetic pathway for phenolic compounds important in wine (see pages 88 and 89).

1994). Late season desiccation, where the physiological integrity of the plant cell is affected, may result in skin-localized anthocyanins 'leaking' out of the plant cell vacuoles and into the pulp of the grape berry.

3.3.2 Management practices and the influence of environment on fruit composition

Given the importance of grape composition on the colour of wine, considerable work has been devoted to understanding how environmental factors can be used to improve wine colour (Downey *et al.*, 2006). For red wine, considerable work has been invested and, from this, a better understanding of how wine colour can be manipulated in the vineyard has been achieved. For white wine, we are still early in our understanding of how grape production practices can be used to influence white wine colour. Ultimately though, wine production practices will likely have a much more profound effect on the final colour of white wines because of the role that oxygen exposure has on wine colour.

As expected, the production of compounds in the grape will be sensitive to both time and temperature. These are probably the two most important factors influencing colour although, within a specific site, other factors including vine water status, light, nutrition, and disease pressure are varied. Understanding the optimal way to manage a site to maximize wine colour quality often begins with a site comparison in order to identify manageable factors (Arozarena *et al.*, 2002).

Using exposure as a critical factor affecting fruit phenolics, additional research has focused on separating the fundamental variables that are affected by exposure, light and temperature. Both of these factors have been found to influence fruit phenolics (Kliewer; 1977; Hunter *et al.*, 1991; Dokoozlian and Kliewer, 1996; Keller and Hrazdina, 1998; Bergqvist *et al.*, 2001; Spayd *et al.*, 2002; Coventry *et al.*, 2005; Joscelyne *et al.*, 2007). Based upon work on anthocyanins, an optimal temperature appears to exist, with excessively high temperatures (in excess of 30–35 °C) leading to altered production (Kliewer and Torres, 1972; Kliewer, 1977; Spayd *et al.*, 2002; Mori *et al.*, 2005; 2007; Yamane *et al.*, 2006). It would also appear that when below the high temperature cut-off, higher temperatures and a reduction in diurnal temperature variation lead to an increase in anthocyanin production (Spayd *et al.*, 2002; Cohen *et al.*, 2008; Tarara *et al.*, 2008).

Vine water status is a factor that is often varied in order to optimize vine balance and reduce disease pressure. As mentioned above, it can also be used to modify grape colour. Many studies have been conducted on the influence of vine water status on grape and wine colour (Matthews and Anderson, 1988; Matthews *et al.*, 1990; Esteban *et al.*, 2001; Kennedy *et al.*, 2002; Roby *et al.*, 2004; Walker *et al.*, 2005; Bindon *et al.*, 2008). In general, a reduction in vine water status will lead to a reduction in berry size with a resulting increase in the concentration in phenolics on a fruit weight basis. It is evident however, that an excessive increase in water deficit severity will adversely affect the biosynthesis of berry phenolics.

A reduction in vine water status reduces vine vigour, and it has been shown that a reduction in vine vigour can lead to an increase in berry and wine phenolics

(Cortell *et al.*, 2005, 2007a,b). Developing a causal explanation for this observation has often been directed to understanding how irrigation/vine vigour influences the environment around the fruit. Concomitant with a reduction in vine vigour, an increase in fruit exposure is generally observed. Increases in exposure lead to an increase in fruit/wine phenolics (Crippen and Morrison 1986a,b; Rojas-Lara and Morrison, 1989; Smart *et al.*, 1990; Reynolds *et al.*, 1994, 1995; Price *et al.*, 1995; Ristic *et al.*, 2007).

General vine balance is an important consideration when managing wine colour (Kliewer and Dokoozlian, 2005). Management practices affecting vine balance include for example: crop load (Bravdo *et al.*, 1984; Reynolds *et al.*, 1994), and canopy management (Reynolds and Wardle 1989; Hunter *et al.*, 1995; Vasconcelos and Castagnoli, 2000; Wolf *et al.*, 2003; Main and Morris, 2004; Reynolds *et al.*, 2005).

The knowledge acquired to date is, as expected, a complex plant response to its environment. Many of the studies point to a situation where an optimal fruit temperature for colour production exists in the grape. Achieving this optimum temperature is site specific in terms of how best to achieve, and may require an increase in fruit exposure (through canopy management, irrigation or vine nutrition for example) or perhaps may require a reduction in fruit exposure in warm climates. Optimizing the conditions necessary for colour in wine must also be balanced with other factors that may affect fruit quality, particularly disease pressure.

3.4 Winery influences on wine colour

Wine colour can be influenced by essentially all aspects of wine production. An understanding of the chemical reactions detailed above provides insight into explaining the effects of various production processes on wine colour. Beginning with the harvest, processes applied to fruit and subsequent wine will potentially affect wine colour. Harvest time can influence the cellular integrity and, hence, the extractability of phenolics from the skin (Fournand *et al.*, 2006), and therefore the permeability of the grape berry cells must also be considered in addition to the total concentration of the solutes. For most white wines, the initial hours after harvest appear to have the greatest impact on eventual white wine colour. For red wines, eventual red wine colour is dictated by practices applied over a longer period of time.

3.4.1 Prefermentation, fermentation and maceration

There are a number of treatments that are applied to harvested grapes prior to fermentation. Once grapes are harvested, it is generally good practice to process them as soon as possible. There are exceptions, however. One such exception is in the production of appassimento style wines, where the grapes undergo dehydration under natural conditions for a period of time before commencing fermentation. In

this case, grape berry desiccation results in a loss of physiological integrity of plant cells and this likely leads to oxidation and condensation reactions, which modify the colour of the resulting wine (Moreno *et al.*, 2008).

For white wine production, grapes are pressed soon after harvest and may or may not undergo prior destemming/crushing. At this stage in processing, the must is particularly susceptible to oxidation, and it is generally advantageous to minimize oxygen exposure. To avoid oxidation, SO_2 is often added to inactivate the oxidative enzyme polyphenol oxidase (Valero *et al.*, 1989). To minimize oxygen exposure to fruit, some processors will press the whole clusters directly. Producers of aromatic white wines, however, generally crush the fruit and allow some skin contact in order to improve the aroma and flavour properties of the wine.

As described above, when white wine phenolics are initially oxidized in must, the yellow oxidation product undergoes further reaction with the reduced form of glutathione to form a colourless product (Singleton *et al.*, 1985; Cheynier *et al.*, 1986). The analysis of this product can give some indication of must oxygen exposure. If further oxidation occurs following glutathione consumption, the resulting white wine will become increasingly yellow in colour. As discussed above, there are numerous factors which will determine the extent to which the must will be oxidized including must pH, oxygen exposure, sulphite addition, glutathione and phenolic concentration, metals which can undergo redox cycling, skin contact time, and temperature (Ramey *et al.*, 1986; Cheynier *et al.*, 1989b; Rigaud *et al.*, 1991; Ricardo-da-Silva *et al.*, 1993; Guyot *et al.*, 1995; Oszmianski *et al.*, 1996). In general, it is considered undesirable to expose must intended for white wine to conditions that promote oxidation. In addition to the production of aromatic white wines, a notable exception is to hyperoxidize the must in order to intentionally promote the oxidation of phenolics (Cheynier *et al.*, 1989a). The rationale for doing so is to oxidatively polymerize the phenolics until they are no longer physically stable in solution and precipitate. The resulting wine would have reduced yellowness and be more colour stable.

For the production of red wines, and although there is some concern for the phenolics common to white wine, the primary attention is with regard to anthocyanins and optimizing their extraction. Given that for most varieties the anthocyanins are restricted to the skin tissue, optimizing diffusion is critically important. Extreme examples of how diffusion plays a critical role in wine style are the production of white sparkling and rosé wines from red grapes.

Once the fruit is crushed, anthocyanins will naturally begin to diffuse out of the skin tissue and into the fermentor. Diffusion is the process by which a compound moves from a region of high concentration toward a region of lower concentration (i.e., from the plant cell into the wine). Considering the generally observed extraction curves for anthocyanins and the effects that wine processing variables have on the rate of the compounds' extraction, the overall process is generally consistent with diffusion. Diffusion is dependent upon the following:

(a) temperature
(b) molecular weight/size and type of molecule

(c) concentration gradient
(d) cell permeability
(e) surface area over the concentration gradient
(f) composition of extraction medium (such as ethanol concentration).

In all instances but molecular size, these variables contribute positively to the rate of diffusion. Time, of course, is an important variable for overall extraction.

From past research, time and temperature are the two critical variables in determining the ultimate amount of anthocyanin present in wine (Berg and Akiyoshi, 1956; Ough and Amerine, 1961; Aubert and Poux, 1969; Timberlake and Bridle, 1976b; Miller and Howell, 1989; Scudamore-Smith *et al.*, 1990; Zimman *et al.*, 2002; Sacchi *et al.*, 2005). For anthocyanins, higher temperatures reduce the time to maximum concentration and increase the maximal amount. Besides the most obvious variables of time and temperature, the other variables undoubtedly also play a role in anthocyanin extraction. Determining their relative importance, however, is difficult because of the compound nature of their affects.

In practice, it is not possible to fully extract all anthocyanins from the grape and, therefore, the rate of extraction and the total amount extracted are of interest to the winemaker. Because tannins, which provide astringency to wine, are also in part derived from the skin tissue (Aron and Kennedy, 2007; Cerpa-Calderón and Kennedy 2008), balancing colour extraction with 'astringency' extraction is an important consideration. Enzymes added prior to fermentation can be used to improve the rate and total phenolic extraction from the skin (Wightman *et al.*, 1997; Guadalupe *et al.*, 2007). Application of high temperature/short time treatment is successful at improving phenolic extraction (Morel-Salmi *et al.*, 2006), as is concentrating the skin of the berry relative to the juice (Singleton, 1972). Sulphur dioxide has been used to enhance anthocyanin extraction, but this may not be ideal for long-term colour quality (Somers *et al.*, 1983)

Once extracted, and prior to fermentation, oxidation and the stability of anthocyanins is a concern (Sarni *et al.*, 1995). In practice, the total quantity of anthocyanin extracted into the fermentor prior to the commencement of fermentation is much less than the quantity extracted during maceration after the commencement of fermentation.

Many of the factors discussed above that influence anthocyanin extraction, are used during fermentation, and various practices have been developed to influence colour extraction (Ough and Amerine, 1961; Timberlake and Bridle, 1976b; Scudamore-Smith *et al.*, 1990; Zimman *et al.*, 2002; Sacchi *et al.*, 2005). The extraction of colour has been shown to have a different extraction curve than tannins (Ribéreau-Gayon *et al.*, 1970; Bertrand *et al.*, 1982). Recent research suggests that colour extraction may track skin tannin extraction (Sampaio *et al.*, 2007). Since wine tannin is a combination of skin and seed tannin, then the difference in extraction behaviour between anthocyanins and total extracted tannin may reflect seed tannin extraction.

As fermentation and maceration continue, extracted phenolic material begins to react. The reactivity of the phenolic compounds as described above should be

considered in this case. For white wine, control of the oxidation process will dictate the colour of the final product. During fermentation, there is sufficient liberation of carbon dioxide to prevent oxidation; following fermentation though care must be given to ensure that oxygen is prevented from contacting the wine. Minimizing air space above the wine is desirable in this case.

For red wine, the anthocyanin reactions that lead to long-term colour stability begin essentially upon extraction. The low molecular weight pyruvic acid adduct of malvidin-3-*O*-glucoside, vitisin A, begins forming during fermentation (Asenstorfer *et al.*, 2003b). Even the formation of pigmented polymers has been found to begin during fermentation (Eglinton *et al.* 2004). In addition, there can be yeast strain variation in the colour properties of young red wine (Hayasaka *et al.*, 2007).

3.4.2 Post-fermentation/maceration treatments

Once a new wine has been produced and fermentation has concluded, it is important in essentially all style variations that oxidation be controlled. Even in the case of sherry production, while there is ullage above the wine during ageing, film forming yeast typically prevents the underlying wine from becoming overly oxidized. For wines intended to be consumed at an early age, in order to maximize the fruity and floral aroma attributes, production practices often dictate that wine should be bottled as soon as possible. This is generally beneficial in terms of colour preservation and reducing wine yellowing. For wines intended to age, barrel storage is often used to oxidize the wine in a very controlled fashion. This is generally beneficial in terms of tannin development (Cheynier *et al.*, 1998). This slow oxidative process is controlled with wine being maintained under less than oxygen saturation conditions. Colour transformations do occur as detailed above, with anthocyanins being modified via oxidation and acid-catalyzed reactions. Variations in pH and oxygen exposure will affect colour development and appearance (Dallas and Laureano, 1994b; García-Viguera *et al.*, 1994; Morais *et al.*, 2002; Walker *et al.*, 2004; de Beer *et al.*, 2008). During this period of time, the optimization of aroma, flavour and wine texture are the desired outcomes of ageing. Wine colour development is typically a secondary effect.

Once wine has been packaged, oxidative reactions slow considerably but ultimately continue. The eventual fate of colour in wine is to become increasingly yellow with age, with a concomitant loss of red colour in the case of red wine. With that said, there are fine examples of red wines whose red colour has persisted for well over the century mark.

3.5 Conclusion

Colour and the other sensory attributes of wines are appreciated together. For the consumer, colour quality is essential to the full enjoyment of wine. It is therefore important as a grape and wine producer to understand how production practices

influence wine colour attributes. A considerable degree of chemistry understanding has taken place since the 1970s, and an understanding of these reactions has led to a better understanding of the effect of specific processes on colour as well as the development of new methods for managing colour.

This chapter has summarized much of our understanding to date, but further developments will surely take place. Future developments in rapid methods of colour assessment both in the vineyard and winery will likely take place in order to improve our ability to manage wine composition more effectively. It is also likely that future research into how colour development influences other wine attributes such as aroma and texture will occur. Finally, the structural determination of new colour compounds and their pharmacologic activities will continue to be an important area of research as we unravel the health-related benefits of wine. There is little doubt therefore that colour will continue be a fascination for wine scientists and producers alike.

3.6 References

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4

Practical methods of measuring grape quality

B. W. Zoecklein, Virginia Tech, USA; K. C. Fugelsang, California State University – Fresno, USA; and B. H. Gump, Florida International University, USA

Abstract: Grape quality depends to a large extent on various metabolites, timing and completeness of ripening and the ripening synchrony of skins, seeds, stems, and pulp. This chapter outlines grape quality issues of importance in stylistic winemaking.

Key words: grape maturity, aroma/flavour, phenols, asynchronous ripening.

4.1 Definition of grape quality

Wines, and the grapes used to produce them, are highly differentiated products influenced by a wide array of factors including variety, growing season, soil, vineyard management, and winemaking characteristics. High-quality wines, regardless of how defined, are the result of the confluence of important attributes, including grape quality. Grape quality is impacted by (i) maturity, purity, and condition, (ii) aroma/flavour and phenolic characteristics, and (iii) harvesting methods, transportation and processing protocols. For the most part, each of these is best evaluated not in isolation, but in combination, to define optimum grape quality for a particular wine type and style.

There are three stages of berry development following flowering: green berry; arrest of green berry development, and pause before the onset of ripening; and fruit ripening or *véraison* (Jackson and Lombard, 1993) (Fig. 4.1). *Véraison* can be divided into stages based upon berry metabolism and transport of substances to the

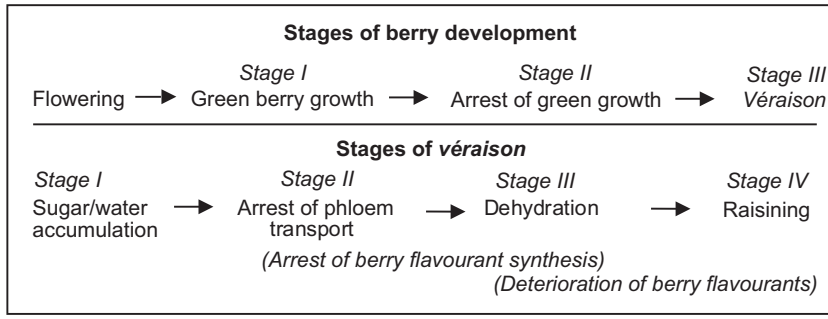

Fig. 4.1 Stages of grape maturation.

vine (Fig. 4.1; Bisson, 2001). It is not well understood if synthesis is controlled by hormonal signals in the vine, or in the fruit independent of other vine influences (Robinson and Davies, 2000). Both changes in phloem transport and the onset of berry dehydration influence fruit composition (Matthews *et al.*, 1990). Overall, the berry approximately doubles in size between *véraison* and harvest (Conde *et al.*, 2007). As a result, many of the solutes accumulated in the fruit during the first period of development have their concentration substantially reduced. However, some compounds are reduced on a per-berry basis, not simply due to dilution. For example, malic acid, which is metabolized and used as an energy source during the ripening phase, is substantially decreased relative to tartaric acid, whose concentration usually remains almost constant after *véraison*. Tannins also decline considerably on a per-berry basis after *véraison*. Some aromatic compounds, including several of the methoxypyrazine compounds, decline after *véraison.*

Fruit maturity influences wine style, and most winemakers understand that timing of grape harvest determines the maximum wine potential thereafter. High-quality wine, regardless of how defined, is the result of the confluence of important fruit attributes, including the development of fruit-derived aroma/flavour components, desirable colour, and desirable tannins. Ideally, these coincide with primary metabolites such as optimum soluble solids concentration (Fig. 4.2). In reality,

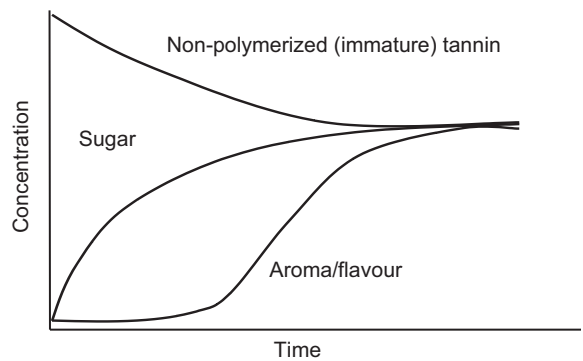

Fig. 4.2 Stylized grape maturation (adapted from Long, 1986).

grape maturity indices seldom align; if they did, maturity evaluations would be an easy task.

4.2 Vineyard factors impacting maturation

A significant volume of research has advanced our understanding of how various viticultural variables and practices, including fruit maturity, crop level, crop exposure (Zoecklein *et al.*, 1998; Bergqvist *et al.*, 2001), leaf area to crop ratio (Kliewer and Dokoozlian, 2005), shoot density, and training systems (Reynolds *et al.*, 1996) affect grape composition and maturation. It is commonly found that higher quality wines from a particular variety within a designated region are made from fruit that reach their targeted maturity earlier. Those that do not are either out of balance, young vines, or are not optimally managed with respect to cropping level, irrigation, pruning, and/or canopy management. Important features impacting fruit maturation include:

- fruit temperature
- humidity
- rainfall
- site characteristics, including soil type and sun exposure
- soil moisture, irrigation management, deficit irrigation
- variety/clone
- training and trellising systems
- row orientation
- canopy management
- rootstock
- yield components: kg fruit per vine, clusters per vine, clusters per shoot, berries per cluster, berry weight.

Table 4.1 shows how viticultural and environmental factors affect grape composition variables.

4.3 Fruit sampling

Fruit sampling is usually begun sometime after berries have reached full *véraison*. Regardless of maturity gauges utilized, an important and universal concern is accurate vineyard sampling. Fruit sampling methodologies have been extensively reviewed (Rankine *et al.*, 1962; Roessler and Amerine, 1963; Jordan and Croser, 1983; Wolpert and Howell, 1984; Kasimatis and Vilas, 1985). There are two basic choices in fruit sampling: cluster sampling or berry sampling. With cluster sampling, a further choice can be made of gathering clusters from throughout the vineyard, or using one or more targeted vines.

If berry sampling is to be employed, two samples of 100 berries each can give accuracy to 1.0 °Brix, and five samples of 100 berries each can give accuracy to

Table 4.1 Grape variables impacted by viticulture and the environment

Quality variable	Soil nutrition	Canopy	Irrigation	Pests and disease
Sugar	Nitrogen excess Potassium	Leaf and fruit exposure Crop load Pruning Summer pruning Crop removal Plant growth regulators	Irrigation RDI[a] PRD[b]	Powdery mildew Viruses
Colour	Nitrogen excess Potassium	Shading Crop removal	RDI Irrigation	*Botrytis* Viruses
Berry size	Nitrogen excess	Pruning Crop removal Plant growth regulators	Irrigation RDI	
pH	Nitrogen excess Potassium	Shading Crop load	Irrigation	
Titratable acidity	Nitrogen excess	Shading Crop load	Irrigation	
Contaminants (including MOG[c] and pests and diseases)	Nitrogen excess Excess chloride	Canopy ventilation Bunch exposure Shading Pruning Crop removal	Saline water	Pests and diseases Chemical residues *Botrytis* Powdery mildew Downy mildew Pests Harvest

[a]RDI = regulated deficit irrigation.
[b]PRD = partial rootzone drying.
[c]MOG = material other than grape.
Source: Krstic *et al.* (2003).

0.5 °Brix. Using cluster sampling, ten clusters can be accurate to 1.0 °Brix (Jordan and Croser, 1983; Kasimatis and Vilas, 1985). It should be noted that there is a general tendency, when examining a cluster prior to berry sampling, to select the most mature berries. Therefore, berry sampling should involve locating the fruit zone, and sampling without examining the clusters or berries. If this does not occur, berry samples will frequently be about 2 °Brix higher than the true value. About 90% of the variation in berry sampling comes from variation in the position of the cluster on the vine, and the degree of sun exposure (Trought, 1996). The vineyard must be sampled based on the degree of fruit exposure (Jordan and Croser, 1983).

4.3.1 Fruit yield components

Many components contribute to grapevine yield (May, 1972). These include the following:

- vines per acre/vines per hectare
- shoots per vine/shoots per metre
- clusters per shoot
- clusters per vine
- cluster weight
- berries per cluster
- berry weight
- fruit weight per vine.

An over-cropped vine is one that has a large crop with insufficient, healthy active leaves; it cannot produce enough sugar to maintain all clusters for desirable ripening, and it fails to produce grapes with sufficient aroma/flavour, and/or desirable phenol compounds. Yield can impact the rate of fruit maturation (Winkler, 1965; Zoecklein *et al.*, 1996). Variation in some of the above-listed components of yield can contribute to variation in the yield at harvest, although the grapevine itself is capable of self-regulation (Clingeleffer, 1983) and yield component compensation (Freeman *et al.*, 1979; Smart *et al.*, 1982). While many yield components cannot be controlled directly, vineyard managers do have the capacity to manipulate some variables in the vineyard. Pruning regulates node number per vine and budburst. Carbohydrate reserves can be modified to influence bud fruitfulness and fruit set (Smith and Holzapfel, 2003). Grapevine canopies can be managed to enhance budburst, bud fruitfulness, and berry growth (Baldwin, 1964; Buttrose, 1974; Smart and Robinson, 1991; Smart, 1992).

The earlier the estimation of average berry weights, the more time the winemaker has to evaluate the crop load, make adjustments, and plan for the season. There is a relationship between berry weight at *véraison* and berry weight at maturity. For Syrah, McCarthy (1997) determined that relationship to be the following: $y = 1.35x + 0.53$, where y = the berry weight at 23 °Brix and x = the berry weight at about 5 °Brix. This relationship will differ by cultivar and site, but can be determined by collecting *véraison* and harvest samples for several seasons. Accurate estimations of yield from precision viticulture techniques, with mapping using global positioning systems (GPS), optical remote sensing, and other tools, may soon be available.

4.3.2 Asynchronous ripening

Variation is an inherent part of biological systems. Variation in the vineyard occurs among berries, bunches, and vines. The wine industry presumes that variation has a negative impact on crop level, fruit composition, and wine quality, although few studies have been conducted to substantiate this assumption (Gray, 2006).

Two components of berry-to-berry variation are significant in grape and wine production: size and berry composition. In extreme cases, this is referred to as 'hen and chicken' or *millerandage* (Winkler, 1965). Variation in berry size affects vineyard yield and wine quality. High levels of variation in the early post-flowering period suggest that variation originated prior to berry set. Such variation most likely results from asynchronous cell division in the floral primordium at

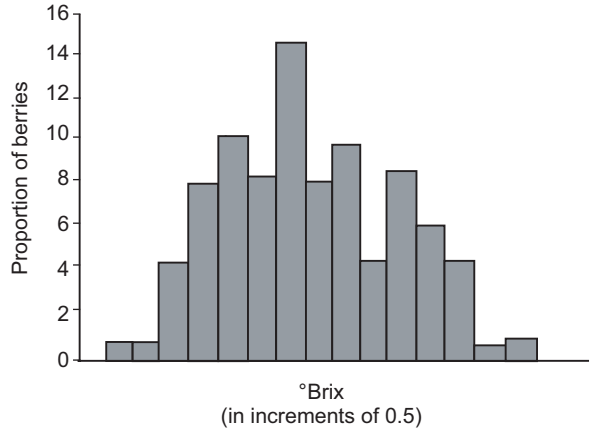

Fig. 4.3 Relationship between °Brix and berry weight at different sampling dates (Long, 1986).

budburst. Decreasing levels of variation may indicate points of re-synchronization in the berry growth cycle: the more synchronized the event, the lower the variation.

A crop with asynchronous clusters or berries has a mixture of developmental stages, resulting in berries with optimal qualities diluted by berries which may be inferior. This can be seen in a frequency distribution, with berry numbers plotted against °Brix (Fig. 4.3). Even before differences arise from processing, it is generally not true that two vineyards or vineyard blocks with the same °Brix values will give similar wines. A juice with Brix of 22° might be composed of a narrow distribution of a few berries at 20° and a few at 24 °Brix, with the majority nearer to 22°. However, there may be a much wider distribution, with berries below 18° and greater than 24°. Because °Brix is a distribution average, juices with similar °Brix values can produce quite different wines, due to variations in aroma/flavour and phenol compounds.

Vine-to-vine variability of visually uniform vines (below), expressed as percentage of the coefficient of variation, was reported by Gray (2006), indicating the inherent nature of vineyard variability. While soluble solids concentrations may be fairly uniform, with a coefficient of variation usually less than 10%, the variance can be much greater if the fruit is not uniform across clusters or if the cluster microenvironment is variable among vines:

- Brix 4–5%
- pH 3–4%
- titratable acidity 10–12%
- berry weight 6–20%
- colour 13–18%.

Vine-to vine-variation

Many variables can be measured at the vine level, including soil characteristics,

carbohydrate reserves, bud fruitfulness, percent budburst, inflorescence primordia number, node number, shoot number, and cluster number.

The inherent variation among individual vines can have a greater impact on yield than external influences such as soil variability, or drainage and fertility irregularities (Strickland *et al.*, 1932). Variation in soluble solids concentrations, titratable acidity, and cluster weight between vines can be much greater than within vines (Rankine *et al.*, 1962). Spatial analysis techniques and GPS have aided our understanding of yield components. Aerial vineyard images, using satellite or aircraft, can be used to calculate a normalized different vegetation index (NDVI) for each vine. These maps can be used to visualize differences in vine vigour or relative biomass on a vineyard scale (Hall *et al.*, 2002).

Variation among clusters

Differences in cluster size are commonplace in most vineyards. Since yield forecasting and maturity testing procedures may rely on cluster sampling, differences in cluster size can be a major source of error. Stratified cluster and berry sampling programs have been devised to overcome some of these problems, but seasonal, varietal, and site-specific considerations confound general sampling protocols (Wolpert and Howell, 1984; Kasimatis and Vilas, 1985). The variables that contribute to variation among bunches include influorescence primordia size, flower number, fruit set, berry number, cluster weight, and cluster position.

Variation among berries

Variation among berries is poorly understood. A typical berry follows a double-sigmoid growth curve during its post-flowering development, but two berries in the same cluster may follow quite different paths (Matthews *et al.*, 1987). The divergence of the growth curves becomes apparent shortly after flowering, and the timing of this divergence is responsible for the extent of the difference between the two berries at harvest. Uneven berry development and its impact on wine quality is largely undocumented. Two studies (Trought, 1996; Trought and Tannock, 1996) determined the extent of variation in the size (weight and volume) and composition (°Brix) of berries within clusters of Chardonnay, Cabernet Sauvignon, and Pinot noir. Seed weight, berry volume, and vascular development of the

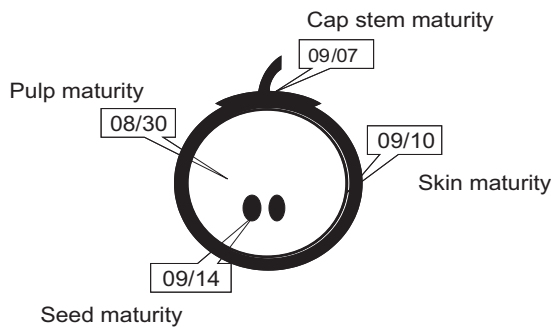

Fig. 4.4 Changes in seed tannin extractability (adapted from Delteil, 1998).

pedicel were correlated. The variables that contribute to variation between berries include berry size, berry composition, seed number, seed size, and berry position.

Relative maturity dates of the important components of a red berry – skin, pulp, seeds, and cap stem – are shown in Fig. 4.4. Given that all parts enter the fermentor in red wine production, the control of stylistic winemaking may be negatively influenced if component parts of the fruit are not at the optimal physiological maturity at harvest.

4.3.3 Measuring vineyard variation

A number of studies have reviewed the factors impacting vineyard variation (Rankine *et al*, 1962; Smart and Robinson, 1991; Trought, 1996; Trought and Tannock, 1996). Prior to fruit sampling, one needs to gain some appreciation of the variation within each vineyard block. Several techniques can be used to quantify the level of dispersion around a population mean, including range, mean deviation, sum of squares, variance, standard deviation, and the coefficient of variation. Expressed as a percentage, the coefficient of variation (CV) is a unitless measure of the sample variability, relative to the sample mean:

$$\text{coefficient of variation (CV)} = \frac{\text{standard deviation } (s) \times 100}{\text{mean } (x)} \qquad [4.1]$$

A sequential comparison of CVs can reveal both the source of variation and the points of re-synchronization in the berry's developmental cycle (Gray 2006).

4.3.4 Vineyard variation management

Zonal management and zonal harvest are appropriate techniques where the grape grower has ready access to the necessary technology. Perhaps the best approach to help minimize vine variation is site selection. Variation may be minimized by choosing a site with limited variation in soil, topography, aspect, and extreme weather events.

Cluster variation may be managed by applying viticultural best-practices or a viticultural hazard analysis and critical control point (HACCP) plan to promote uniform budburst, shoot growth, flowering, cluster exposure, and berry development (Coombe and Iland, 2004). The specifics of berry-to-berry variation are not well understood. Factors that may contribute include variations in cluster architecture, the role of vascular function in berry growth and development, the relationship between seed development and berry development, and the relative importance of cell division and cell expansion throughout the entire developmental cycle (Gray, 2006).

4.4 Fruit maturity gauges

Maturity evaluation should be viewed in the context of stylistic goals. If the fruit

does not contain desirable aroma/flavour, colour, and tannin characteristics, these features will likely not be optimized in the resultant wine. If a clear descriptive analysis of the quality target exists, the time of harvest can aid in meeting those goals. Maturity evaluations usually involve several to many of the following (Zoecklein *et al.*, 1999):

- aroma/flavour and intensity of aroma/flavour
- grape skin tannins and tannin extractability
- stem lignification or 'ripeness'
- seed numbers per berry
- seed 'ripeness' or tannin extractability
- sugar per berry
- red fruit colour
- °Brix
- acidity
- pH
- berry softness
- berry size/weight
- berry shrivel
- potential for further ripening, general fruit condition.

Maturity variables often change with time. It is not completely understood how each of the above relate to one another, and the importance of their individual or collective values as predictors of wine quality. The time to harvest is prior to deterioration of desirable fruit characters or components. However, the factors that control the loss of berry aroma/flavour compounds, for example, and when degradation may be initiated is not well understood. As such, a chemical marker of the onset of fruit aroma/flavour deterioration would be ideal as a maturity gauge (Bisson, 2001).

4.4.1 Berry size/weight

There is evidence suggesting that smaller berries may yield richer must, in terms of colour intensity and tannin composition. However, Matthews and Kriedemann (2006) reported that the cause of berry size is more important in determining must composition and wine sensory properties than berry size *per se*. They suggested that how the change in size came about is important, making a distinction between environmental factors versus biological processes that underlie variation in reproductive development. For example, smaller berry size in red varieties, such as Cabernet franc, commonly yields a richer must if berry size is reduced by environmental factors such as deficient irrigation. By contrast, Shiraz berries that are smaller for developmental reasons, and have fewer seeds, do not necessarily produce musts that are richer (Walker *et al.*, 2005). High yield reduces the weight of individual fruit, but generally causes lower, rather than higher, concentrations of solutes (Bravdo *et al.*, 1985). Increased light exposure increases both berry size and solute concentration (Dokoozlian and Kliewer, 1996). Additionally, the

timing of water deficits prior to *véraison* often, but not always, increases Brix. The question remains whether that is solely the consequence of a reduction in berry size. Knowledge of berry size may allow for adjustments in wine processing methodologies such as cap management, *saignée*, etc. to reach stylistic goals.

4.4.2 Sugar evaluation

Methods of expressing sugar concentration

Sugar is usually expressed as °Brix, °Baumé, total soluble solids concentration (TSS) or by specific gravity. °Brix, named after the German scientist Adolf Brix, is defined as grams of soluble solids per 100 g of solution. It is a measure of all soluble solids, including pigments, acids, glycerol, and sugar. Generally, the fermentable sugar concentration of grape must accounts for 90–95% of the total soluble solids. Therefore, determination of °Brix provides only an approximate measurement of sugar concentration. The vast majority of grape sugar consists of the two monosaccharides glucose and fructose. The ratio of these two is dependent upon the variety and the extent of fruit maturity, with glucose dominating during early berry development. Overripe fruit has a low glucose to fructose ratio, which can have implications with regard to fermentation completion (Zoecklein *et al.*, 1999).

Baumé, often used in Europe and Australia, named from the French pharmacist Antoine Baumé, is a measure of the sugar concentration of fruit and the potential alcohol that can be achieved by complete fermentation. Thus, °Brix and °Baumé naturally relate to each other: 1.0 °Baumé is equivalent to 1.8 °Brix. Grapes with a 13 °Baumé if fermented completely would produce a wine with about 13% (v/v) alcohol. °Brix, or its equivalents, is commonly used for the estimation of potential alcohol, important for stylistic winemaking and as a method for grower compensation.

A number of studies have shown a correlation between sugar accumulation and grape berry aroma/flavour compounds; however, the strength of the association depends on a number of variables (Robinson and Davies, 2000). The synthesis of many grape aroma/flavour compounds requires energy, but the factors leading to cessation of synthesis have not been well defined. In cold to cool heat summation regions, °Brix is generally more strongly correlated to aroma/flavour than in warmer regions (Jackson and Lombard, 1993). Strauss *et al.* (1987) demonstrated that one group of aroma/flavour compounds, norisoprenoids, are strongly correlated to grape sugar. Norisoprenoids, 13-carbon terpenoids, are derived from the degradation of carotenoids, and are associated with descriptors such as grassy, tobacco, smoky, kerosene, tea, and honey (Strauss *et al.*, 1987). The norisoprenoids appear to be more stable than the compounds associated with fruity aroma notes. Thus, while sugar can indicate general maturity level, it is not a clear estimation of aroma/flavour. It is commonly found that higher quality wines from a particular variety within a region are made from grapes that reach their targeted soluble solids earlier.

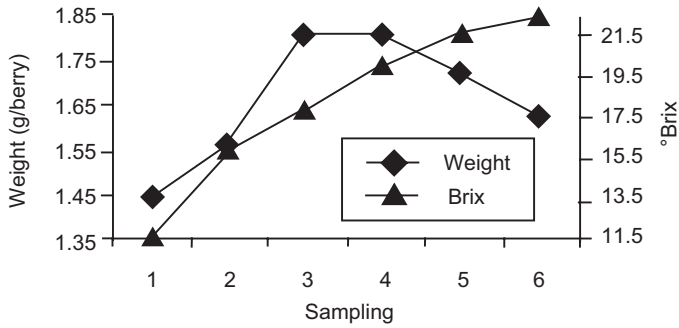

Fig. 4.5 Relative grape maturation (Kennedy, 2000).

Potential for further ripening; hang time

A typical sugar profile during ripening shows an initial rapid accumulation but, at some point during development, the vine ceases transport of sugar to the fruit. Further increases in sugar concentration are due to dehydration. °Brix, berry aroma/flavour, and phenol maturity are not always strongly correlated. This has resulted in extended fruit 'hang time' to allow for desirable changes in secondary metabolites. The results may include the loss of fruit weight, increases in °Brix and, thus, elevated levels of potential wine alcohol. Figure 4.5 illustrates the relationship between berry weight and °Brix at several sampling dates. As maturation continues, berry weight increases, then declines. This decline frequently occurs prior to harvest. °Brix can increase in late stages of maturity, either due to the production of sugar by the plant or to dehydration of the berry.

Berry shrivel and weight

Grape maturation can be evaluated by assessing physical properties of the berry, such as weight, firmness, and deformability. Berry softening is due to changes in composition of cell walls of the fruit, particularly due to pectin and xyloglucan depolymerization, which accompanies arrest of xylem flow to the fruit (Rogiers *et al.*, 2006).

Berry shrivel is an important attribute impacting yield and, frequently, wine style. Shrivel is particularly notable in some varieties such as Shiraz, where shrinkage begins in warm regions at about 80–90 days post-flowering (McCarthy and Coombe, 2001). The decline in berry weight is more closely related to the time from flowering than to °Brix. Symptoms include loss of berry turgidity and wrinkling of the skin. The rate of berry shrinkage varies as a result of region, season, and/or climatic conditions, and among vines within blocks (Rogiers *et al.*, 2006).

Between the maximum berry weight and time of harvest, there can be substantial decline in weight. Research indicates that the maximum rate of production of aroma/flavour compounds occurs at about the time the berry stops importing water from the phloem, or shortly thereafter. Therefore, maximum aroma/flavour occurs sometime after the berry reaches maximum weight in most instances, suggesting

the importance of this as a stylistic winemaking tool. In one study, McCarthy and Coombe (2001) determined optimum harvest weight for maximum secondary metabolite concentration in an Australian Syrah to be 1.2 g per berry. The incidence of berry shrivel and degree of shrivel is used as a maturity gauge for some varieties.

Brix-to-alcohol ratio

Producing balanced, harmonious wines is an important industry goal. Balance refers to the relative concentrations of volatile and structural/textural components. Making wine in a warm growing region or vineyard site may pose a challenge with regard to avoiding excessive alcohol concentration, where increased 'hang time' can result in alcohol levels that negatively impact wine balance.

Theoretically, a given weight of fermentable sugar will yield 51.1% alcohol by weight. The actual alcohol yield is generally different from the theoretical. In the past, winemakers used the conversion factor of 0.55 multiplied by the °Brix to estimate the potential alcohol produced in a dry wine. However, the actual conversion rate can vary from 0.54 to 0.62, or higher. These differences are the result of several factors listed below. For example, softening of grapes occurs from *véraison* to harvest as a result of changes in pectin polysaccharides. Increases in deformability occur with increases in the water-soluble polysaccharide concentration, which can increase the non-sugar-soluble solids concentration:

- variety
- season
- maturity level/soluble solids
- fermentation temperature
- open versus 'closed' fermentors.

Sugar per berry

The °Brix of grape must accounts for 90–95% of the fermentable sugars. However, this measurement is a ratio (wt/wt) of sugar to water and may change due to physiological conditions in the fruit. A potential problem encountered in °Brix, °Baumé, or any soluble solids measures used as a fruit maturity index occurs with changes in fruit weight. Over time, soluble solids readings may show no change, but in fact there may be substantial changes in the fruit weight, either increases or decreases (Table 4.2).

Sugar accumulation may cease due to unfavourable environmental conditions, such as very high or low vineyard temperatures, but resume once conditions have changed. It is important to be able to distinguish transient effects from the permanent cessation of transport of photosynthates. Once phloem transport has ended, any further increases in °Brix will be due to loss of water, not continued synthesis, and translocation of sugar.

Assessing changes in berry weight, and noting the point at which average berry weight starts to decrease, while °Brix increases, can indicate the onset of dehydration. However, this can be difficult to monitor where fruit maturity is not uniform across clusters or berries.

Table 4.2 Determination of sugar per berry

	Changes in berry weight		
Changes in sugar/berry	Decreases	No change	Increases
Increases	Maturation and dehydration	Maturation	(a) Major increase: maturation and dilution (b) Minor increase: maturation
No change	Dehydration	No change	Dilution
Decreases	Dehydration and sugar export	Sugar export	Sugar export and dilution

Source: Long (1984).

The concept of sugar per berry utilizes a soluble solids evaluation, such as °Brix, and takes into account the weight of a berry sample. For example, if data were taken from the same vineyard at five-day intervals and the soluble solids (°Brix) of both sample dates measured 22 °Brix, it might be concluded that there had been no change in fruit maturity. However, sugar per berry calculations could lead to a different conclusion if there were changes in berry weight (Table 4.2). Sugar per berry calculations yield considerably more information than that available by evaluation of °Brix measurements alone.

4.4.3 pH and acidity

pH and potassium

Assessments of acidity and pH are used to help define the optimal time of harvest. Both fruit acidity and pH have a significant impact on wine. pH plays a major role in winemaking, affecting the following (Zoecklein *et al.*, 1999):

- colour
- oxidation rate
- ability to clarify
- biological stability
- protein stability
- tartrate stability
- metal complexation
- sensory attributes.

The pH values for white wines may be 3.5 or less. Higher values usually are observed for red wines, largely because of contact of juice and skins before and during fermentation. Changes in fruit pH are complex, and a result of a number of environmental and viticulture management factors.

Grapes are very rich in potassium, an essential macronutrient for growth and development. Potassium (K^+) is the main cation in must and wine (Blouin and Cruège, 2003). Potassium is absorbed by the roots and distributed to all parts of the vine. Early in the season, when the growth rate is high, much of the K^+ accumulates

in the leaves. After *véraison*, a sharp increase in berry K^+ is observed as a result of K^+ redistribution from leaves to berries (Ollat and Gaudillère, 1996; Blouin and Cruège, 2003).

Excessive K^+ concentration in the fruit at harvest may result in increases in pH and thus negatively impact potential wine quality, particularly in red wines (Davies *et al.*, 2006). The stoichiometric exchange of tartaric acid protons with K^+ cations results in the formation of largely-insoluble potassium bitartrate, leading to a decrease in free acid and tartrate:malate ratio (Gawel *et al.*, 2000). The overall result is an increase in pH. High K^+ levels in the berry may decrease the rate of malate degradation by impairing malate transport from the storage pools in the vacuole to the cytoplasm. Grape skin contains from three to 15 times more K^+ than is present in the pulp. Therefore, berry K^+ levels are often more important to red than to white wines, due to skin contact in red wine production (Mpelasoka *et al.*, 2003).

The levels of K^+ in grape berries may be affected by numerous factors including K^+ level in the soil, antagonistic elements in the soil such as magnesium and calcium, grape variety, and viticultural practices (Mpelasoka *et al.*, 2003; Davies *et al.*, 2006). A detailed knowledge of the mechanisms involved in K^+ transport from the soil, xylem and phloem translocation through the vine, and its accumulation in the berry is crucial in order to develop strategies leading to reduction of excessive accumulation in grape berries and, subsequently, improve fruit and wine quality.

Potassium uptake occurs by multiple mechanisms, both a passive low-affinity K^+ transport across membranes and a high-affinity uptake (Davies *et al.*, 2006). External K^+ levels are thought to determine which mechanism is used. Several vineyard management considerations impact K^+ uptake and pH evolution. Severe stress late in the season can increase K^+ uptake. Crop and overall vine balance are also important in helping to manage pH evolution. Over-cropping may delay the rate of fruit maturity, which can result in increases in pH.

Titratable acidity

The acid concentration of fruit and resultant wine is important to structural/textural balance. Titratable acidity (TA) in grapes normally ranges between 5.0 and 16.0 g/L as tartaric acid; these values are influenced by variety, climatic conditions, cultural practices, and maturity of the fruit.

The organic acid concentration of a wine is traceable to four sources. The grape contributes tartaric, malic, and, to a much lesser extent, citric acid. By comparison to tartaric and malic acids, which are present at concentrations ranging from 2.0–10 g/L and 1.0–8.0 g/L, respectively, citric acid is found in unfermented grapes at 0.2–3.0 g/L (Amerine and Ough, 1980). Alcoholic fermentation results in formation of lactic, acetic, and succinic acids, in addition to very small quantities of other acids from the tricarboxylic acid cycle. Bacterial involvement may produce substantial amounts of lactic and acetic and, on occasion, propionic and butyric acids. Lastly, mould growth on the grape may result in gluconic acid concentrations of 1.5 –10 g/L in a finished wine (McCloskey, 1974).

The reduction in TA during fruit ripening is partly related to the respiration of malic acid in the berry and is, therefore, related to temperature. Grapes grown in warmer regions (heat summation units) mature earlier and have a lower TA at the same soluble solids concentration, when compared to fruit grown in a cooler climate (Gladstones, 1992). A characteristic of cooler growing regions is lower daily temperature fluctuations during the late stages of fruit ripening, an important contributor to acid retention (Gladstones, 1992).

A historic index of ripeness involves the product of °Brix times the square of the pH (Amerine and Joslyn, 1970). Another historical scale relates TA and sugar; in this case the °Brix value is divided by the TA (Gallander, 1983). This is designed to indicate the optimal sugar/acidity balance. Others have suggested that this value can be higher for late harvest fruit (Amerine *et al.*, 1980).

There are several problems associated with using only sugar and acid as primary maturity gauges. For example, the sugar-to-acid ratio is variable across different varieties and growing conditions and, therefore, may be difficult to use as a general predictive value. However, changes in TA may be useful in assessing maturity and the changes in the rate of maturity.

Organic acids

Malate is consumed as an energy source in the berry during *véraison*, and the concentrations decrease relative to tartrate (Jackson and Lombard, 1993). Tartrate concentrations generally remain constant during *véraison*, but may rise slightly during grape dehydration. Malate concentrations decrease with maturity, and may plateau at a low level, roughly 2–3 g/L (Jackson and Lombard, 1993). Grapes may catabolize sugar if malate concentrations decline too much, depending upon the variety (Conde *et al.*, 2007).

Generally, the malate-to-tartrate ratio does not appear to correlate well with aroma/flavour production in the fruit (Amerine *et al.*, 1980). Its use as a maturity gauge is confounded by varietal and seasonal differences. However, there is a strong correlation between the malic acid concentration and the concentration of an important group of grape-derived aroma compounds, the methoxypyrazines. Methoxypyrazines such as IBMP (2-methoxy-3-isobutylpyrazine), a nitrogen-containing plant metabolite, can impart a vegetal aroma to some varieties including Cabernet Sauvignon, Cabernet franc, and Sauvignon blanc. Described as bell- or green pepper-like, excessive concentrations of IBMP can negatively impact the aromatic quality of wines. Decreases in pyrazines are the result of fruit maturation and temperature (Allen, 2006). The decrease in IBMP is correlated to malic acid decline (Roujou de Boubee *et al.*, 2000).

4.4.4 Phenolic compounds

The quantitative and qualitative measurement of phenolic compounds is used as a maturity gauge in stylistic winemaking (González-san José *et al.*, 1991). Increases in the total phenolic concentration are associated with maturity, as is grape anthocyanin pigment concentration and general fruit colour.

Tannins are located mainly in the skin, stems, and seeds of grapes, which contain different types of tannins. The two important tannin phases in the grape berry include accumulation and maturation. In skins, tannin accumulation starts around flowering and is completed before *véraison*. In seeds, tannin accumulation starts around flowering and is completed one to two weeks after *véraison*. Tannin maturation occurs during ripening and results in progressively decreased extractability, coinciding with perceived softening and 'ripening' of tannins. Tannin perception is complex and depends not only on tannin composition, but on the matrix in which the tannin is present.

The methods of measuring fruit colour, and the correlation between colour and other grape quality parameters, continue to be evaluated. Environmental and vineyard management practices have a large impact on fruit pigment accumulation. However, the concentration of one pigment, malvidin-3-glucoside, has been shown to be relatively unresponsive to growing conditions, with concentrations increasing as a function of maturity only (Keller and Hrazdina, 1998). Anthocyanins, as a group, have an optimum temperature range of about 17–26 °C, suggesting that berry colour would be more difficult to achieve in extremely warm and extremely cool regions. Excessive berry exposure and excessive canopy shade can also impact the rate of berry maturity and, thus, colour development. Excessive irrigation, too much nitrogen, calcium deficiency and *Botrytis* growth can negatively impact grape colour.

Berry colour is used as a harvest gauge, predictor of potential red wine quality, and as a means of grower compensation. Using fruit colour as a predictor of ultimate wine colour or quality may not be easy, due to several factors (Boulton, 2005). Grape and wine colour relate to aroma/flavour, but are not strongly correlated. Anthocyanins and aroma/flavour are produced by different biochemical mechanisms, and therefore their concentrations are not strongly correlated. Levengood (1996) reported that total phenols correlated more strongly to red wine colour than total anthocyanin concentration. Forty percent of the variation in red wine colour was explained by vineyard variables. Red wine colour is dependent upon a number of factors including:

- amount of colour produced by the fruit prior to harvest
- uniformity of ripening: clusters, berries, and fruit components
- fungal degradation of fruit
- berry size and skin-to-pulp ratio
- extraction during processing
- extent of loss during processing and fermentation due to laccase, yeast adsorption, etc.
- amount of colour lost via extended maturation
- pH
- degree of interaction between anthocyanins, cofactors, and polymeric phenols.

4.4.5 Grape aroma/flavour and maturity evaluation

Major aroma/flavour components in fruit are present in low concentrations, in the

order of 10–6000 μg/kg fresh weight (Winter, 2004). For example, the concentration of a methoxypyrazine is generally in the range of 8–20 ng/L (Allen *et al.*, 1995). Such small concentrations have profound implications with respect to both analytical measurement and sensory evaluation as a maturity gauge.

Most varieties have a spectrum of five to 20 aroma/flavour volatiles that may be sufficient to characterize them (Winter, 2004). The pool of free aroma components and their precursors increases rapidly in the advanced stages of fruit maturity, a process referred to as 'engustment' (Coombe and McCarthy, 1997). Many, but not all, varietal aroma/flavour compounds are chemically bound, odourless precursors. Hydrolysis, as a result of heat, acidity, UV, or fungal enzyme activity, can convert a percentage of aroma/flavour precursors to their odour-active forms (Günata *et al.*, 1988; Francis *et al.*, 1992; Sefton *et al.*, 1993). Analysis of the total and/or non-phenolic precursor concentration, by assessment of the glycoconjugates (glycosyl–glucose or GG analysis), is used by some to evaluate fruit aroma/flavour potential (Williams and Francis, 2000; Zoecklein *et al*., 2000).

Many juice aroma evaluation methods recommend addition of pectolytic enzymes to aid in the conversion of a portion of the bound glycosidic precursors to their odour-active forms. Additionally, salivary enzymes, which contain lyase, may be an important reason for tasting fruit such as Sauvignon blanc, versus simple evaluation of processed juice aroma. It has been reported that cysteine-bound conjugates may be hydrolyzed by yeast lyases, thus releasing volatile thiols that contribute to varietal aroma/flavour (Dubourdieu *et al.*, 2000).

As with many primary metabolites, aroma/flavour components may be dramatically affected by growing conditions and viticulture practices (Zoecklein *et al.*, 1992, 1996). As such, any aroma/flavour index of ripeness must be customized to site-specific factors and cultural practices. For example, cluster microclimate may exert more of an influence than the vine environment (Bureau *et al.*, 2000). Cluster and vine shading decrease the concentration of norisoprenoid glycoside conjugates, while light exposure increases the levels of compounds such as 2-methoxy-3-isopropyl and 2-methoxy-3-isobutyl pyrazines in unripe grapes. Light also catalyzes photodecomposition of these compounds in mature grapes (Hashizume and Samuta, 1999). Nitrogen and water availability also exert a strong impact on grape flavourant composition, as does the length of the ripening period (Keller *et al.*, 1998; Sipiora and Granda, 1998).

4.4.6 Additional chemical maturity gauges

Arginine has been reported as a maturity gauge (Jackson and Lombard, 1993). A decline in arginine may signal maturation. However, arginine concentrations are variable and influenced by varietal and seasonal differences. More recently, there has been some interest in measuring another amino acid, proline, as a maturity gauge and a monitor of vine water stress.

The role of glutathione as an antioxidant is well established. Increased attention has focused on the positive correlation between glutathione concentration and preserving some thiol-based wine aroma/flavour compounds (Dubourdieu *et al.*,

2000). The glutathione concentration of grapes increases at the onset of *véraison* and during ripening, but it is unclear if this compound is correlated with aroma/flavour development in the fruit (Okuda and Yokotsuka, 1999).

4.5 Berry sensory analysis (BSA)

Berry sensory analysis (BSA) follows a standardized set of 20 descriptors, assessing the ripeness of wine grapes by judging fruit stems, skin, pulp, and seeds separately (Winter *et al.*, 2004). It uses a four-point scoring system to determine relative ripeness and the change in ripeness over time. As with any maturity analysis, this system is most advantageously used in conjunction with other assays.

An aroma evaluation of the fruit is important in assessing relative maturity. A typical progression of aroma descriptors for Cabernet Sauvignon grapes includes the following:

- green, underripe
- lightly herbaceous
- herbaceous
- minty–blackcurrant
- blackberry
- jam–prune-like.

Aroma/flavour masking, and the fact that many compounds are present as conjugated bound precursors, makes fruit aroma/flavour evaluation only a rough approximation of the aroma/flavour potential of the wine. The following should be noted:

- most aroma/flavour compounds are likely synthesized independently of each other in the berry
- high concentration of one aroma volatile is not necessarily correlated with high concentration of another
- synthesis of most aroma/flavour molecules varies dramatically with the season and vineyard management practices
- grape aroma/flavour compounds have different rates of loss in the fruit.

Because of differences in detection thresholds among evaluators, it is important to have as many evaluators as possible. It is also important to use contrasts when evaluating fruit. The best approach is to freeze a sub-sample of the fruit collected. At the next sensory evaluation, the frozen sub-sample from the previous review is thawed, and the sensory features compared with the current sample. Contrasting allows for the detection of changes occurring with time, and the presence or absence of undesirable aroma/flavour, textural, and visual characteristics.

Optimal sensory evaluation involves an understanding of the following:

- standardized and controlled environment
- representative sample
- optimal sample temperature

- elimination of bias
- importance of sample contrasts
- use of skilled evaluators
- number of evaluators and evaluations required to gain a true picture
- minimize presentation effects (adaptation)
- minimize physiological effects (time of day, not tasting for a period after eating or drinking)
- using the proper testing method.

In addition to aroma/flavour, cluster stems can also be evaluated to aid in the assessment of berry ripeness. Stems undergo a change from green unripe, to brown or ripe stems, to overripe or brittle. These changes are seasonal and varietal-specific. Green or un-lignified stems, including cap stems, which enter the fermentor, can negatively influence the tannin profile of the resultant wine.

During fruit maturation, seeds may 'mature' at a different rate than Brix changes. As seeds mature, they change colour from green to brown to dark brown. This colour change represents oxidative reactions and corresponds to the degree of extractable tannins (Fig. 4.6). Tannin extractability decreases during phases II and III of berry development. In conventional red wine production, seed tannins make up over 60% of the total tannin concentration (Singleton, 1988). It should be noted that changes in tannin maturity can occur late in the season, when it would appear that no additional ripening can transpire.

Some winemakers taste seeds in order to assess grape maturity. However, seed bitterness may be overwhelming, and many are not able to distinguish levels of seed bitterness. The physical characteristics of the seeds, including colour, uniformity of colour, brittleness, and texture, are important indicators of fruit maturity. Because of the quantitative and qualitative role of seed tannins in red wines, seed evaluation is highly important.

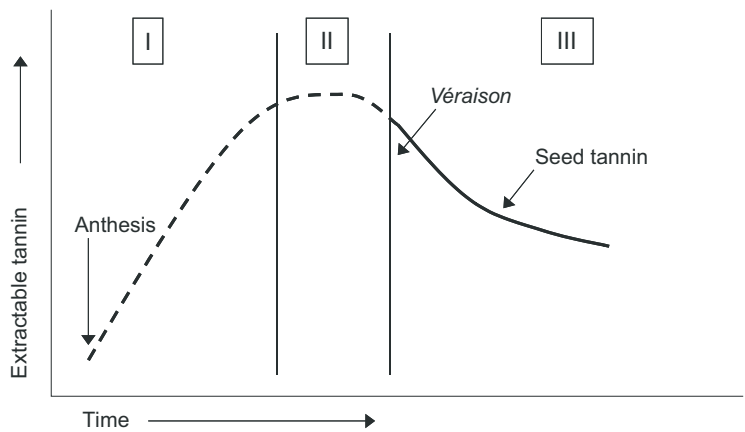

Fig. 4.6 Changes in seed tannin extractability (Kennedy, 2000).

4.6 Non-conventional maturity evaluation tools

Because of the difficulties associated with sensory evaluation, there is a need for a simple, reliable, and objective technique for evaluation of fruit maturity. If the optimal aroma composition of fruit at harvest could be defined, instrumentation analysis, such as an electronic nose, would be a useful tool for routine assessment of optimal maturity.

A major challenge for the grape and wine industry is to replace time-consuming laboratory analyses, used in process and control quality monitoring, with new application techniques that are fast, precise, and accurate. For example, red grape colour measurements represent the need for rapid analytical methods that may be used as objective indicators of grape quality, grape ripeness, and/or uniformity of ripeness. Substantial progress has been made in this area. Many of the new technologies being developed for component analysis are spectroscopic techniques that operate in the visible (Vis), near infrared (NIR), and mid-infrared (MIR) wavelength regions of the electromagnetic spectrum. Additionally, research in non-invasive testing using fluorescence or photoluminescence, T-rays (terahertz radiation, or the far infrared region of the spectrum just before microwaves), X-ray, and gamma rays for some grape and wine components may prove successful (Smith, 2006).

The analysis of grape and wine volatiles represents a substantial challenge. Conventional analyses of volatiles are mostly conducted using gas chromatographic (GC), GC mass spectrometry (GC–MS), and GC olfactory (GCO) methods, and involve very expensive equipment, time- and labour-intensive steps, methods development, sample preparation, separation of specific volatile compounds using appropriate chromatographic columns, and chromatogram interpretation.

Electronic nose (Enose) technology represents a possible alternative to volatile measurement, at least in some applications. These are multi-sensor arrays designed to measure head-space volatiles. Each sensor type has a greater or lesser affinity for a particular chemical class or group of compounds. The adsorption of volatiles on the sensor surface causes a physical or chemical change in the sensor, allowing a specific reading for that sample in a unique pattern or 'fingerprint' of the volatiles (Mallikarjunan, 2005). Using chemometric techniques and multivariate statistical analysis, it is possible to distinguish among groups of samples, to possibly identify individual sample components. Electronic nose systems are so-named because their methods of operation are analogous to the way the human sense of smell operates, where multiple nerve cells in the olfactory epithelium provide responses so the brain can identify and characterize aromas.

4.7 Grape sample processing

There are three distinctive juice zones in the fruit (Fig. 4.7). Due to compartmentalization within the fruit, it is essential that growers and winemakers standardize fruit sample processing. Without such standardization, it is impossible to compare results.

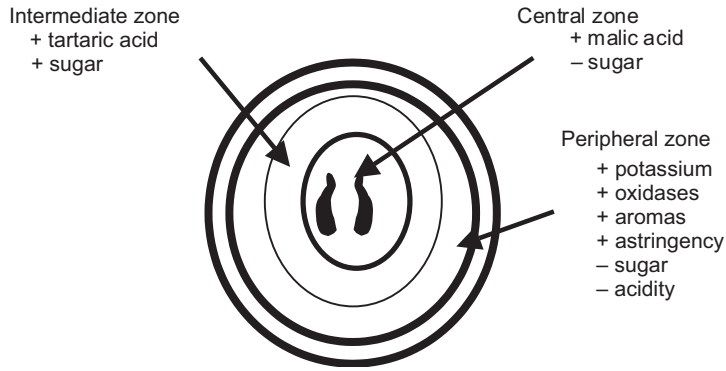

Fig. 4.7 Three zones of a grape berry, showing relative concentration of berry components (adapted from Dunsford and Sneyd, 1989).

Sample processing should be performed to duplicate what is expected to occur in the cellar. Therefore, the use of a laboratory hand press would duplicate whole cluster pressing, while a blender may provide a level of extraction similar to red fruit fermented on the skins to dryness. Common systems used to process fruit samples include the following:

- stomacher bag
- blender
- press.

4.7.1 Fruit quality evaluation

At the winery, representative grape samples are collected and examined for material-other-than-grapes (MOG; e.g. leaves, cane fragments), rot, fruit composition, juice aroma, and flavour.

4.7.2 Diseases and fruit rots

Moulds are saprophytic filamentous fungi. When conditions permit, their growth may lead to fruit deterioration, as well as exposing fruit to secondary activity of spoilage yeast and bacteria. Common moulds involved in vineyard spoilage include *Penicillium*, *Aspergillus*, *Mucor*, *Rhizopus*, and *Botrytis*.

The nature and concentration of microbial metabolites differ as a function of biotic and abiotic factors. Quantification of mould, yeast, and bacterial metabolites in collected juice samples is the best procedure for evaluation of potential impact on wine quality factors. Key indicators of fruit rot, such as the presence and concentration of ethanol, glycerol, gluconic acid, galacturonic acid, citric acid, laccase, acetic acid, ethyl acetate, ochratoxin A (OA), can be determined by contract laboratory services. Many grape growers attempt to quantify rot based on visual assessment of the incidence. This is frequently done as a percentage of clusters impacted, or a percentage of incidence per cluster. Regardless, most

premium wineries, in regions where fruit rot potential is great, conduct fruit sorting. This is generally a combination of field culling and winery sorting.

Mould growth on grapes is considered undesirable, except for the association of *Botrytis cinerea* in the production of certain sweet wines. In some cases, mould growth and associated degradation of fruit stimulate the activity of native yeast, and acetic and lactic acid bacteria, producing a wide range of metabolites. Evaluation of the potential impact of fruit rots on subsequent wine is best achieved by the quantification of rot metabolites.

Penicillium *and* Aspergillus

After early fall rains, *Penicillium* may develop in berry cracks, making the fruit unfit for wine production. *Penicillium* spp. are frequently referred to as 'cold-weather moulds', growing well at temperatures between 15 °C and 24 °C (59–76 °F).

Aspergillus is a common vineyard fungus found on damaged fruit. This mould is more abundant in warmer climates, and can metabolize sugars to produce citric acid, increasing the acid content of the juice. Mould metabolites may not only impact wine quality, but can make wines unacceptable, with regard to health concerns. For example, some species of *Aspergillus* can produce OA.

Botrytis cinerea

Botrytis cinerea is unique in its parasitology. Frequently, its development results in a decrease in grape quality, referred to by the French as 'pourriture grise' (grey rot), or 'graufaule' by German winemakers. Only under certain conditions does *Botrytis* produce an overmaturation termed 'noble rot' or 'edelfaule', indispensable in the production of the great sweet Sauternes in France, Trockenbeerenausleses in Germany, and Tokay Aszu in Hungary. Under cold and wet conditions, *Penicillium*, *Mucor*, and *Aspergillus* spp., as well as other fungi and yeast, may overgrow *Botrytis*, causing 'vulgar rot' ('pourriture vulgaire'). Breakdown of the grape integument provides a substrate for the growth of native yeasts and acetic acid bacteria, and may produce a condition called 'pourriture acide', or 'sour rot'.

Botrytis and other moulds use ammonia nitrogen, reducing the levels available for yeast metabolism. Additionally, thiamine (vitamin B_1) and pyridoxine (vitamin B_6) are depleted. Like other fungi, *Botrytis cinerea* produces laccase (Dubernet *et al.*, 1977), which catalyzes phenolic oxidation. Ewart *et al.* (1989) reported significant reduction in total anthocyanins in Pinot noir infected with *Botrytis*, even when the laccase activity was low. In addition to laccase, pectolytic enzymes and esterases, produced by the mould, break down grape tissue (Sponholz and Dittrich, 1985). Additionally, formation of polysaccharides produced by fruit rots can create wine clarification problems.

4.7.3 Agrochemical residue

Spray diaries should be kept by all grape growers and be part of the viticultural HACCP plan. Such records help assure compliance with regard to maximum

residue limits, and help provide the winemaker with the knowledge that fermentations will not be compromised by spray residues.

4.8 Conclusion

The knowledge of grape quality parameters is of cardinal importance, since wine quality is directly and strongly correlated to the quality of the vintage. This review outlined fruit components which may influence wine, particularly in regard to fruit maturity. While it is understood that grape maturity can have a profound impact on wine, other factors impacting fruit composition, including cultivar, climate, soil, and notably vine water status, vineyard management, and winemaking protocols are also important.

The challenges for the grape and wine industry include prediction of optimal fruit maturity for the types and styles of wines desired, and understanding the relationships between fruit composition and consumer wine preferences. Additional challenges include replacing time-consuming grape sampling and evaluation methods with new techniques that are fast, precise, and accurate. Technologies, including remote sensing, may provide objective, non-destructive measures of grape composition, grape ripeness, and/or uniformity of ripeness.

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5

Instrumental analysis of grape, must and wine

D. Cozzolino and R. G. Dambergs, The Australian Wine Research Institute, Australia

Abstract: Rapid instrumental methods such as spectroscopy and electronic nose are used in the food and beverage industries to monitor and assess the composition and quality of products. Similar to other food industries, the wine industry has a clear need for simple, rapid and cost-effective techniques for objectively evaluating the quality of grapes, wine and spirits. An examination of the literature reveals that ultraviolet (UV), visible (VIS), near-infrared (NIR) and mid-infrared (MIR) spectroscopy are applied in many different steps during wine production. More recently electronic nose (EN) devices have been used to analyse wines to determine their geographical origin or to predict sensory characteristics. This chapter highlights the most recent applications of VIS, NIR, MIR spectroscopy and EN to analyse grape, must and wine samples.

Key words: near-infrared, mid-infrared, spectroscopy, electronic nose, grape, wine.

5.1 Introduction

It is common industry practice to monitor grape quality by measuring total soluble solids (TSS) and acidity by visual assessment and also by taste (Dambergs *et al.*, 2003, 2006; Gishen *et al.*, 2005). Acidity and soluble solids measures are insufficient as quality indicators, and it is not possible to adequately assess quality by tasting alone. Therefore, there is a strong need in the modern wine industry for timely information that can be used for grape berry maturity assessment, identification of vineyard blocks or sections of a vineyard that should be segregated and

load quality assessment when grapes are delivered to the winery (Dambergs *et al.*, 2003; Gishen *et al.*, 2005; Cozzolino *et al.*, 2006a).

Existing analytical methods for measurement of grape and wine composition are not appropriate for the demands of production in a global market (Dambergs *et al.*, 2003; Gishen *et al.*, 2005). Factors like promptness and low cost of analysis, minimal sample preparation and environmental friendly methods are of paramount importance in the modern wine industry. Even simple analyses currently require samples to be sent to a geographically separate laboratory, with inherent delays in achieving results. More complex analyses for important grape parameters, such as anthocyanins, flavour compounds, yeast assimilable nitrogen (YAN), tannin and phenolics are not considered as serious options by industry because of their cost and slow turnaround time (Dambergs *et al.*, 2003; Gishen *et al.*, 2005; Cozzolino *et al.*, 2006a).

Spectroscopic techniques such as near-infrared (NIR) and mid-infrared (MIR) offer possibilities for simple, rapid and cost-effective analysis throughout the wine industry production chain, starting with grapes and finishing with wines and spirits, as well as their potential to be used in environmental monitoring. Although the use of spectroscopic methods is widely applied in the food industry in general, acceptance of this technology in the grape and wine industry has been relatively slow and mainly restricted to large wineries (Cozzolino *et al.*, 2006a; Bauer *et al.*, 2008).

An initial application of NIR spectroscopy in the wine industry was the measurement of alcohol content using filter instruments with two or three wavelengths (Gishen *et al.*, 2005; Cozzolino *et al.*, 2006a; Bauer *et al.*, 2008). However, since the availability of new instruments (e.g. monochromator or diode array spectrophotometers, Fourier transform), the availability of faster computers and the development of new algorithms and software for chemometric analysis, new applications of both NIR and MIR spectroscopy in the wine industry are now evidenced (Cozzolino *et al.*, 2006a; Bauer *et al.*, 2008).

This chapter highlights the most recent applications of NIR, MIR spectroscopy and electronic nose to analyse the composition of grapes, juice and wines.

5.2 Near- and mid-infrared spectroscopy (NIR and MIR)

Infrared radiation is in the region of the electromagnetic spectrum between the visible (VIS) and the microwave wavelengths. The nominal range of wavelengths for NIR is between 750 and 2500 nm (13 400–4000 cm^{-1}), while for the MIR, the spectral range is from 2500–25 000 nm (4000–400 cm^{-1}) (McClure, 2004; Workman, 2004; Cozzolino *et al.*, 2006a) (see Fig. 5.1).

Solid, liquid or gaseous samples can absorb some of the incoming infrared radiation at specific wavelengths resulting in a 'fingerprint' or spectrum. Spectral 'signatures' in the MIR result from the fundamental stretching, bending and rotating vibrations of the sample molecules, whilst NIR spectra result from complex overtones of the fundamental vibrations. Although NIR intensities are

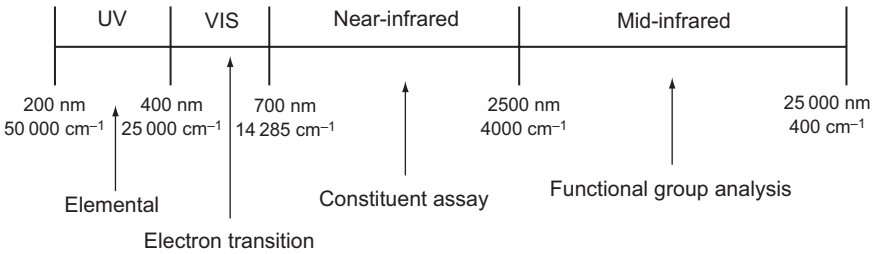

Fig. 5.1 Electromagnetic spectrum.

10–1000 times lower than for the MIR range and the peaks concomitantly smaller, highly sensitive spectrometers can be built by several means including the use of efficient detectors and brighter light sources (McClure, 2004; Nicolai *et al.*, 2007). This allows concentrated bulk or even aqueous materials to be scanned and analysed quickly and easily.

Although spectral peaks in the MIR frequencies are often sharper and better resolved than in the NIR, all the higher overtones of the OH, NH, CH and SH bands from the MIR wavelengths are still observed in the NIR region, although much weaker than the fundamental frequencies in the MIR (Miller, 2001). In addition, the existence of combination bands (e.g. CO stretch and NH bend in protein) gives rise to a crowded NIR spectrum with strongly overlapping bands (McClure, 2004). A major disadvantage of this characteristic overlap and complexity in NIR spectra has been the difficulty of quantification and interpretation of data from NIR spectra. On the other hand, the broad overlapping bands can diminish the need for using a large number of wavelengths in calibration and analysis routines. In recent years, new instrumentation and computer algorithms have taken advantage of this complexity and have made the technique much more powerful and simple to use. NIR spectroscopy is characterised by low molar absorptivities and scattering, which allows evaluation of pure materials without detector saturation. The NIR region of the electromagnetic spectrum, once regarded as having little potential for analytical work, has now become one of the most promising for molecular spectroscopy. The advent of inexpensive and powerful computers has contributed to the surge of new NIR applications (Nicolai *et al.*, 2007, Woodcock *et al.*, 2008).

5.3 Spectrophotometers

There are currently over 65 different commercially available NIR instruments which include benchtop, on-line and portable formats. Instruments can be split up into instruments working with a limited number of fixed frequencies (e.g. filter instruments, light-emitting diodes (LEDs)) and scanning instruments (McClure, 2004; Nicolai *et al.*, 2007). Scanning instruments can be divided into monochromators (grating, diode-array, acousto-optical tunable filter (AOTF)) and those working with Fourier transform (FT) techniques (Michelson or polarisation interferometers) (McClure, 2004; Nicolai *et al.*, 2007). The highest spectral resolution

Table 5.1 Commercial available instruments for the analysis of grape, grape juice and wine samples by developer

Commercial name		Mode	Sample type	Developer
WineScan™/ GrapeScan™/ OenoFoss™	MIR	Transmission cell	Clear liquid samples (wine or grape juice)	www.foss.dk
Foss NIR systems 6500	VIS–NIR	Reflectance and transmission	Grapes, grape juice and wine	www.foss.dk
Bacchus	UV–VIS, MIR	Transmission	Clear liquid samples	www.thermo.com
Corona	VIS–NIR	Reflectance	Grape samples	www.zeiss.de
Luminar	AOTF–NIR	Reflectance	Grape and wine	www.brimrose.com
Ocean Optics	UV, VIS, NIR	Reflectance and transmission	Grape and wine	www.oceanoptics.com

MIR: mid-infrared; NIR: near-infrared; UV: ultraviolet; VIS: visible. Note that this is not an exhaustive list and the information is sourced from the web page of the developer.

($< 0.1\ cm^{-1}$) can be obtained with Fourier transform Michelson interferometer instrumentation, but the disadvantage is that VIS wavelengths cannot be acquired (Workman, 2004).

Speed of data acquisition is for most of the scanning instruments in the range of a few seconds. Extremely high scanning rates (> 100 scans per second) can be achieved with AOTF and diode-array spectrophotometers; however, this speed comes usually at the expense of spectral resolution. Specifications could differ in terms of wavelength scanning range (400–2500 nm, 400–1700 nm, 400–1100 nm or 1100–2500 nm), wavelength data point interval, noise, stability and measurement time (Workman, 2004). Considerations when buying instruments would include the type of sample to be analysed, wavelength range needed, spectral resolution needed, the need for pre-calibrations, network requirements and, most importantly, after sales support (Workman, 2004; Nicolai *et al.*, 2007). In MIR instruments transmission cells or attenuated total reflectance (ATR) attachments are the most common sample presentation modes used for the analysis of grape and wine samples. Table 5.1 presents some of the commercial spectrophotometers available in the market used to analyse grape, grape juice and wine samples.

5.4 Chemometrics

The NIR spectrum is essentially composed of a large set of overtones and combination bands. This, in combination with the complex chemical composition

of natural plant products, causes that the NIR spectrum to be highly convoluted (McClure, 2004). Additionally, the spectrum might further be complicated by wavelength dependent scattering effects, tissue heterogeneities, instrumental noise, ambient effects and other sources of variability (Williams, 2001; McClure, 2004). As a consequence, it is difficult to assign specific absorption bands to specific functional groups, let alone chemical components (Williams, 2001; McClure, 2004).

The combination of NIR spectroscopy and multivariate techniques like partial least squares (PLS) and principal component regression (PCR) provides a powerful tool for analysis. Multivariate statistical techniques (also called chemometrics) are required to extract the information about quality attributes which is hidden in the spectra (Siebert, 2001; Naes *et al.*, 2002).

Chemometrics covers quite a broad range of methods such as exploratory data analysis, pattern recognition and statistical experimental design (Siebert, 2001; Naes *et al.*, 2002). The most commonly used multivariate data analysis techniques applied to NIR spectra are PCA, PLS, PCR, discriminant analysis (DA) and artificial neural networks (ANN) (Adams, 2004; Siebert, 2001; Naes *et al.*, 2002). Chemometrics, unlike classic statistics, considers multiple variables simultaneously and takes collinearity (the variation in one variable, or a group of variables, in terms of co-variation with other variables into account) (Naes *et al.*, 2002). Calibration development can mathematically describe the co-variation (degree of association) between variables, or find a mathematical function (regression model), by which the values of the dependent variables are calculated from values of the measured (independent) variables (Naes *et al.*, 2002). However, when applying any of the data modelling techniques presented above, it is important to select an optimum number of variables or components. If too many are used, too much redundancy in the X-variables (wavelengths) is used and the solution can become overfitted – the model will be very dependent on the dataset and will give poor prediction results (Naes *et al.*, 2002). On the other hand, using too few components will cause underfitting and the model will not be large enough to capture the variability in the data (Naes *et al.*, 2002). This 'fitting' effect is strongly dependent on the number of samples used to develop the model and, in general, more samples give rise to more accurate predictions (Naes *et al.*, 2002).

Typical NIR (this also applies for MIR and electronic noses) procedures to develop a calibration start from the collection of NIR spectra (reflectance or transmittance) over the 400–2500 nm (VIS and NIR) spectral region of a suitable number of samples with known value. The data, both spectra and reference, are usually inspected using PCA in order to visualise outliers or atypical samples, as well as inspect the structure of the data set. Thus, calibration models are produced using PLS or PCR. Data pre-treatment using mathematical transformation of the NIR spectra can be applied to enhance spectral features and/or remove or reduce unwanted sources of variation during the development of the calibration models. After the calibration, a validation set (samples not included in the calibration) is used to test the robustness and accuracy of the model.

5.5 Applications of near- and mid-infrared spectroscopy in grapes and wine

5.5.1 Measurement of grape composition

Grape composition at harvest is one of the most important factors that determining the future quality of the wine (Dambergs *et al.*, 2003; Gishen *et al.*, 2005; Cozzolino *et al.*, 2006a). Traditionally, grapes are harvested based on the concentration of TSS, which is an estimate of sugars, mainly glucose and fructose, usually determined using a refractometer. The prediction of quality variables in red grapes using NIR spectroscopy is usually conducted by scanning homogenised grape samples using a research grade laboratory NIR spectrophotometer, but other sample presentation modes (including whole grapes) and cheaper, more adaptable instruments have been used (Gishen *et al.*, 2005; Cozzolino *et al.*, 2006a; Nicolai *et al.*, 2007).

It is well known that NIR spectroscopy is able to measure TSS in other fruits, and several authors have reported the use of this method to determine TSS in grapes and musts (Jarén *et al.*, 2001; Arana *et al.*, 2005; Cozzolino *et al.*, 2006a; Larrain *et al.*, 2008). Table 5.2 shows the standard error of prediction (SEP) values obtained for TSS, total anthocyanins and pH measured in grapes using NIR spectroscopy reported by different authors, in different varieties, wavelength regions, vintages and geographical regions.

It has been reported that grape total anthocyanin concentration (colour) is a good predictor of red wine composition and quality and is widely used by the Australian wine industry (Dambergs *et al.*, 2003; Gishen *et al.*, 2005; Cozzolino *et al.*, 2006a). Total anthocyanins, TSS and pH can be measured in homogenates of red grape berries scanned in reflectance over the wavelength range of 400–2500 nm (Dambergs *et al.*, 2003, 2006; Cozzolino *et al.*, 2005a). With calibrations for total anthocyanins in red grapes, it has been observed that for large data sets incorporating many vintages, regions and grape varieties, PLS calibrations show pronounced non-linearity (Cozzolino *et al.*, 2005a; Dambergs *et al.*, 2006). The SEP values reported for total anthocyanins vary from 0.05 to 0.18 mg g^{-1}, and increase with diverse sample sets in comparison to sample sets restricted on the basis of growing region and/or variety (Dambergs *et al.*, 2006). These observations may be related to non-linearities encountered in the calibrations produced with the diverse sets (Dambergs *et al.*, 2006). An alternative strategy to mitigate the effects of non-linearity on the NIR calibrations for total anthocyanins is to use LOCAL regression (Dambergs *et al.*, 2006). The same studies demonstrated that TSS calibrations were not significantly affected by the sample matrix (Dambergs *et al.*, 2006). The use of ANN as an alternative method for non-linearity was also reported when red grapes were analysed using VIS–NIR spectroscopy (Janik *et al.*, 2007).

The use of NIR spectroscopy has now been put into practice by several large Australian wine companies for the determination of the concentration of total anthocyanins (colour) in red grapes for payment purposes. In 2003, it was estimated that 20% of the total crush in Australia used VIS and NIR spectroscopy

Table 5.2 Statistics reported by various authors for the analysis of total soluble solids (TSS), total anthocyanins and pH in red grape samples by visible and near-infrared spectroscopy

Reference	Variable	*N*	Wavelength range (nm)	Type	Range	SECV/SEP	RPD
Dambergs *et al.*, 2006	TSS (°Brix)	> 3000	800–1600	Red grapes	11–37	0.27	11.04
	Total anthocyanins (mg g^{-1})		550–1600		0.2–2.6	0.12	4.17
	pH		550–1600		2.8–4.1	0.08	3.0
Larrain *et al.*, 2008	TSS (°Brix)	> 500	640–1100	Red grapes	26–29	1.24	3.7
	Total anthocyanins (mg g^{-1})				1.3–2.7	0.30	1.6
	pH				3.3–4.1	0.14	2.0
Arana *et al.*, 2005	TSS (°Brix)	> 30	900–2500	Chardonnay		1.27	1.88
				Viura		1.89	1.54

N: number of samples; SECV: standard error of cross validation; SEP: standard error of prediction; RPD: SD/SEP; SD: standard deviation.

Table 5.3 Comparison of the standard error of prediction for total soluble solids (TSS) and total anthocyanins in homogenates and whole red grape samples

Variable	Homogenates	Whole grapes
TSS (°Brix)	0.12	1.03
Total anthocyanins (mg g^{-1})	0.12	0.27

to assess the composition of the grapes harvested (Dambergs *et al.*, 2003, 2006; Cozzolino *et al.*, 2006a). Similar NIR applications have been reported by private wineries and research groups in Chile (Herrera *et al.*, 2003), Italy (Carlini *et al.*, 2000), Spain (Torres winery, pers. comm.), South Africa, Portugal and USA (Sethuramasamyraja *et al.*, 2007).

The possibility of simplifying the sample presentation (e.g. using whole grapes instead of homogenates) could dramatically increase sample throughput in the winery (Cozzolino *et al.*, 2004a, 2006a). Scanning of single berries is also a possibility; however, high coefficients of variation (up to 40%) in the VIS–NIR spectra were observed when samples were rotated or scanned in different positions relative to the spectrophotometer (Cozzolino *et al.*, 2004a, 2006a; Gishen *et al.*, 2005). This variation within the same berry might be due to variations in chemical composition and surface contamination (e.g. dust, soil) and to different degrees of sun exposure (shading) (Cozzolino *et al.*, 2004a, 2006a; Gishen *et al.*, 2005).

Investigations of whole grape berry presentation using a diode-array spectrophotometer indicated that NIR may have potential for use at the weighbridge or for in-field analysis of total anthocyanins, TSS and pH (Cozzolino *et al.*, 2004a). The major challenges relating to the measurement of whole grapes concern sample presentation, instrument availability and cost, and the desirable accuracy for the prediction of chemical composition that might be achieved. Table 5.3 compares the SEP values obtained when homogenates and whole red grape berries were analysed using VIS–NIR spectroscopy. As a direct consequence of the sample presentation (homogenised versus whole) the SEP values varied for total anthocyanins from 0.20–0.12 mg g^{-1} and for TSS from 1–0.24 °Brix. The use of both VIS–NIR and MIR spectroscopy as reported to measure glycosylated compounds (GG) in white grape juice (Schneider *et al.*, 2004; Cynkar *et al.*, 2007a).

As well as measuring chemical composition, the use of VIS–NIR was reported as a tool to measure physical properties (firmness, elasticity, touch resistance) in batches of Cabernet Franc grapes (Le Moigne *et al.*, 2008). The method based on VIS–NIR spectroscopy was able to discriminate ripening and parcel effects, and to predict ripening stages and parcel type using discriminant methods (Le Moigne *et al.*, 2008).

5.5.2 Fungal diseases in grapes

In measuring grape quality, there is also a need for objective measures of negative quality parameters such as the degree of mould contamination, particularly with mechanically harvested grapes, where visual assessment can be difficult (Dambergs

et al., 2003; Gishen *et al.*, 2005; Cozzolino *et al.*, 2006a). Assessment of grapes for fungal infection at the weighbridge would normally be done by visual inspection, but this can be difficult with mechanically harvested fruit. In this context, the use of VIS–NIR spectroscopy was reported for the detection of powdery mildew (*Erysiphe necator*) in wine grapes (Dambergs *et al.*, 2007). Samples of Chardonnay grapes with varying degrees of powdery mildew infection (classified visually) were homogenised then scanned in reflectance mode over a 400–2500 nm wavelength range and analysed for powdery mildew DNA content (Gishen *et al.*, 2005; Dambergs *et al.*, 2007). Powdery mildew DNA content correlated with the visual infection classification and strong spectral correlations with infection level were also observed. The implication of this work is that it might be possible to discriminate infected fruit at the weighbridge to provide a 'go/no-go' test to highlight suspect fruit for further detailed analysis to determine suitability for winemaking (Gishen *et al.*, 2005; Dambergs *et al.*, 2007). The use of NIR for detecting fungal infection is discussed in more detail in Chapter 13.

5.5.3 Measurement of wine composition

Much of the NIR applications in the wine industry have concentrated on the measurement of ethanol (Kaffka and Norris, 1976; Osborne *et al.*, 1993; Cozzolino *et al.*, 2006a). Currently there are a number of dedicated NIR-based alcohol analysers in wine laboratories, and this technique has become a routine analysis method for alcohol content in wine. Ethanol has a strong NIR absorbance signal in alcoholic beverages, usually second only to water, but accuracy and robustness of calibrations can be limited by matrix variations, particularly variations in sugar concentration (Kemeny *et al.*, 1983; Davenel *et al.*, 1991). However, water tends to dominate the wine NIR spectra and may obscure minor wine components. Figure 5.2 shows the NIR raw spectrum of white and red wine samples analysed in transmission (1 mm path length). Two large absorption bands were observed corresponding to the O–H bonds around 1400 and 1900 nm (water and ethanol), respectively.

To overcome this issue, some studies reported the use of dry extracts as sample preparation (DESIR: dry extract spectroscopy by infrared absorption) (Chandley, 1993; Dambergs *et al.*, 2004) to measure total phenols and sugar concentration in fortified wines. Medrano and collaborators (1995) examined the use of NIR spectroscopy and DESIR to measure total phenols, in addition to ethanol and sugar in Porto wine. The SEP obtained were 3.14 g L^{-1}, 5.92 g L^{-1} and 1.30 for D-glucose, D-fructose and total phenolics, respectively (Medrano *et al.*, 1995).

The use of NIR spectroscopy has been reported to measure several wine compositional parameters such as alcohol content, pH, volatile acidity, organic acids, malic, tartaric and lactic acids, reducing sugars and sulphur dioxide in a set of red, rosé and white wines (see Table 5.4) (Cozzolino *et al.*, 2006a). Accurate NIR calibrations were obtained for the determination of alcohol ($R^2 = 0.98$, SEP = 0.24% v/v), pH ($R^2 = 0.81$, SEP = 0.07), reducing sugars ($R^2 = 0.71$, SEP = 0.33 g L^{-1}) and lactic acid ($R^2 = 0.81$, SEP = 0.41 g L^{-1}) (Urbano-Cuadrado, 2004,

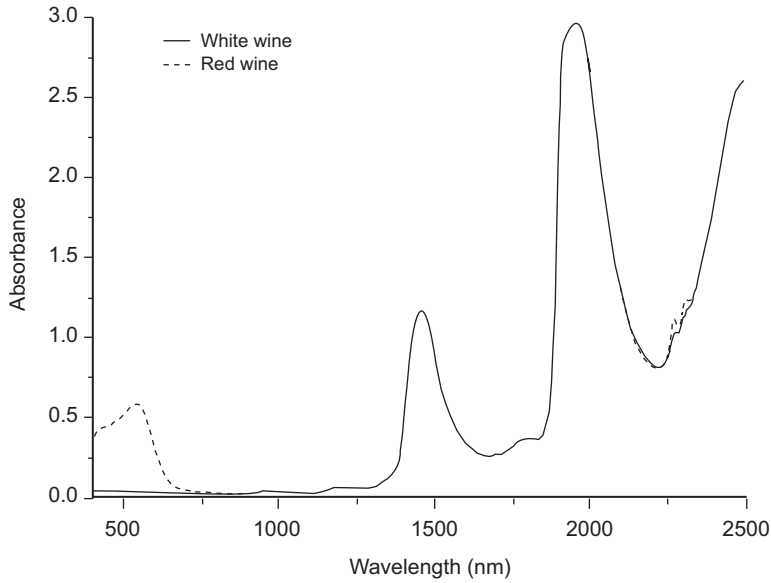

Fig. 5.2 Near-infrared transmission spectrum of white and red wine samples.

2005a,b; Guggenbichler *et al.*, 2006). The same authors compared the use of NIR and Fourier transform (FT) MIR spectroscopy to measure several wine variables (Urbano-Cuadrado, 2004, 2005a,b; Guggenbichler *et al.*, 2006). The authors concluded that, for most of the wine compositional variables measured, the NIR calibration results were better than those obtained by FT–MIR mainly because of the high signal-to-noise ratio of the MIR method (Urbano-Cuadrado, 2004, 2005a,b). The use of NIR spectroscopy has also been reported for the determination of the concentration of sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), iron (Fe) and copper (Cu) in both white and red wine samples (Sauvage *et al.*, 2002; Cozzolino *et al.*, 2008a). Sweet wines made from botrytised-grapes represent a complex matrix in that they have very high sugar, acid and glycerol content in comparison with standard wines (Garcia-Jares and Medina, 1987). NIR spectroscopy was explored in combination with a number of multivariate calibration routines to analyse ethanol, glycerol, glucose and fructose in botrytis-affected style wines (Garcia-Jares and Medina, 1987). The use of NIR spectroscopy was also evaluated to build calibrations for free amino nitrogen (FAN) in grape must and malo-lactic fermentation status of wines (Manley *et al.*, 2001). Although calibrations could not accurately quantify the concentrations of the compounds of interest (malic acid, lactic acid, FAN), classification models based on SIMCA (Soft Independent Modelling by Class Analogy) as a discriminant method could distinguish between groups of high, medium and low concentration. The classification rates obtained ranged between 80 to 88% (Manley *et al.*, 2001).

Although NIR spectroscopy has already found widespread applications in quality control and process analysis in the grape and wine industry, due to the fact

Table 5.4 Analysis of grape juice, must, wine and spirits by visible and near-infrared spectroscopy in transmission mode by various authors (source: Cozzolino *et al.*, 2006a)

Reference	Parameter	*N*	Varieties	Wavelength range (nm)	Range	SECV/SEP	RPD
Urbano-Cuadrado *et al.*, 2004	Alcoholic degree (%, v/v)	24	Red, rosé and white wines	400–250010–15	0.24	5.7	
	Total acidity (meq L^{-1})				3.5–8.7	0.48	2.27
	pH				3.2–4.0	0.07	2.4
	Glycerol (g L^{-1})				2–12.4	0.72	4.0
	Reducing sugars (g L^{-1})				0.6–9.7	0.33	10.3
Sauvage *et al.*, 2002	Sodium (mg L^{-1})	24	White wine	400–2500	5–118	9.22	2.8
	Potassium (mg L^{-1})				265–1100	79.0	2.8
	Magnesium (mg L^{-1})				78–718	14.5	2.7
	Calcium (mg L^{-1})				30–120	8.09	2.8
Manley *et al.*, 2001	Sugar (°Brix)	97	White varieties (must and wine)	1000–250017–27	0.31	5.95	
	Malic acid (g L^{-1})				0–4.8	1.02	1.2
	Lactic acid (g L^{-1})				0–5.6	1.34	0.96
Cozzolino *et al.*, 2004b	Malvidin 3 glusoside (mg L^{-1})	> 350	Red varieties (must and wine)	400–250014–427	28	3.5	
	Pigmented polymers (mg L^{-1})				4–103	5.9	3.1
	Tannins (mg L^{-1})				12–991	131.2	1.8
Dambergs *et al.*, 2002	Methanol (g L^{-1})	123	Grape spirit	1200–2450 0	0.02–20.1	0.06	12.5

N: number of samples; SECV: standard error of cross validation; SEP: standard error of prediction; RPD: SD/SEP; SD: standard deviation.

Table 5.5 Analysis of grape juice, must and wine by mid-infrared spectroscopy in transmission mode by various authors

Reference	Variable	*N*	Varieties	SD	Range	SEP/SECV
Soriano *et al.*, 2007	Malvidin-3-glucoside (mg L^{-1})	336	Red wines	59.4	–	22.2
	Total anthocyanins (mg L^{-1})	323		96.5		34.31
Patz *et al.*, 2004	Alcohol (vol %)	52*	Several varieties		7.4–14	0.02
	Alcohol (g L^{-1})				58.7–110.7	1.96
	Volatile acidity (g L^{-1})				0.14–1.4	0.008
	pH				2.5–3.9	0.01
	Total acid (g L^{-1})				3.7–14.1	0.06
	Fructose (g L^{-1})				0–167	1.21
	Glucose (g L^{-1})				0.2–63.5	0.49
Urbano-Cuadrado *et al.*, 2005	Glycerol (g L^{-1})	140	Several varieties	2.88		0.68
	Total acid (g L^{-1})			1.09		0.54

N: number of samples; SD: standard deviation; SECV: standard error of cross validation; SEP: standard error of prediction; *German wines only.

that NIR absorptions reflect overtone and combination bands, NIR spectra are much less distinct that MIR. Therefore, the increasing use of MIR in wine analysis was of special interest due to the presence of sharp and specific absorption bands of some wine constituents (Bauer *et al.*, 2008). Recently the employment of dedicated FT–MIR instruments – e.g. WineScan™, GrapeScan™ (FossA5) and Bacchus (Microdom) – are now available and are used extensively in routine analysis of grape juice and wine samples by the wine industry worldwide (Cozzolino *et al.*, 2006a; Bauer *et al.*, 2008). Several wine compositional parameters such as alcohol content, volatile acidity, pH, tartaric acid, lactic acid, glucose plus fructose, acetic acid, tannins, polysaccharides, volatile compounds, polyphenols and glycerol have been proposed and implemented for routine analysis using FT–MIR instruments (Gishen and Holdstock, 2000; Coimbra *et al.*, 2002; Rousseau *et al.*, 2002; Edelmann *et al.*, 2003; Kupina and Shrikhande, 2003; Moreira and Santos, 2004, 2005; Nieuwoudt *et al.*, 2004; Patz *et al.*, 2004; Urbano-Cuadrado *et al.*, 2004; Cocciardi *et al.*, 2005; Dixit *et al.*, 2005, Lletí *et al.*, 2005; Sáiz-Abajo *et al.*, 2006; Versari *et al.*, 2006; Boulet *et al.*, 2007a,b; Fernandez *et al.*, 2007; Soriano *et al.*, 2007; Bauer *et al.*, 2008; Jensen *et al.*, 2008; Smyth *et al.*, 2008). Table 5.5 summarises some of the applications of FT–MIR on the measurement of wine composition.

5.5.4 Monitoring wine fermentation

Wine fermentation is a complex process in which grape juice is transformed by microbial action into a high-value product, wine. The modern wine industry needs both fast and reliable process quality control methods and techniques that operate in real time in order to assure the quality of the final product to the consumer (Cavinato *et al.*, 1990; Davenel *et al.*, 1991; Vandenberg *et al.*, 1997; Cozzolino *et al.*, 2004b; Jorgensen *et al.*, 2004; Gishen *et al.*, 2005; Zeaiter *et al.*, 2006). Control of the wine fermentation process is a very important step during wine production in order to monitor accurately and rapidly control both substrate conversion (e.g. sugars to ethanol, malic acid to lactic acid) and product quality (Cavinato *et al.*, 1990; Davenel *et al.*, 1991; Cozzolino *et al.*, 2004b, 2006b,c; Gishen *et al.*, 2005).

Several studies report the use of both NIR and MIR spectroscopy to monitor wine fermentation such as the use telecommunications grade fibre optics to measure ethanol in wines of different grape varieties (Buchanan *et al.*, 1988), the measurement of sugar and ethanol using different fermenter sampling systems and temperature equilibration (Bouvier, 1988), the comparison of a filter-based NIR instrument with a FT–NIR scanning instrument to measure sugars and ethanol (Davenel *et al.*, 1991) and the use of ATR and MIR to monitor wine fermentation (Bellon-Meurel, 1993).

In recent years, research has been conducted in order to assess the potential of VIS–NIR spectroscopy to predict the concentration of and monitor the extraction and evolution of phenolic compounds during red wine fermentation (Cozzolino *et al.*, 2004b). Results showed that VIS–NIR spectroscopy could predict the concentration of major anthocyanins such as malvidin-3-glucoside ($R^2 = 0.91$ and SECV

= 28.0 mg L^{-1}), pigmented polymers (R^2 = 0.87 and SECV = 5.9 mg L^{-1}), and tannins (R^2 = 0.83 and SECV = 131.1 mg L^{-1}) in Cabernet Sauvignon and Shiraz wines during fermentation (Cozzolino *et al.*, 2004b). The use of FT–MIR and fibre optics to monitor large-scale wine fermentations has also been reported (Urtubia *et al.*, 2004; Uturbia *et al.*, 2008). In recent years, Fernández-Novales *et al.* (2008) studied the prediction of density and sugars in white wine fermentation using a miniature fibre optic NIR instrument.

5.5.5 Wine quality grading

The ability to accurately assess wine quality is an important part of the wine-making process, particularly when allocating batches of wines to styles determined by consumer requirements (Francis and Newton, 2005; Gishen *et al.*, 2005). Wine quality, in terms of sensory characteristics, is normally a subjective measure, performed by experienced winemakers, wine competition judges or wine tasting panellists (Gishen *et al.*, 2005) and involves the measurement, interpretation and understanding of human responses to the properties perceived by the senses such as sight, smell and taste (Cozzolino *et al.*, 2005b, 2008b; Smyth, 2005).

In general, there are two types of methods for evaluation of food quality: subjective and objective. Subjective methods are based on the human assessment of the quality characteristics of the food (Huang *et al.*, 2004; Francis and Newton, 2005; Smyth, 2005). These methods usually involve the perception of texture, flavour, odour, colour and touch (Martens, 1999; Smyth, 2005). However, even though human evaluators can be highly trained, their opinions may vary because of mental and physical variability. Subjective sensory methods can be time-consuming and are susceptible to large sources of variation. By nature, such assessments can be biased by individual preferences and may be subject to day-to-day variation (Francis and Newton, 2005; Smyth, 2005). Objective methods for assessment of quality include instrumental analysis and could be very beneficial for numerous reasons as they are non-subjective, highly repeatable and reproducible, and most of all the fact that instruments do not suffer from fatigue or adaptation (Martens, 1999; Jellema *et al.*, 2005; Smyth, 2005; Cozzolino *et al.*, 2008b).

Given the complex nature of wine, there are many advantages to developing instrumental methods to describe quality. However, to be of practical use by the wine industry, instrumental methods must be objective, cost-effective and provide rapid, reproducible results, with continuous operation. To date, instrumental methods for sensory analysis have lacked the ability to consistently perceive all of the key sensory attributes of interest, and have been inconsistent in predictive relationships between sensory and instrumental measurements depending on the food analysed (Martens, 1999). Flavour compounds are often present in concentrations below the detection limit of NIR spectroscopy, but the more abundant organic compounds in the wine matrix offer potential for objective quality grading by this technique.

It has been demonstrated that wine quality rankings (as the score or allocation

assigned to wines by sensory panels) for red and fortified wines could be discriminated by VIS–NIR spectroscopy (Gishen *et al.*, 2005). Studies carried out in a commercial winery in Australia, demonstrated that VIS–NIR spectroscopy can predict wine quality as judged by both commercial wine quality rankings and wine show scores (Gishen *et al.*, 2005). Correlations between NIR spectra and sensory data obtained using wine show samples were less significant in general, in comparison with the commercial grading data. The R^2 and SECV obtained using a small set of samples ($n = 20$) to predict tawny port wine score were 0.84 and 0.97, respectively. The commercial samples were all from one major producer, from one growing area and were graded immediately ex-vintage, with minimal oak treatment (Francis and Newton, 2005; Gishen *et al.*, 2005; Dambergs *et al.*, 2007). For dry red wines, the best calibrations were obtained with a class of Pinot noir – a variety that tends to be produced in limited areas in Australia and would represent the least matrix variation. Similar to the winemakers' quality allocation study, the highest loadings were observed predominantly in the VIS region around 520 nm (wine pigments) (Gishen *et al.*, 2005; Dambergs *et al.*, 2007). Grading of wine by VIS–NIR spectroscopy could provide a rapid assessment or pre-screening tool to add to the range of analyses available to winemakers. It could allow preliminary blend allocation of large numbers of batches of wines prior to sensory assessment. Winemakers may be able to develop 'profiles' for their blends as in-house VIS–NIR calibrations. Calibrations based on sensory scores will tend to be difficult to obtain due to variation between individual wine tasters and may not pick up compounds that are present at low concentrations, yet have strong sensory properties. Nevertheless, interpretation of spectral data may provide valuable insight into the more abundant parameters affecting wine quality and highlight the interactions that occur within the complex wine matrix in governing sensory properties.

A recent study has demonstrated that VIS–NIR spectroscopy could predict quality scores derived from a panel of winemakers in a set of red wine samples. Most of the NIR calibrations developed accounted for more than 70% ($R^2 > 0.70$) of the variation described by the sensory panel (Cozzolino *et al.*, 2008b).

With regard to white wines, the use of VIS–NIR to measure aroma (honey, estery, lemon, caramel, toasty, perfumed floral, passionfruit) and palate properties (overall flavour and sweetness) in commercial available bottles of Australian Riesling and unwooded Chardonnay was reported (Cozzolino *et al.*, 2005b; Smyth, 2005). The results showed good correlation between spectra and sensory properties ($R > 0.70$) for estery, honey, toasty, caramel, perfumed floral and lemon, while poor correlations ($R <$ about 0.55) were found in most of the cases for passionfruit, sweetness and overall flavour, respectively (Cozzolino *et al.*, 2005b, 2008b; Smyth, 2005).

As in other foods, it is likely that no single compositional characteristic explains the differences in sensory properties between the wines, rather a combination of different variables such as grape and yeast derived compounds (e.g. volatile and non-volatile compounds), oxidation products and other constituents present in the wine matrix. However, no information is currently available concerning possible correlations between spectral data and volatile components in the wine varieties

analysed. Therefore these methods (VIS, NIR and MIR) might indirectly explain the variations in the sensory characteristics of the wines analysed. Taking into account the nature of the characteristics measured, such calibrations might be useful as rapid instrumental screening tools when large numbers of wines need to be rated for quality prior to formal sensory analysis in order to reduce the cost and resources required for such analyses, and particularly for those assessments where the purpose is to assess the degree and incidence of bottle aged development in a set of wines.

The practical implications for the wine industry are that instrumental measurements such as VIS, NIR and MIR spectroscopy are complementary to sensory analysis and can facilitate the task at early stages of product development, making high-throughput screening of novel products feasible or to maintain the consistency of the product. Alternatively, spectroscopy may be useful as a rapid method for the determination of approximate quality category estimation as a pre-screening mechanism (low, medium and high) of some sensory properties in wine shows (Cozzolino *et al.*, 2008b).

5.5.6 Measurement of methanol and ethanol in grape distillates

Grape spirit is produced by distillation of wine or wine/grape derived process waste, and is used in the production of fortified wines. Methanol concentrations in grape marc, one of the major sources of distillation raw materials, can be high due to the action of mould and bacteria in the raw product (Dambergs *et al.*, 2002). The methanol concentration in the final product must be minimised to comply with food regulations, and operating continuous stills can be difficult without rapid methanol analysis to allow fine tuning of the stills in a timely manner (Dambergs *et al.*, 2002). In comparison to wine, the distillation process streams represent relatively simple matrices, consisting of predominantly ethanol, water and minor quantities of other volatile organic compounds. Two key compounds that are routinely monitored during the distillation process are ethanol and methanol, which have characteristic NIR spectra based on differences in relative concentrations of CH_3 groups, wavelength shifts for OH groups and a CH_2 group unique to ethanol. NIR calibrations have been developed using both PLS and MLR methods with transmission spectra of wine fortifying spirit using gas chromatography (GC) as the reference method. The PLS calibrations approached the accuracy of the reference methods, with an R^2 of 0.998 and a SECV of 0.06 g L^{-1} for methanol and an R^2 of 0.96 and SECV of 0.08% v/v for ethanol. The calibrations were very robust as indicated by high values for the ratio of the standard deviation of the reference data to the standard error of prediction of the calibration (RPD > 12). MLR calibrations were less accurate, but they were robust across vintages (Dambergs *et al.*, 2002).

5.5.7 Product authenticity

Verification of authenticity of food in general, and wine in particular, has become

a potential application of NIR and MIR spectroscopy (see Chapter 8). Adulteration can take many forms, including the addition of sugars, acids, volatile oils, over-dilution of concentrate, addition of juices of other fruits, use of concentrate in a 'fresh' product and use of low-quality product recovered from what are normally waste products of manufacture (Arvantoyannis *et al.*, 1999). Food adulteration has been practised since ancient times but has become more sophisticated in the recent past. Foods or ingredients most likely to be targets for adulteration include those which are of high value or are subject to the vagaries of weather during their growth or harvesting. The practice of adulteration commonly arises for two main reasons: firstly it can be profitable, and secondly adulterants can be easily mixed and are subsequently difficult to detect. To counter this problem manufacturers subject their raw material and by-products to a series of quality controls which include high performance liquid chromatography (HPLC), thin layer chromatography (TLC), enzymatic tests and physical tests to establish their authenticity and hence guarantee the quality of the products manufactured for the consumers (Cordella *et al.*, 2002).

The use of VIS–NIR was investigated to discriminate two Australian white wine varieties, namely Riesling and unwooded Chardonnay, with accuracy of up to 95% (Cozzolino *et al.*, 2003). Both VIS and NIR spectroscopy combined with multivariate analysis were used to classify commercial Tempranillo wines from Australia and Spain (Liu *et al.*, 2006; Liu, 2007). Multivariate methods such as PCA, discriminant partial least square discriminant analysis (PLS–DA) and linear discriminant analysis (LDA) were used to classify Tempranillo wines according to their geographical origin. PLS–DA models correctly classified 100% and 84.7% of the Australian and Spanish Tempranillo wine samples, respectively (Liu *et al.*, 2006). Similar results were obtained when Riesling wines samples from Australia, New Zealand, France and Germany were analysed (Liu, 2007; Liu *et al.*, 2008).

FT–MIR has been used to explore the possibility of grading wine samples from the Qualified Denomination of Origin (QDO) 'Rioja' (Lletí *et al.*, 2005). The results showed that the calibration procedures using spectra were adequate to quantitatively classify wine samples from QDO and to qualitatively distinguish between 'adequate' and 'abnormal' wine samples (Lletí *et al.*, 2005). A similarity index (SI) based on MIR spectra of wines was developed (Bevin *et al.*, 2006). The classification of red wines made from different grape varieties using FT–MIR was reported by several authors (Edelmann *et al.*, 2001; Picque *et al.*, 2005; Bevin *et al.*, 2008) as well as the use of ATR–FT–MIR as a tool to differentiate Greek red wines on the basis of grape variety (Tarantilis *et al.*, 2008).

5.5.8 In bottle measurement

An ideal method for the determination of chemical composition of wine in a routine manufacturing schedule should be non-invasive, non-destructive and rapid to ensure timely processing of the food being analysed (Cozzolino *et al.*, 2007). Classical univariate spectroscopy methods in the ultra violet (UV) and VIS regions of the electromagnetic spectrum require physical separation of the constituent of

interest from the matrix, usually by dissolution in a solvent. NIR spectroscopy combined with chemometrics offers the advantages of simplicity of sample presentation, speed of collecting the information (spectra) and low cost.

In NIR short wavelengths (700–1600 nm) the spectrum has less intense peaks and can tolerate longer path lengths (5 up to 30 mm) but yields poor spectral resolution, while peaks at longer wavelength regions (> 1500 nm) are more intense and require short optical path length but provide better spectral resolution (Murray and Cowe, 2004).

Glass is transparent to NIR radiation – a property that has allowed the pharmaceutical industry to use NIR to analyse liquids through the bottle in order to determine moisture in lyophilised sucrose through intact glass vials as well as real-time monitoring in solid phase synthesis of a resin-bound alcohol (Kamat *et al.*, 1989). Recently, the use of NIR spectroscopy was reported by other authors to analyse foods and beverages such as sufu, beer and whisky, non-destructively, in their point of sale containers (Iñon *et al.*, 2005; Lu and Han, 2005; Nordon *et al.*, 2005).

Combining both the VIS and NIR regions in one instrument provides a vast improvement in efficiency related to instrumental, sampling and analytical cost (Cozzolino *et al.*, 2007). Skouroumounis and co-workers at the Australian Wine Research Institute (AWRI) demonstrated that single wavelengths in the VIS region could be used to assess oxidation (browning) of white wines in either coloured or non-coloured bottles (Skouroumounis *et al.*, 2003). The potential of VIS–NIR to determine wine composition (e.g. ethanol, pH) in the bottle was also reported by researchers at AWRI (Cozzolino *et al.*, 2007). Although the accuracy and precision suggest that it is not sufficient for analytical purposes, the possibility of monitoring wine quality variables through the bottle non-intrusively gives a new dimension to the analysis of wine composition. New commercially available spectrophotometers in the market (e.g. diode array instruments) with simple sample presentation extend this potential for at/on-line applications. Although these results are promising, the authors believe that this type of instrument or sample presentation can be used only as an indicative rather than a quantitative analytical tool to monitor process because of limitations in analytical accuracy.

It is expected that future development of such applications will provide the wine industry with a very fast and non-destructive method to monitor composition or changes and to detect unwanted problems in bottled wine prior to retail sale (e.g. oxidation), and provide a rapid means of qualitative rather than quantitative analysis (Cozzolino *et al.*, 2007).

5.6 Electronic noses

The concept of electronic nose (EN) as a device to mimic the discrimination of the mammalian olfactory system was introduced in the early 1980s (Röck *et al.*, 2008). The term EN is often associated with the detection of odours or the attempt to smell with a technical device (Röck *et al.*, 2008). The EN offers the capability to detect

some important gases with no odour activity, and is not necessarily adapted only to substances of importance to mammalian life such as the scent of other animals, foodstuff or spoilage (Röck *et al.*, 2008).

The classical EN, consisting of an array of sensors, is still the most common approach, although new technologies have recently entered in the market (Röck *et al.*, 2008). A diverse numbers of technologies have been used such as metal oxide sensors (MOS) (Berna *et al.*, 2008), mass spectrometry (MS), ion mobility spectrometry (IMS) and gas chromatography (GC) sensors (Hurst, 1999; Guadarrama *et al.*, 2001; Deisingh *et al.*, 2004; Gutierrez *et al.*, 2007; Röck *et al.*, 2008).

The application of EN has been investigated by several authors as a means of differentiating food samples on the basis of both aroma and volatile compounds in the food industry (Bartlett *et al.*, 1997; Deisingh *et al.*, 2004). Food product characterisation based on the analysis of their aroma properties is a widely used technique (Bartlett *et al.*, 1997; Deisingh *et al.*, 2004). Nowadays, analytical solutions for food and wine composition often involve the use of gas chromatography–mass spectrometry (GC–MS) techniques, but analysis can be time-consuming, due to sample preparation steps and complex data interpretation (Guadarrama *et al.*, 2001; Deisingh *et al.*, 2004; Gutierrez *et al.*, 2007).

Recent research has shown that rapid analysis of volatile fractions in wine by MS without chromatographic separation produces signals containing useful information that can be used to produce a fingerprint of wine based on its aroma profile (Deisingh *et al.*, 2004; Smyth, 2005; Cozzolino *et al.*, 2006b; Cynkar *et al.*, 2007b; Gutierrez *et al.*, 2007). Few studies have examined the use of EN or gas sensors to characterise the aroma of wine, mainly because major compounds in the samples headspace, such as ethanol, cause interference with the gas sensor (Di Natale *et al.*, 1995, 2000, 2004; Gutierrez *et al.*, 2007). However, this limitation does not exist with MS–EN instruments where the wine headspace is monitored and the whole spectra are analysed (Smyth, 2005; Cozzolino *et al.*, 2006b; Cynkar *et al.*, 2007b; Gutierrez *et al.*, 2007).

5.7 Applications of electronic noses in grape and wines

5.7.1 Grape ripeness

The ability of EN to classify grape samples based on maturity was investigated in Cabernet Sauvignon (*Vitis vinifera* L.) (Athamneh *et al.*, 2007). Results were compared using discriminant and canonical discriminant analysis with analysis of headspace volatiles using a hand-held EN instrument. The EN was able to determine the difference in ripeness among sample groups in two seasons, demonstrating the ability of EN to distinguish maturity levels. Likewise, field measurements demonstrated the potential of EN as a rapid, non-destructive tool for evaluating grape maturity (Athamneh *et al.*, 2007).

5.7.2 Wine aroma

Most of the references in the literature on the application of EN in wine are

related to monitoring aroma and other volatile compounds in either ferments or in wine (Deisingh *et al.*, 2004; Smyth, 2005; Cozzolino *et al.*, 2006b; Cynkar *et al.*, 2007b). The use of MS–EN was explored as a rapid technique for fingerprinting of volatiles in wines before and after malo-lactic fermentation, in wine stored in oak barrels and in Semillon wine bottled with different closures (Di Natale *et al.*, 1995, 2000, 2004; Pinheiro *et al.*, 2002; Lozano *et al.*, 2005, 2006; Gutierrez *et al.*, 2007). It was also reported that a single MS–EN mass spectrum might provide a means of characterising complex features of wine including aroma, stability during storage (e.g. protein or heat stability), oxidation, quality grading or blending (Smyth, 2005; Cozzolino *et al.*, 2006b; Cynkar *et al.*, 2007b).

The use of MOS was tested for the ability to characterise Ontario-produced fruit wines (McKellar *et al.*, 2005). Eight fruit wines (blueberry, cherry, raspberry, blackcurrant, elderberry, cranberry, apple and peach) and four grape wines (red, Chardonnay, Riesling and ice wine) were each obtained from a minimum of five Ontario wineries. Replicates of each wine sample were dried onto membrane filters to remove ethanol and analysed by MOS (McKellar *et al.*, 2005). According to the authors, it was possible to separate completely each wine variety based on differences between wineries. However, when all wine data were pooled, classification by variety was poor (58.7% correctly classified), determining that analysis of different wine varieties from a single winery revealed some misclassification. The results show that MOS can discriminate fruit and grape wines into natural and useful groupings and may become an important tool for standardisation of wine quality (McKellar *et al.*, 2005).

An application of EN for the identification of typical aromatic compounds present in white and red wines (e.g. fruity, floral, herbaceous, vegetative, spicy, smoky) was reported (Lozano *et al.*, 2005, 2006). Both PCA and LDA show that data sets of these groups of compounds are clearly separated, and a comparison among several types of ANN has been also performed. The results confirmed that the system has good performance in the classification of typical red and white wine aromas (Lozano *et al.*, 2005, 2006).

5.7.3 Wine discrimination and classification

Several reports suggested that EN together with chemometric methods might be used by the wine industry for the identification of white wine varieties or their blends (Smyth, 2005; Cozzolino *et al.*, 2006b; Cynkar *et al.*, 2007b). Even though the conventional analysis based on GC–MS provides fundamental information about the volatile compounds presents in the wine, the use of EN has advantages of simplicity of sample preparation and reduced time of analysis (Smyth, 2005; Cozzolino *et al.*, 2006b; Cynkar *et al.*, 2007b).

The use of MS–EN was reported to classify Riesling and unwooded Chardonnay wines (Smyth, 2005; Cozzolino *et al.*, 2006b; Cynkar *et al.*, 2007b), to discriminate between different wines, regions and vintages (Buratti *et al.*, 2004; Penza *et al.*, 2004; Garcia *et al.*, 2006; Aleixandre *et al.*, 2008).

5.7.4 Wine spoilage

Taints caused by *Brettanomyces* spp. spoilage are of concern to winemakers and consumers (Berna *et al.*, 2008). The comparison of metal oxide-base EN and MS–EN for the prediction of red wine spoilage was reported (Berna *et al.*, 2008). The use of direct head-space analysis was reported as a method for fast screening of 2,4,6-trichloroanisole (TAC) in wines (Marti *et al.*, 2003).

5.8 Conclusions

Instrumental spectroscopic techniques are increasingly being used by research scientists as cost-effective quantitative and qualitative analytical tools. Infrared and EN can effectively represent a 'fingerprint' of the grape or wine sample being analysed and can be used to simplify methods and reduce analytical times for many grape and wine analytes. These advantages, together with the ability to provide detailed chemical information and simultaneously measure several analytes, have been the impetus for developing such methods.

Key factors contributing to the use of these methods in the wine industry have been advances in instrument reliability, readily available chemometrics software and improved computing power – these have enabled a paradigm shift in rapid analytical methods.

Compared to traditional laboratory methods, instrumental spectroscopic techniques often give new and better insight into complex problems by measuring a great number of chemical compounds at once, thus enabling the 'fingerprinting' of each sample. They can be used for continuous and non-destructive measurements on grape and wine products and processes over time, and in many cases give an understanding of the chemical properties of materials *in-situ* during the production of various wine products. These methods are attractive due to their inherent features of versatility, flexibility, effectiveness and richness of information.

In the near future, we can imagine that red and white grapes will be analysed with small-scale, inexpensive, portable hand-held instruments for total anthocyanins (colour), tannins, sugars (TSS), pH and acidity, and yeast assimilable nitrogen. Wine fermentation can be monitored using fibre optics, at-line or by the use of attenuated total reflectance (ATR) cells, yeast strains can be identified using NIR or MIR, and their aroma profile measured by hand-held EN instruments. Wine is analysed routinely for alcohol content, pH, sugars and tannins using NIR or FT–MIR instruments. Further, the technology offers the exciting prospect of potentially providing for the development of control management systems, which would be of great benefit to the whole supply chain of the wine industry.

Grape growers and winemakers could benefit if the compositional quality of grapes could be rapidly and non-destructively assessed using instrumental spectroscopic techniques at the weighbridge or even whilst still on the vine. As the technology of spectroscopic instrumentation and chemometrics advances further, the resulting spin-offs may further assist the wine industry in its quest to define and

objectively measure grape and wine quality, and to assure consumers of the quality of the final product to be enjoyed.

However, the lack of formal education in both instrumental spectroscopic techniques and chemometrics is still a barrier for the widespread use of this technology as an analytical tool for the analysis of aromatic plants, natural products and essential oils. The potential savings, reduction in time and cost of analysis and the environment friendly nature of the technology has positioned instrumental spectroscopic techniques as the most attractive techniques with a bright future in the arena of the analysis of grape and wine.

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6

Advances in microbiological quality control

J. P. Osborne, Oregon State University, USA

Abstract: During the winemaking process, the quality of a wine can quickly be affected by microbial spoilage due to a number of yeast and bacterial species present in wine. In order to prevent the microbial spoilage of wine it is essential to be able to recognize it when it occurs, understand where it is most likely to occur, and which wine microorganisms are involved. To do this, traditional microbial detection methods such as plating and microscopy are beginning to be combined with powerful new detection methods based upon molecular biology techniques utilizing polymerase chain reaction (PCR). The control of microbial growth in wine is still largely achieved through the use of SO_2. However, new options such as the use of lysozyme, dimethyldicarbonate, bacteriocins, and ozone, may allow a reduction in the amount of SO_2 required. The design and use of a quality control program such as hazard analysis and critical control point (HACCP) can be effective in integrating all the information regarding wine microbial spoilage that is now available to a modern winemaker. This systematic approach allows the identification of key spoilage problems, corrective actions that will be undertaken if a problem is found, verification of the impact of the corrective action, and documentation of the whole process.

Key words: microbial spoilage, traditional and modern microbial detection methodologies, alternatives to SO_2 for microbial control, sanitation, quality control programs.

6.1 Introduction

The fermentation of grapes into wine represents a complex biochemical process involving many microorganisms. Unlike many other food production systems,

there is minimal effort given to fully eliminating unwanted microorganisms from the starting ingredients (grapes/must/juice). For example, during the brewing process, microorganisms are killed through the heating of the wort prior to fermentation. This prevents any unwanted organisms from causing spoilage problems. However, during the winemaking process it is neither practical nor necessary to eliminate all of the microorganisms present on the grapes. This is because there are only a limited number of microbial species that can survive and grow during the fermentation process and those that can are not considered pathogenic. Therefore, winemakers are concerned with controlling microorganisms that may spoil their wine rather than microorganisms that may cause disease or sickness. This has meant that the focus on managing and controlling microbial populations during the winemaking process rather than complete elimination. For example, at certain stages of the winemaking process efforts are made to encourage the growth of certain yeast and bacterial species, while at other stages techniques are employed to minimize or eliminate microbial growth. For a winemaker to successfully guide their wine through this process, they must have an understanding of which microbial species are present at each winemaking stage, what their populations are, and how best to encourage, control, or prevent, their growth.

When grapes are received at the winery there are several genera of yeasts and bacteria naturally present on the grapes or on winery equipment (Kunkee *et al.*, 1965; Lafon-Lafourcade *et al.*, 1983; Fleet *et al.*, 1984; Wibowo *et al.*, 1985). During the course of alcoholic and malo-lactic fermentations, successional growth of microorganisms occurs relative to their differing tolerances of inhibitory substances and varying growth requirements (Costello *et al.*, 1983; Fleet *et al.*, 1984; Wibowo *et al.*, 1985; Davis *et al.*, 1986b). For example, non-*Saccharomyces* yeasts are usually present on sound grapes in numbers ranging from 10^3–10^5 cfu/mL. However, during the early stages of alcoholic fermentation, their viability rapidly decreases due to lack of oxygen and elevated ethanol concentrations. This leaves *Saccharomyces cerevisiae*, a more ethanol-tolerant yeast, as the dominant yeast species to complete the fermentation (Fleet *et al.*, 1984; Heard and Fleet, 1985; Hansen *et al.*, 2001; Nissen and Arneborg, 2003). Towards completion of alcoholic fermentation when *Saccharomyces* enter stationary/death phase, populations of *Oenococcus* can increase to conduct malo-lactic fermentation (MLF). During the conservation or ageing of wine, several other bacteria such as *Acetobacter*, *Lactobacillus*, and *Pediococcus* can grow as can spoilage yeast such as *Brettanomyces*. All of theses microorganisms have varying growth requirements and tolerances to inhibitory compounds, and these factors play a role in determining the microbial ecology of the wine.

Traditionally there was little thought given to identifying particular microbial species or populations during the winemaking process. Alcoholic fermentation was allowed to occur naturally with no addition of yeast starter cultures, and the natural succession of microorganisms took care of the rest. However, more modern winemaking practices include the use of yeast starter cultures to induce alcoholic fermentation and bacterial cultures to induce MLF (Nygaard *et al.*, 2002). Under these more controlled conditions, knowledge regarding the population of *Saccha-*

romyces in the fermenting must or the abundance of malo-lactic bacteria conducting the MLF can be crucial. In addition, current winemaking trends in many parts of the world are towards reducing the number of chemical additives used during winemaking. For example, many winemakers are looking to minimize the addition of SO_2 as this antimicrobial can be a health concern for certain people. However, in order to reduce the amount of SO_2 added to a wine, or any other antimicrobial, it is most efficient to make additions only when necessary. To do this the winemaker needs to have the tools to detect, identify, and enumerate microorganisms in the wine. Only then can he/she make appropriate decisions to control the growth of undesirable microorganisms in their wine. This chapter summarizes current information regarding microbial quality control in the winery including major spoilage microorganisms and emerging problems, detecting and identifying microorganisms during winemaking, microbial control and sanitation, and the development of quality control programs in the winery.

6.2 Microbial spoilage of wine

The most effective way to control microbial spoilage during winemaking is to understand which spoilage microorganisms are present during which stages and the potential spoilage issues that could occur. A brief overview of important wine spoilage yeast and bacteria incorporating some of the latest information will be given here, but for more detailed discussions readers are directed to recent articles regarding wine spoilage yeast (Loureiro and Malfeito-Ferreira, 2003), acetic acid bacteria (Du Toit and Pretorius, 2002), and wine lactic acid bacteria (Osborne and Edwards, 2005).

6.2.1 Spoilage yeast

There are a number of yeast species present on grapes and winery equipment that can play a role in the spoilage of wine. Non-*Saccharomyces* yeast species such as *Candida*, *Kloeckera*, *Cryptococcus*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Debaryomyces*, and *Metschnikowia* dominate the yeast population on grapes with *Saccharomyces* being rarely found in the vineyard (Fleet *et al.*, 1984; Heard and Fleet, 1986, 1988; Fleet and Heard, 1993). These non-*Saccharomyces* yeast dominate the early part of the alcoholic fermentation but their numbers decrease as ethanol levels increases and oxygen levels decrease (Fleet *et al.*, 1984; Heard and Fleet, 1986t, 1988). However, some species such as *Candida*, *Pichia*, and *Hanseniaspora* can survive at low levels during the alcoholic fermentation (Heard and Fleet, 1986) and may grow during wine storage and ageing. While there is some debate regarding whether these yeast can improve the aroma and flavour of a wine (Egli *et al.*, 1998; Henick-Kling *et al.*, 1998; Jolly *et al.*, 2003), there is no doubt that uncontrolled growth of some of these yeast species can cause spoilage of a wine.

During the early stages of the alcoholic fermentation or during pre-fermentation

maceration (cold soak), apiculate yeast such as *K. apiculata* and *Hanseniaspora* may produce large amounts of sensorially important compounds such as acetic acid and ethyl acetate (Sponholz, 1993; Plata *et al.*, 2003). Ciani and Maccarelli (1998) reported that *K. apiculata* strains produced between 166 to 763 mg/L of ethyl acetate. This compound at low levels in wine (<50 mg/L) may not be objectionable, but at concentrations greater than 150 mg/L ethyl acetate may give an objectionable 'fingernail polish' aroma (Jackson, 2000).

After alcoholic and malo-lactic fermentation is complete, certain oxidative film yeast may cause spoilage problems if the wine is improperly stored. A thin, dry, white film on the surface of the wine may form and is usually caused by the oxidative yeast *Candida vini* (formerly *Candida mycoderma*), but other yeast such as *Pichia membranefaciens* may also contribute (Baldwin, 1993). *C. vini* can produce acetaldehyde and ethyl acetate via the oxidation of ethanol and can also oxidize some organic acids producing a decrease in acidity (Zoecklein *et al.*, 1995). Maintaining topped tanks and barrels and keeping free SO_2 levels sufficiently high will help prevent spoilage by *C. vini* while low cellar temperatures (<15 °C/60 °F) can also help (Fugelsang and Edwards, 2007).

Another yeast that may cause spoilage problems during storage/ageing of wine is *Zygosaccharomyces*. These yeast are considered particularly dangerous as they are osmophilic, resistant to ethanol, SO_2, and sorbate (Thomas and Davenport, 1985). They are rarely found on sound grapes and most infections can be traced to the use of contaminated grape juice concentrates used for making sweet or sparkling wines (Loureiro and Malfeito-Ferreira, 2003). Spoilage is caused by the formation of gas, sediment, or cloudiness in the wine (Loureiro and Malfeito-Ferreira, 2003).

The most important wine spoilage yeast worldwide are of the *Dekkera/Brettanomyces* genus. These yeast have caused serious economic losses in the worldwide wine industry (Fugelsang, 1997) and have therefore been the subject of recent intensive research efforts. *Brettanomyces* is the asexual, non-sporulating form while *Dekkera* is the sexual, sporulating form. *B. bruxellensis* and *B. anomalus* are the two species most commonly found in wine, with *B. bruxellensis* being most frequently identified in *Brettanomyces* spoiled wines (Sponholz, 1993; Connell *et al.*, 2002; Conterno *et al.*, 2006). Until recently, it was thought that *Brettanomyces* was not present on the surface of wine grapes and that contamination occurred in the winery through importation of spoiled wine, poor sanitation of hoses, tanks, and barrels, and contamination by fruit flies (Licker *et al.*, 1999; Connell *et al.*, 2002; Loureiro and Malfeito-Ferreira, 2003). However, a recent study utilizing specific enrichment medium suggested that *B. bruxellensis* can be present on wine grapes (Renouf and Lonvaud-Funel, 2007) but usually in very low numbers. Despite this, the most frequently cited place where *Brettanomyces* is found in the winery is wood cooperage (Fugelsang and Edwards, 2007).

Compared to most other wine microorganisms, *Brettanomyces* is very slow growing and is usually only detected in significant numbers during the ageing or storage of a wine. Fugelsang and Zoecklein (2003) reported a bell-shaped growth pattern with maximum populations occurring five to seven months after initial

inoculation of Pinot noir wines. However, significant variation in growth rates and population changes were noted amongst the six strains tested. Very low levels of sugars (glucose, fructose, galactose, and trehalose) are required for its growth with Chatonnet *et al.* (1995) reporting that as little as 275 mg/L of sugar was sufficient to support the growth of the yeast and cause spoilage of wine. In addition, *Brettanomyces* can use ethanol as a sole carbon and energy source (Silva *et al.*, 2005), and Blondin *et al.* (1982) reported that *Brettanomyces* could utilize cellobiose, a disaccharide present in wood barrels. Because of its ability to utilize these alternative carbon sources, *Brettanomyces* is capable of surviving and causing spoilage even in wines that are considered dry.

The major spoilage problem associated with *Brettanomyces* is the production of the volatile ethylphenols 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG) (Heresztyn, 1986; Chatonnet *et al.*, 1992, 1995). Elevated levels of 4-ethylphenol in red wine are associated with aromas described as 'horsy', 'smoky', 'medicinal', or 'leather' while 4-ethylguiacol has been described as 'clove' or 'spice'. Although the sensory threshold of 4-EP and 4-EP in red wine is 605 µg/L and 110 µg/L, respectively (Chatonnet *et al.*, 1992), the concentration at which these compounds become objectionable in wine can vary greatly. This variation is due primarily to the type of wine and the relative concentrations of the two compounds. For example, Phister and Mills (2003) reported that the detection threshold of 4-EP was lower in a Tempranillo wine than a Cabernet Sauvignon wine. In addition, Chatonnet *et al.* (1992) reported that the sensory threshold of 4-EP is lower when both 4-EP and 4-EG were present in the wine together, a finding confirmed by Coulter *et al.* (2003). A number of researchers also reported large differences between the amount of 4-EP and 4-EG produced by different strains of *Brettanomyces* (Coulter *et al.*, 2003; Fugelsang and Zoecklein, 2003; Joseph and Bisson, 2004). In addition to producing volatile ethylphenols, *Brettanomyces* can produce large amounts of acetic acid when growing on glucose (Ciani and Ferraro, 1997; Freer *et al.*, 2003) and can also produce isovaleric acid, a compound described as 'rancid' (Licker *et al.*, 1999).

6.2.2 Spoilage bacteria

Acetic acid bacteria

Acetic acid bacteria (AAB) are aerobic, spherical to rod shaped, Gram negative bacteria that can produce acetic acid via the oxidation of ethanol (Holt *et al.*, 1994; Saeki *et al.*, 1997). Two AAB genera are important to the wine industry, *Acetobacter* and *Gluconobacter*. *Gluconobacter* species are commonly isolated from grapes and musts but disappear as alcoholic fermentation begins while *Acetobacter* are more ethanol tolerant and may survive through alcoholic fermentation (Drysdale and Fleet, 1984, 1988; Joyeux *et al.*, 1984). The population of these bacteria on grapes differs according to grape health. For example, on healthy grapes their populations are low, below 10^2 cfu/mL, while on damaged or *Botrytis* infected grapes their populations can reach over 10^5 cfu/mL (Joyeux *et al.*, 1984; Drysdale and Fleet, 1989).

Sound winemaking practices that minimized exposure of wine to air and correct use of SO_2 were considered to be enough to inhibit the growth of AAB due to their aerobic nature. However, it has become increasingly evident that these bacteria are present during all stages of winemaking and can even multiply under the anaerobic or semi-anaerobic conditions present during winemaking (Joyeux *et al.*, 1984). For example, Millet and Lonvaud-Funel (2000) and Du Toit *et al.* (2005) both demonstrated that *Acetobacter* species could survive for long periods of time in wine at low populations under anaerobic conditions. However, when oxygen was added to the wine, the populations rapidly increased demonstrating the risk of introducing air during racking and pumping operations. Therefore, minimizing the exposure of wine to air as well as maintaining a low pH (<3.50 (Du Toit and Lambrechts, 2002)), low temperatures (Joyeux *et al.*, 1984), and adequate SO_2 levels (0.7–1 mg/L molecular (Du Toit and Pretorius, 2002)) are the best strategies to control their growth.

If growth does occur in wine, AAB can cause a number of spoilage problems. The main spoilage issue is the excessive production of acetic acid. This can occur prior to fermentation on damaged grapes where yeast may metabolize grape sugars producing ethanol which is then oxidized to acetic acid by AAB (Joyeux *et al.*, 1984). Acetic acid concentrations as high as 3.9 g/L may be found in juices made from infected grapes (Drysdale and Fleet, 1989). During storage and/or ageing of wine, growth of AAB can quickly render the wine spoiled through the production of acetic acid (Drysdale and Fleet, 1989; Sponholz, 1993), ethyl acetate (Drysdale and Fleet, 1989), and acetaldehyde (Du Toit and Pretorius, 2002). AAB tend to produce acetaldehyde under low-oxygen conditions and have been shown to produce up to 250 mg/L in wine (Du Toit and Pretorius, 2002), well above the compounds' sensory threshold of 100 mg/L. While the production of these compounds may greatly decrease wine quality, there are also legal consideration as the legal limit for acetic acid in wine is 1.2–1.4 g/L (Drysdale and Fleet, 1989; Sponholz, 1993).

Lactobacillus

Lactobacillus represents a highly diverse group of species that are Gram positive, microaerophilic bacteria that vary from long to short rods or even coccobacilli (Kandler and Weiss, 1986). The most common species of *Lactobacillus* isolated from grapes and wines include *L. brevis*, *L. casei*, *L. hilgardii*, *L. plantarum*, and *L. trichodes* (Costello *et al.*, 1983; Lafon-Lafourcade *et al.*, 1983; Davis *et al.*, 1986a,b; Kandler and Weiss, 1986; Dicks and Van Vuuren, 1988). In addition, Edwards *et al.* (1998, 2000) recently isolated two novel *Lactobacillus* spp. from commercial grape wines undergoing sluggish/stuck alcoholic fermentations. Based on phenotypic and phylogenetic evidence *L. kunkeei* and *L. nagelii* were proposed as new species (Edwards *et al.*, 1998, 2000).

Lactobacillus species may be present on grapes and winery surfaces, and certain species are more prevalent during different stages of the winemaking process. Regardless of initial population levels, most *Lactobacillus* species decrease in population during the alcoholic fermentation (Fugelsang and Edwards,

2007). A couple of exceptions to this rule are *L. kunkeei* and *L. nagelii* (Edwards *et al.*, 1998, 2000). Huang *et al.* (1996) reported that *L. kunkeei* could grow rapidly during the alcoholic fermentation causing stuck or sluggish fermentations. After the alcoholic fermentation and/or the malo-lactic fermentation, other *Lactobacillus* species such as *L. hilgardii*, *L. brevis*, and *L. casei* may grow causing spoilage problems during the ageing or storage of wines (Davis *et al.*, 1986b; Costello *et al.*, 2001). The sequential dominance of different *Lactobacillus* species is partly due to their varying pH and ethanol tolerances (Davis *et al.*, 1986b). For example, *L. plantarum* ceases growth at ethanol concentrations of 5–6% v/v, while *L. casei* and *L. brevis* are more ethanol tolerant (Wibowo *et al.*, 1985).

Aside from causing stuck or sluggish alcoholic fermentations (Huang *et al.*, 1996), uncontrolled growth of certain *Lactobacillus* species can cause spoilage through the production of acetic acid (Davis *et al.*, 1986b; Huang *et al.*, 1996; Edwards *et al.*, 1998) or other adverse odours or flavours. Certain strains of *L. brevis*, *L. buchneri*, and *L. collinoides* have been implicated in the production of acrolein, a compound that may react with phenolic compounds in wine to produce bitter compounds (Sponholz, 1993). The bacteria do not produce acrolein directly but rather produce the precursor compound 1, 3-propanediol from the metabolism of glycerol (Schultz and Radler, 1984; Claisse and Lonvaud-Funel, 2000; Sauvageot *et al.*, 2000). Certain *Lactobacillus* species have also been associated with the 'mousy' defect in wines (Heresztyn, 1986; Costello *et al.*, 2001). This type of spoilage is characterized by the development of an offensive odour that renders a wine unpalatable (Costello and Henschke, 2002). Lactobacilli associated with this taint are *L. brevis*, *L. hilgardii*, and *L. cellobiosus* (Costello *et al.*, 2001).

Pediococcus

Pediococci are characterized as being spherical, Gram positive, non-motile, catalase negative, aerobic to microaerophilic microorganisms that divide in two planes to form tetrads or large clumps of cells (Garvie, 1986; Axelsson, 1998). The most common species found in wine are *P. damnosus*, *P. parvulus*, and *P. pentosaceus* (Lafon-Lafourcade *et al.*, 1983; Davis *et al.*, 1986b; Garvie, 1986; Edwards and Jensen, 1992). *Pediococcus* spp. have been isolated from wines worldwide (Costello *et al.*, 1983; Fleet *et al.*, 1984; Edwards and Jensen, 1992; Manca de Nadra and Strasser de Saad, 1995) and yet their prevalence and impact on wine quality has still not been clearly defined.

Pediococcus may enter wine through being present on soil, grapes, or winery equipment but their survival is favoured when pH is greater than 3.50 (Wibowo *et al.*, 1985; Davis *et al.*, 1986a). As with other lactic acid bacteria (LAB) species present on the grapes, their numbers remain low during the alcoholic fermentation. Once alcoholic and/or MLF is complete, they may grow in wine resulting in spoilage (Davis *et al.*, 1986b). Even in wines that have undergone MLF, sufficient sugars such as glucose, fructose, arabinose, and trehalose may remain (Liu and Davies, 1994) allowing bacterial growth and subsequent spoilage problems. Some recent studies have also demonstrated that *P. pentosaceus* can use glycerol as its sole carbon source (Salado and Strasser de Saad, 1995,

1996) increasing the possibility of *Pediococcus* growth even in wines considered 'dry' (that is <0.5 g/L).

Growth of *Pediococcus* spp. in wine has been considered undesirable due to the production of off-aromas and flavours. Pediococci can produce excessive acetoin and diacetyl giving an undesirable buttery aroma and flavour at high concentrations (Sponholz, 1993). As with some *Lactobacillus* species, certain Pediococci species have also been implicated in the production of bitter taint via the degradation of glycerol (Davis *et al.*, 1988; Sponholz, 1993; Du Toit and Pretorius, 2000). Furthermore, *P. damnosus* and *P. pentosaceus* have both been shown to cause 'ropy' wines via the production of extra-cellular polysaccharides characterized as β-D-glucans (Llaubéres *et al.*, 1990). This results in an increase in viscosity and can render the wine visually unappealing (Manca de Nadra and Strasser de Saad, 1995; Fugelsang, 1997). In general, wines that have higher pHs (>3.50) and contain glucose and nitrogen sources are most at risk of this problem (Walling *et al.*, 2005).

An additional spoilage issue associated with *Pediococcus* is the production of biogenic amines. Biogenic amines are low molecular weight organic bases that have undesirable physiological effects on humans when absorbed at too high a concentration (Silla Santos, 1996; Arena and Manca de Nadra, 2001; Lonvaud-Funel, 2001). These compounds are formed by decarboxylation of the corresponding amino acids by microorganisms such as LAB (Halasz *et al.*, 1994; Arena and Manca de Nadra, 2001). *Pediococcus* as well as *Lactobacillus* have been implicated in the production of these compounds (Delfini, 1989; Arena and Manca de Nadra, 2001). In particular, production of biogenic amines has been associated with wines that have undergone spontaneous malo-lactic fermentations (Soufleros *et al*, 1998).

6.2.3 Viable but non-culturable wine microorganisms

Our understanding of wine spoilage microorganisms and particularly their growth and control in wine has been further complicated by recent discoveries that some wine microorganisms can exist in a state known as 'viable but non-culturable' (VBNC). Microorganisms in this state do not grow on conventional microbiological medium but still remain intact and viable (Roszak and Colwell, 1987). At a later stage when growth conditions are favourable, these microorganisms may begin growing again. This state is usually brought about in response to stresses such as temperature, oxygen concentration, and exposure to antimicrobial agents. For example, Millet and Lonvaud-Funel (2000) reported that acetic acid bacteria survived in a VBNC state when oxygen levels in a wine were very low but that the bacteria rapidly recovered from this state when oxygen was available. This finding was confirmed by Du Toit *et al.* (2005) where oxygen addition to wines revived *Acetobacter aceti* populations that had fallen to below detectable methods using standard plating methods. In addition, Du Toit *et al.* (2005) also suggested that *Brettanomyces* may enter a VBNC state when exposed to SO_2.

Currently, the microorganisms in wine believed to be able to enter a VBNC state are *Acetobacter aceti*, *Brettanomyces bruxellensis*, *Candida stellata*, *Lactobacil-*

lus plantarum, *Saccharomyces cerevisiae*, and *Zygosaccharomyces bailii* (Millet and Lonvaud-Funel, 2000; Divol and Lonvaud-Funel, 2005; Du Toit *et al.*, 2005; Oliver, 2005). Many of these microorganisms are considered spoilage microorganisms and so the difficulty in detecting them due to being in a VBNC state presents a problem when developing microbial control strategies in the winery. For example, prior to bottling a winery may plate out a sample of wine to detect any spoilage microorganisms. If, for example, *Brettanomyces* is present in the wine in a VBNC state then it will not be detected. *Brettanomyces* may then grow in the bottle at a later date when conditions are more favourable as demonstrated by Renouf *et al.* (2007). Of further concern is the observation reported by Millet and Lonvaud-Funel (2000) that some bacteria and yeast present in a VBNC state could pass through a 0.45 μm filter. It was suggested that microorganisms in this VBNC state may be reduced in size and so able to pass through the membrane. No further incidences of this occurring in wine have been reported but, given the importance of sterile filtration to the wine industry, this is a subject that requires further investigation.

6.3 Detecting microorganisms during the winemaking process

The identification and quantification of various wine spoilage microorganisms is crucial to their control and management during winemaking. For example, it is important to know which microorganisms are present in the wine as wine spoilage microorganisms have varying degrees of susceptibility to antimicrobials used in the wine industry. In addition, while the presence of a few hundred cells per mL of *Brettanomyces* may be of concern and require action, the detection of *Oenococcus oeni* at similar populations may not be worrisome at all. The following section details some of the classical methods employed to detect, identify, and quantify microbial populations in wine. However, the focus is on some of the latest techniques being utilized as well as methods currently being used at the research level that may soon be more widely utilized in the wine industry.

6.3.1 Traditional methods

Traditional methods utilized to identify microorganisms during winemaking typically include microscopic examination and culturing. A juice or wine may be examined under a microscope and information regarding the shape, size, and arrangement of cells can be gained. Microscopy can be used to quickly scan multiple wine samples, although concentration (usually by centrifugation) may be required as a minimum of 10^3–10^4 cells per mL are usually needed for detection. Tentative identification using a microscope can be performed with some training (especially identification of bacterial species). However, errors can easily be made as yeast may look different depending on the medium they are growing on or the age of the culture. For more definitive identification, other methods must be employed.

The growth of a microorganism on a specific media coupled with microscopic examination can be a powerful tool to identify microorganisms. For example, most bacterial species present during the winemaking were identified by traditional microbiological techniques based on cell morphological and physiological differences as described in Bergey's Manual (Kandler and Weiss, 1986). In addition, the ability of microorganisms to utilize certain carbohydrates or sources of nitrogen can also be used to aid in the identification of a microorganism (Fugelsang and Edwards, 2007) as can growth at certain temperatures or growth under aerobic or anaerobic conditions. However, many of these techniques are time-consuming and laborious and are rarely used in a winery situation. Instead, a more common technique utilized is the direct plating of wine samples onto selective media and microscopic examination of colonies that grow. Wine can be plated onto a medium that allows growth of all microorganisms in the wine or can be plated onto a medium that only allows growth of specific microorganisms. For example, a medium rich in nutrients containing cycloheximide and ampicillin is selective for *Brettanomyces* in wines as ampicillin will prevent bacterial growth and cycloheximide will prevent *Saccharomyces* from growing. *Brettanomyces* is naturally resistant to cycloheximide and so will be able to grow on the plate. Selective media for certain wine microorganisms are available from commercial laboratories.

Although growth on selective media can be a useful method to employ when identifying wine microorganisms, it is still time-consuming and labour-intensive. Some microorganisms may take up to 14 days to grow and so results are not readily available. Some level of training and equipment is also required in order to plate samples and interpret the results. In addition, culture-based assays will not detect VBNC microorganisms and often exhibit biases resulting in an incomplete representation of the true diversity of microorganisms present (Amann *et al.*, 1995; Hugenholtz *et al.*, 1998). As a result, a large amount of research has been undertaken to develop more specific, rapid, and reliable techniques to identify microorganisms present during winemaking.

6.3.2 Modern and emerging detection methods

Modern detection methods have focused on providing highly specific and rapid results capable of detecting microorganisms at very low populations. Many of these methods utilize molecular biology techniques based on the similarity and dissimilarity of DNA sequences (Lonvaud-Funel *et al.*, 1991). Others rely on immunological assays or the detection of metabolites or proteins unique to a particular wine microorganism (Dewey *et al.*, 2000). These methods usually need specialized and expensive equipment and require extensive training to use and interpret results. Therefore, most of the detection methods described in the following section are currently utilized only by research or commercial laboratories. However, many of these methods do have the potential to be modified and simplified for use in a winery setting in the future.

A technique to monitor microbial populations on surfaces that has been successfully applied in the food industry is the use of a bioluminescence to detect

ATP (Davidson *et al.*, 1999). This technique utilizes the luciferin–luciferase assay where the reaction of ATP with the enzyme and luciferin results in light being emitted that can be measured by a light meter. An estimation of viable cell numbers is possible as a higher population of microorganisms should result in more ATP being present. Kits are available that include a light meter, swabs, and reagents, and this method has been used successfully in the alcoholic beverage industry (Thompson, 2000). The major drawback with this method is that it does not provide specific information regarding what types of microorganisms are present and it can also be difficult to correlate the measurement of ATP to viable cell counts (Fugelsang and Edwards, 2007).

An immunological technique that is being explored for its application in the wine industry is the enzyme-linked immunosorbent assay (ELISA). This assay relies on the interaction between an antigen and an antibody specific for that antigen. Antibodies to a specific microbial cell wall, cellular component, or metabolite can be developed offering a high degree of specificity. The method can include the use of a secondary antibody–chromophore complex that will produce a colour when the initial coupling of the antigen and antibody occurs. This allows quantification. The assay has been evaluated for use in the wine industry for the detection of *Botrytis* on grapes (Dewey *et al.*, 2000, 2008), the detection of the mycotoxin ochratoxin A in wine (Flajs *et al.*, 2009), as well as the enumeration of *Brettanomyces* (Kuniyuki *et al.*, 1984). The method can give results within 24 hours but has the disadvantage of being unable to distinguish between dead and alive cells. However, this technology is being widely adopted in other food and beverage industries (Li *et al.*, 2000; Franek and Hruska, 2005) and so will undoubtedly have some useful applications in the wine industry.

Perhaps the area where the most advances have been made in recent years is in the use of molecular biology and the utilization of the polymerase chain reaction (PCR) for microbial detection. Lonvaud-Funel *et al.* (1991) utilized this technology in the development of methods to direct molecular characterization of lactic acid bacteria strains through DNA–DNA hybridization. However, some interference from cross-hybridization between closely related species was reported. For example, this method did not allow species segregation among the *Pediococcus* genus. Other techniques were developed that attempted to solve this problem such as the use of restriction fragment length polymorphism (RFLP). Le Jeune *et al.* (1995) utilized RFLP to identify wine bacterial species while Guillamon *et al.* (1998), Granchi *et al.* (1999), and Mercado *et al.* (2007) used RFLP to identify specific wine yeast species. In addition, Cocolin *et al.* (2002) and Baleiras-Couto *et al.* (2005) followed yeast ecology during red wine fermentations using RFLP.

Other techniques that have been investigated include the use of real-time PCR (Q-PCR) where specific amplification products formed during the PCR process are detected in real time. Specific fluorescently labelled nucleotides increase in proportion to the amount of PCR product being formed during the PCR reaction and allow real-time analysis of results. Q-PCR has been used to detect and quantify *Brettanomyces* (Phister and Mills, 2003; Delaherche *et al.*, 2004),

Zygosaccharomyces (Rawsthorne and Phister, 2006), *Hanseniaspora* (Hierro *et al.*, 2007), and wine lactic acid bacteria including *Pediococcus* (Delaherche *et al.*, 2004; Renouf *et al.*, 2007), *Lactobacillus*, and *Oenococcus* (Pinzani *et al.*, 2004; Renouf *et al.*, 2007).

The use of denaturing gradient gel electrophoresis (DGGE) has also been explored as a way to identify specific microbial species in wine and differentiate between closely related species. PCR amplified sequences are subjected to an increasingly denaturing environment during electrophoresis. This allows the separation of PCR amplified sequences of the same size but different sequences (Renouf *et al.*, 2007), making this technique a very sensitive tool for species separation and identification. For example, Renouf *et al.* (2007) followed the evolution of the wine microbial ecosystem identifying 52 different yeast species and 40 bacterial species that were present on grapes prior to fermentation. This diversity dramatically dropped during winemaking leaving *Brettanomyces*, *Oenococcus*, and *Pediococcus* as the dominant species during wine maturation. PCR–DGGE has also been used to analyze *O. oeni* and *L. plantarum* populations in red wine (Spano *et al.*, 2007) as well as yeast and bacterial species present on grape, wine, and cellar equipment (Renouf *et al.*, 2007).

Probe-based detection systems are also being developed that allow the rapid identification and quantification of wine microorganisms. These systems usually rely on oligonucleotide probes that contain a fluorescent dye. When binding of the probe to the target DNA occurs, this causes the probe to fluoresce allowing detection and quantification (Fugelsang and Edwards, 2007). One such probe, the Scorpion® probe, is currently being used by a commercial laboratory that services the wine industry.

Although these modern methods of detecting microorganism in wine offer many advantages over traditional methods, such as being less time-consuming and laborious, providing rapid results, having high specificity, and the ability to detect VBNC cells, they still have some drawbacks. A possible problem with the direct use of microbial DNA from wine is the quantification of dead cells (Hierro *et al.*, 2006). Another is the ability to differentiate between closely related species (Renouf *et al.*, 2007). In response, there are a number of studies being performed to optimize techniques such as PCR–DGGE to differentiate between important wine microorganisms such as *P. damnosus* and *P. parvulus* (Renouf *et al.*, 2006) or *Pediococcus* and *Oenococcus* (Renouf *et al.*, 2007) as well as the detection of RNA by reverse transcription-PCR which is considered to be a better indicator of cell viability (Hierro *et al.*, 2006). Currently very few of these methods are available for utilization in a winery. However, this will change as methods are simplified and equipment costs decrease. The key for a winery in the future will be the integration of these powerful detection tools into a quality control program where microbial populations can be monitored and managed during every phase of winemaking. If the type and populations of specific microorganisms are known then control strategies can be developed to prevent or minimize wine spoilage issues.

6.4 Microbial control and sanitation in the winery

Controlling the growth of microorganisms during the winemaking process involves not just identifying what microorganisms are present but, more importantly, what actions can be taken to prevent their growth and minimize potential spoilage issues. This can involve the use of chemical additives as well as physical treatments such as filtration. Where sanitation practices seek to minimize or prevent contamination of the wine, microbial control involves reducing or eliminating populations of microorganisms in wine as well as preventing growth and the production of undesirable compounds. Current or traditional methods employed to control microbial growth and sanitize the winery will be discussed in the following section as well as emerging methods that are beginning to be used in wineries and future tools that may be utilized.

6.4.1 Controlling microbial growth

Current standard methods

The most widely used tool in the winery to control microbial growth is sulphur dioxide. SO_2 has been used for thousands of years during winemaking as an antimicrobial and antioxidant agent. It is very effective in these roles, is readily available, and is relatively cheap and easy to use. It may be added to a must or wine in the form of a compressed gas, or in solid form as potassium metabisulphite (usually dissolved in water). When added to a must or wine, sulphur dioxide either ionizes to free SO_2 ($SO_2{\cdot}H_2O$, HSO_3^-, and/or SO_3^{2-} depending on pH) or can become bound SO_2 by reacting with acetaldehyde, glucose, pyruvic acid, α-ketoglutaric acid, or glucose on a 1:1 molar ratio (Burroughs and Sparks, 1973; Amerine and Ough, 1980; Romano and Suzzi, 1993; Zoecklein *et al.*, 1995). Of these forms, the molecular variety of free SO_2 ($SO_2{\cdot}H_2O$) is thought to be the most antimicrobial (Rahn and Conn, 1944; Macris and Markakis, 1974; King *et al.*, 1981; Edinger, 1986; Zoecklein *et al.*, 1995). The amount of free SO_2 required to maintain a given molecular SO_2 level is directly related to the pH with an increase in pH requiring high concentrations of free SO_2. Therefore using one universal free SO_2 level as a target concentration for ensuring the protection of wine is inadequate if you do not take pH into consideration also.

Microorganisms in wine have differing degrees of sensitivity to SO_2. In general, yeast are less sensitive to SO_2 than bacteria with LAB being more sensitive than AAB (Du Toit *et al.*, 2005; Fugelsang and Edwards, 2007). However, there is some discrepancy in the literature regarding the sensitivity of the various wine LAB and this area requires clarification. For example, Davis *et al.* (1988) suggested that *O. oeni* was more sensitive to SO_2 than *Pediococcus* while Hood (1983) reported that *Pediococcus* was more sensitive to SO_2 than *Lactobacillus* and *Oenococcus*. Furthermore, while some yeast such as *Brettanomyces* may be controlled with molecular SO_2 levels of 0.8 mg/L (Du Toit *et al.*, 2005), other yeast such as *Zygosaccharomyces* are tolerant to much higher levels of SO_2 up to 2 mg/L molecular (Warth, 1985).

Although use of SO_2 is critical to the successful control of microorganisms in wine, there is a worldwide trend towards reducing its use due to health concerns (Yang and Purchase, 1985) as well as the desire to minimize chemical additives during the winemaking process. This has lead to a lot of investigation into possible replacements of SO_2 and the use of alternative antimicrobial agents. It most cases these alternatives do not replace the use of SO_2 as an antimicrobial, but rather they minimize the amount of SO_2 required to control microbial growth.

Emerging control methods

An alternative antimicrobial agent that has been investigated is the enzyme lysozyme. Lysozyme is an enzyme present in hen eggs and has lytic activity against Gram + bacteria including wine LAB (Delfini *et al.*, 2004). Activity against Gram – bacteria found in wine such as *Acetobacter* is minimal and the enzyme has no effect on yeast or moulds. Use of lysozyme can be helpful in reducing the populations of bacteria in the must prior to alcoholic fermentation, in preventing *Lactobacillus* spoilage during a stuck or sluggish alcoholic fermentation (Gao *et al.*, 2002), as well as preventing MLF from occurring (Daeschel *et al.*, 2002; Nygaard *et al.*, 2002). Lysozyme is more active in white wines than reds as being a protein it will interact with the phenolic compounds present in a red wine (Daeschel *et al.*, 2002; Bartowsky *et al.*, 2004; Delfini *et al.*, 2004). In addition, it may cause protein instability in a white wine that will require fining to remove.

An additional group of antimicrobial agents currently being studied for use in the wine industry are the bacteriocins. Bacteriocins are proteinaceous substances that have a narrow spectrum of activity against closely related species. Many excellent reviews on bacteriocins have been published, and the reader is referred to those for more in-depth information (De Vuyst and Vandamme, 1994; Jack *et al.*, 1994; Nes *et al.*, 1996). Bacteriocin production is a characteristic typical of many LAB and a number of researchers have reported the production of bacteriocins by LAB species present in wine (Strasser de Saad and Manca de Nadra, 1993; Navarro *et al.*, 2000; Yurdugul and Bozoglu, 2002). A bacteriocin produced by a non-wine LAB, *Lactobacillus lactis*, that has been successfully used in the food industry is nisin. Nisin is an effective inhibitor of Gram + bacteria although Radler (1990a,b) reported that species differed in their sensitivity. Daeschel *et al.* (1991) demonstrated that nisin could be used to control MLF in Chardonnay while Rojo-Bezares *et al.* (2007) reported that nisin was an efficient antimicrobial agent against wine LAB with *O. oeni* being the most susceptible species. The authors also noted a synergistic effect on LAB growth inhibition by nisin and SO_2. In addition, Knoll *et al.* (2008) showed that nisin activity was not negatively affected by phenolic compounds found in red wine, demonstrating that it could be used in both red and white wines. Despite these promising results, the cost-effectiveness of using nisin and the fact it is not approved for use in wine in the USA have meant that nisin is not currently used in the wine industry. However, given its potential, nisin could be a future alternative to other chemical preservatives currently used during wine production.

Aside from the addition of purified bacteriocins to wine, researchers have

investigated the use of bacteriocin-producing wine microorganisms to control growth of spoilage microbes. Many examples of wine bacteria inhibiting other bacteria have been documented. For example, Navarro *et al.* (2000) isolated nine strains of *L. plantarum* from Rioja red wine that showed antibacterial activity, while Yurdugul and Bozoglu (2002) identified an isolate of *Leuconostoc mesenteroides* subsp. *cremoris* from wine that produced a bacteriocin-like inhibitory substance. Furthermore, Strasser de Saad and Manca de Nadra (1993) isolated two strains of *P. pentosaceus* that produced an inhibitory substance against strains of *Lactobacillus*, *Oenococcus*, and *Pediococcus*. Researchers have also investigated the use of recombinant DNA technology to transfer the bacterial genes encoding for bacteriocins to the wine yeast *S. cerevisiae*. For example, Schoeman *et al.* (1999) developed bactericidal yeast strains by expressing the *pedA* pediocin gene from *P. acidilactici* in *S. cerevisiae*. The authors proposed that development of such bactericidal strains could lead to the use of *S. cerevisiae* strains capable of acting as biological control agents to inhibit the growth of spoilage bacteria. While this technology shows some promise for the future, current consumer aversion to the use of genetically modified organisms in food means the use of genetically modified wine yeast is presently not an viable option (Beringer, 2000; Dequin, 2001; Akada, 2002; Boyazoglu, 2002).

A microbial control agent that is being used with more frequency in the wine industry is dimethyldicarbonate (DMDC). This compound is sold under the trade name Velcorin™ and is approved for use in the USA up to the cumulative amount of 200 mg/L (Ough, 1993). DMDC enters the cell and causes death through inactivation of key enzymes. It is hydrolyzed to carbon dioxide and methanol in aqueous solutions within a few hours and yields no residual odours or flavours. Ough (1993) reported that in a wine containing 10% v/v ethanol, 25 mg/L DMDC was sufficient to control the growth of *Saccharomyces*, *Brettanomyces*, and *Schizosaccharomyces*. However, a higher rate of 100 mg/L was reported by Costa *et al.* (2008) as necessary to prevent growth of *Saccharomyces*, *Brettanomyces*, and *Schizosaccharomyces*. In addition, Delfini *et al.* (2002) reported that 150 mg/L DMDC was required to delay the growth of *S. bayanus* in a grape must while higher rates of 250–350 mg/L was needed to inhibit *Candida*, *Pichia*, *Kloeckera*, and *Hansenula* species in grape must. Bacteria also appear to be more resistant to DMDC with reported values of over 1000 mg/L being required to prevent their growth (Delfini *et al.*, 2002; Costa *et al.*, 2008). While DMDC appears to be an effective in the control of yeast growth in wine, it is less effective against bacteria and because of its low solubility in water it requires specialized equipment and training to use. It also has no residual effect and so may be most effective as an additive before bottling to eliminate populations of yeast that could cause spoilage in the bottle.

6.4.2 Sanitation

A crucial step in preventing microbial spoilage is the cleaning and sanitization of the winery. An effective sanitization program will minimize the risks of contami-

nating wines with spoilage microorganisms as well as background populations of these microorganisms in the winery, thus reducing the need for the addition of antimicrobial agents. This section will outline some of the current knowledge regarding winery cleaning and sanitization as well as some emerging tools that can be used in this area. For a more detailed discussion of winery cleaning and sanitization readers are directed to Fugelsang and Edwards (2007).

While cleaning refers to the physical removal of organic matter, debris, or minerals from a surface, sanitization is the reduction or elimination of microorganisms. Proper cleaning is critical to a successful sanitization program as excess organic matter will greatly reduce the effectiveness of most sanitizing agents. Sanitization is not the same as sterilization as sterilization implies the elimination of 100% of the viable microorganisms while sanitization results in the reduction of microbial populations. Traditional sanitizing agents used in the wine industry include acidulated SO_2 (for example, 100 mg/L SO_2 solution prepared in combination with 3 g/L citric acid), hot water (>82 °C) (Coggan, 2003) and steam, and chlorinated cleaners. While acidulated SO_2, and hot water and steam are still widely used, concern over the use of chlorinated sanitizers and the formation of 2, 4, 6-trichloroanisole (TCA) (Pena-Neira *et al.*, 2000; Sefton and Simpson, 2005; Simpson and Sefton, 2007) has led to the recommendation to eliminate chlorine use in the winery.

Replacing the use of chlorinated sanitizers in the winery are a number of other sanitizing agents. These include quaternary ammonium compounds (QUATS), iodophores, and peroxides. QUATS are effective against yeast and Gram + bacteria in wine but are less effective against *Acetobacter* (Fugelsang and Edwards, 2007). Iodophores (a mixture of iodine and acid) are widely used in other food industries and are easy to use with a broad spectrum (Wirtanen and Salo, 2003). However, they may foam excessively, are expensive, and there are concerns over residual flavours and/or aromas (Wirtanen and Salo, 2003). Peroxide-based sanitizers include hydrogen peroxide (H_2O_2), sodium percarbonate, and peroxyacetic acid (PAA). H_2O_2 has limited uses in the winery and has a very short shelf life during storage due to chemical decomposition. Sodium percarbonate is widely used to treat barrels and is much more stable than H_2O_2. PAA is a highly reactive oxidant and special training and handling is required when using it in its concentrated form (40% w/v). It is, however, a very effective sanitizer, is biodegradable, and has minimal corrosive properties.

A sanitizer that is being increasingly adopted in the wine industry is ozone (O_3) (Coggan, 2003; Marko *et al.*, 2005; Pascual *et al.*, 2007). This strong oxidizer is very unstable and rapidly degrades to O_2. This means it cannot be stored and must be generated when needed. Ozone is dissolved in water and commonly used for barrel cleaning and sanitation, tank cleaning and sanitation, clean-in-place systems, and general surface sanitation (Pascual *et al.*, 2007). Special equipment is required to generate and use ozone, and portable ozone generators are often used in wineries. While ozone is very effective against a broad spectrum of wine microorganisms (Khadre *et al.*, 2001), it does not have any residual activity and special training is required before it can be safely utilized in a winery.

6.5 Quality control programs

The previous sections have outlined the spoilage certain wine microorganisms can cause, the methods used to detect, identify, and quantify them, and control methods that can be used to prevent or minimize their growth during the winemaking process. However, without a systematic approach to applying this information in the winery, preventing microbial spoilage of wine may become a haphazard affair. The hazard analysis and critical control points (HACCP) system is an example of such a systematic approach that has been widely applied in the food industry (Kourtis and Arvanitoyannis, 2001; Christaki and Tzia, 2002; Min and Min, 2006). HACCP programs identify potential problems, critical points where monitoring is needed, corrective actions that can be taken, verification of actions taken, and documentation of the process. HACCP programs in the food industry focus primarily on minimizing risks that may cause human illness or death, but some of the same principles can be applied to winemaking when addressing wine microbial spoilage issues (Christaki and Tzia, 2002).

The first step in developing a quality control program for a winery is developing a flow diagram that identifies the key steps of winemaking from the vineyard to the bottle. This will be different for different wine styles (for example red versus white versus sparkling) and can be customized for any sized operation. Potential dangers to wine quality are then identified, for example high volatile acidity (VA) from bacterial growth. Once these quality issues are established then critical control points at each step of the process can be determined relevant for the particular issue. For VA the critical control points may be the incoming fruit, the cold soak, alcoholic fermentation, oak maturation, and bottling. At each critical control point key parameters or critical limits are then determined and analyses to be performed are identified. For VA this could be the temperature of fruit when it is received at the winery as high temperatures will encourage microbial growth. A critical limit for temperature would then be established. A standard protocol for measuring fruit temperature would be put in place so that it could be determined whether the incoming fruit was a desirable temperature. If analysis indicated a problem then established corrective measures will be implemented. In the case of high fruit temperatures a corrective measure would be cooling the fruit to minimize microbial growth.

An important step in any quality control program is the documentation of what actions have been taken. This ensures that corrective actions have in fact occurred and, just as importantly, that the corrective actions are not repeated by accident. This information also provides technical data for each fruit lot passing through the winery and can be useful when making decisions about future vintages. For example, documentation of variables such as pH, nitrogen, and sugars of grapes from a certain vineyard may help guide decisions a winemaker will make when using grapes from the same vineyard in future years. Documentation is also important from a legal standpoint. Some wine additives have legal limits and so documentation of their use and how much has been added during winemaking is critical.

Verification is the final step that is required for a successful quality control program in the winery. This involves verification of the accuracy of the analysis performed, the effectiveness of actions taken, as well as the impact of actions taken. Verification is particularly important when the quality program is first adopted and acts as a feedback mechanism for the whole system. For example, verification may indicate that a particular treatment was not effective and needs modification or that more detailed analysis is required. A simplified example of a quality control program is shown in Fig. 6.1. Here a basic flowchart for red winemaking is shown along with some key components of a quality control program. The program illustrated in Fig. 6.1 has been designed to prevent spoilage by LAB. A few key analyses are listed as are some suggested critical limits, corrective actions, and verification steps. Many of these steps, such as critical limits, type and frequency of analysis, and corrective actions to be taken, would be decided by each individual winery. This allows wineries to tailor-make quality control programs suited to their specific needs and develop plans for preventing and controlling the various microbial threats to wine quality.

6.6 An integrative approach to microbiological quality control in the winery

The microbial spoilage of wine can have large impacts on the quality of a wine. High-quality wines can quickly be spoiled by the growth of certain microorganisms causing large economic losses. Due to the fact that winemaking is not a sterile process, the winemaker seeks to control and prevent the growth of the few yeast and bacterial species that can grow in wine. Spoilage yeast and bacteria present more immediate threats at different stages of winemaking and understanding when and under what conditions these spoilage microbes grow can be critical in their control. An understanding of the type of spoilage that these microorganisms can cause is also important when diagnosing what the problem may be.

Traditional microbiological techniques have been used for decades to aid in the identification and quantification of wine microorganisms in the winery. This includes direct plating and microscopy. These are still powerful tools as part of a strategy to control microbial spoilage. However, newer detection methodologies have been developed that allow rapid and specific analysis of microbial populations. These include the use of PCR, ELISA, and bioluminescence. As these methodologies become more available to the winemaker, the key will be how to integrate these powerful detection tools into the quality control program of wineries.

The specific identification of microbial species during winemaking will also impact the efficiency of methods used to control their growth in wine. The predominant way in which microbial growth in wine is controlled currently is through the use of SO_2. This valuable tool has yet to be replaced as an antimicrobial and antioxidant, but other antimicrobials are now available that can minimize the use of chemicals. These include the use of lysozyme, DMDC, and potentially bacteriocins. In addition, as we increase our understanding of how certain yeast

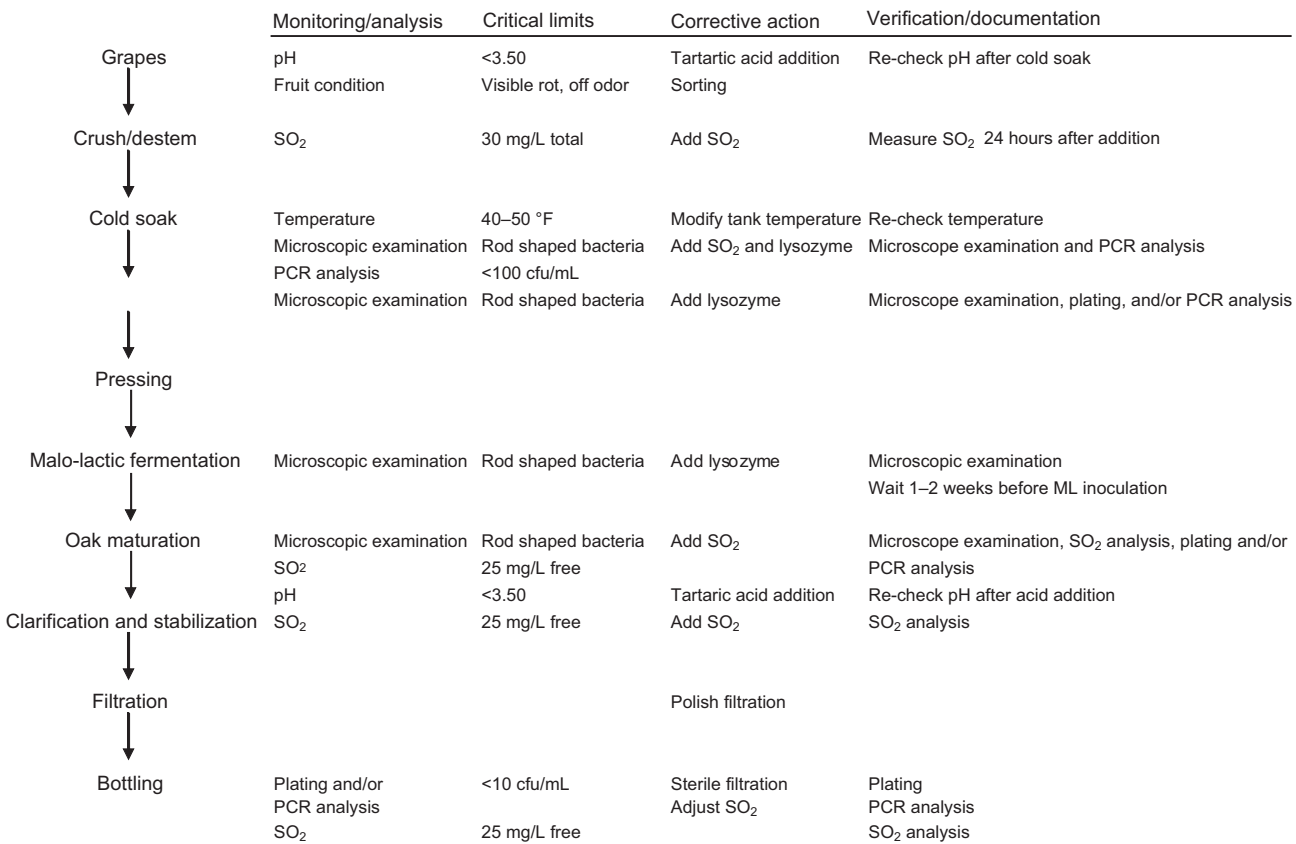

Fig. 6.1 Example of a simplified red wine processing scheme and quality control program for preventing wine spoilage by lactic acid bacteria.

and bacterial species interact with each other in wine, potential new solutions to microbial control may present themselves. The development of yeast strains that can prevent the growth of certain spoilage bacteria is being explored and may offer an alternative to the use of chemical preservatives in the future.

Research is continuing into how, why, and when microorganisms cause spoilage of wine. A lot of new information regarding this topic is now available to a winemaker. However, without an understanding of what this information means and how it can be applied, this information becomes of little value. In addition, the amount of data that a modern winemaker can gather regarding their wine can be daunting if there is no system in place to place the data in context within the winemaking process. The use of quality control programs such as HACCP-based programs is one way that a winery can utilize information regarding wine spoilage microorganisms, detection and identification methods, and control methods. This systematic approach can be utilized to design plans to control or prevent microbial spoilage problems during winemaking. Depending on the needs of the winery, methodologies ranging from sensory analysis to PCR analysis can be incorporated into a plan that includes when and how to monitor microorganisms, what specific actions should be taken if analysis reveals there is a problem, verification to ensure the corrective action was effective, and documentation of the whole process.

6.7 References

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7

Sensory analysis of wine

I. Lesschaeve, Vineland Research and Innovation Centre, Canada; and A. C. Noble, University of California, USA

Abstract: The main advantage of sensory techniques over traditional wine tasting is to ensure that tasting data are collected in the least biased way that can be analyzed and interpreted statistically. The present chapter reviews current methodologies and best practices for successful implementation of sensory techniques within winery operations, whether these aim at characterizing wine sensory properties, ensuring wine production quality, or developing new wine styles according to consumer sensory preferences. Although the use of sensory evaluation seems to remain the prerogative of large wineries, several realistic options are proposed to demonstrate how small operations can adopt good sensory practices with limited resources and support their business decisions.

Key words: sensory evaluation, wine, analytical test, hedonic test, business decisions.

7.1 Introduction

Tasting wine has always been part of winery operations. Oenologists and winemakers have long used their senses to assess wine quality at every stage of production. These professionals are often referred to as experts; they tend to rely on their own holistic assessment, the result of objective and subjective considerations and tasting experience. Sensory science is a young discipline that emerged in the 1940s, from food acceptance experiments conducted by the US Army (Meiselman and Schutz, 2003) and the search for quality control methods in beer production with the development of the triangle test in Scandinavia (Pangborn, 1964). Wine has been one of the early applications of sensory science at the University of California at Davis and elsewhere (Amerine *et al.*, 1965; Amerine and Roessler, 1983). The main advantage of sensory techniques over traditional

wine tasting is the ability to collect wine assessments in the least biased way. This is accomplished by using protocols minimizing physiological and psychological factors known to affect human sensory responses and utilizing assessors which are highly sensitive to sensory stimuli and able to evaluate their perception analytically and objectively. Sensory professionals often make the analogy between a sensory panel and a high-tech analytical instrument, similar to what a gas chromatography–mass spectroscopy (GC–MS) could represent for flavour chemists. Analytical data collected from sensory panels are expected to be accurate, sensitive, repeatable and reproducible.

Another branch of sensory science includes hedonic tests, designed to determine consumer preference between two or more products, and also requiring *ad hoc* protocols minimizing any factors that could influence consumer appreciation.

Sensory techniques have been used mainly to support research efforts in viticulture and oenology (e.g. Noble *et al.*, 1995; Heymann and Noble, 1987; Noble and Shannon, 1987; Francis *et al.*, 1992, 2003; Cliff and Dever, 1996; Reynolds *et al.*, 1996). Commercial applications have increased since the 1990s with the emergence of preference mapping techniques, able to identify sensory attributes driving consumers' wine preference (Lesschaeve, 2007b).

The purpose of this chapter is to discuss the scope and breadth of application of wine sensory evaluation to manage quality and safety in wineries of all sizes. The first part will describe the material conditions required to implement sensory techniques. The second part will review applications of sensory evaluation in a business context. Particular emphasis will be placed on small wineries with limited resources. The chapter concludes with some perspectives for the future.

7.2 Tasting environment and best practices

Sensory evaluation is a scientific discipline used to evoke, measure, analyze and interpret reactions to stimuli perceived through the senses (ASTM, 2000). Similar to wine chemical analysis (e.g. alcohol, titratable acidity), sensory tests must be conducted under standardized and controlled conditions minimizing psychological and physiological biases affecting human responses. Those factors are described in Table 7.1 along with existing sensory practices which aim to minimize them. Implementation of good sensory practices is therefore critical in maintaining the integrity of the sensory test and ensuring the objectivity of the results. Recommended practices in wine sensory evaluation are summarized below.

7.2.1 Good sensory evaluation practices

It is important to remove all marketing cues that can alter perception of quality. Samples are always presented uniformly, in identical containers coded with random numbers to prevent bias from extraneous clues such as brand or treatment. Standard tulip-shaped clear wine glasses are optimal for evaluation of aroma, while plastic cups or beakers can be used when only taste or mouthfeel are being

evaluated. Providing watch glasses or Petri dishes as sample lids during aroma evaluations increases aroma intensity and reduces odours in the tasting facility. For evaluation of flavour, differences in appearance are normally masked by serving samples in opaque containers (e.g. black ISO glasses) or under red light.

Analytical sensory tests must be conducted in facilities that prevent any visual, audio or olfactory distractions. For example, the company break room is not a good location since reminiscent odours of coffee or food could disturb wine evaluation. The environment in which sensory tests are conducted should be temperature-controlled, quiet and odour-free. Positive pressure inside the tasting room is recommended although not mandatory if the ambient air can be quickly regenerated through air cleaners or by opening windows (assuming the outdoor air is not contaminated). Controlled lighting as described by Eggert and Zook (1986) is critical in wine appearance evaluation for hue and clarity assessments in particular.

Hedonic tests are typically conducted in a central location. Central location settings should simulate a natural context of consumption as much as possible, as recent research showed that liking scores can vary substantially between a laboratory (clinical) environment and a real restaurant (King *et al.*, 2007; Petit and Sieffermann, 2007). With the increasing presence of computers in the home and the use of the Internet for domestic purposes, on-line surveys are becoming popular among consumer researchers. Most traditional sensory software packages now have web-based applications, allowing the use of sensory methods and respective experimental designs for on-line tests with or without product evaluation.

Whether their participation involves simple or complex tasks, panellists should not be the experimenters (who administer the tests). In cases in which panellists are required to pour or distribute their own samples, error can be introduced by non-uniform sample sizes and by mix-up of samples. Further, panellists may learn or speculate about the design of the experiment. Asking panellists to perform complex tasks on their own, such as initiating timing with a stopwatch, tasting a sample and turning a device on and off at specified times, distracts from their ability to concentrate on rating intensity and introduces error.

Instructions given to panellists to perform the test must be clear and non-ambiguous. Experimenters should not assume written instructions are meaningful by themselves; moreover, experienced panellists tend to partially read instructions, especially if a task looks familiar, although the procedure might have changed.

Studies must be designed to account for sequence effects in tasting wines that influence human responses. In all analytical sensory tests, samples are usually expectorated rather than being swallowed to reduce any change in response due to satiety, fatigue or, in the case of alcoholic beverages, increase in blood alcohol. For affective tests, consumers are instructed to consume wine as they would normally do, which entails swallowing the product.

First position and carry-over effects may alter sensory perceptions when evaluating wine. For example, astringency and bitterness are perceptions that can build up over repeated ingestions, leading to over-estimated intensity rating of these attributes (Noble, 2002). Use of a pectin solution (followed by thorough

Table 7.1 Psychological and physiological factors affecting sensory perceptions in wine evaluation

Bias type	Bias name	Definition	Sensory evaluation practice
Psychological	Central tendency	Panellist tends to avoid the use of scale endpoints or to choose middle sample in a choice test (e.g. triangle)	Use at least a 10-point scale and randomize order of sample presentation among panellists
	Contrast	Panellist tends to overrate the perceived intensity when a strongly intense sample follows a series of weakly intense samples	Avoid monotonous presentation of samples or balance the order of presentation for the carry-over effect
	Dumping	Panellist uses another attribute to describe one perception	Make sure the descriptor list is exhaustive or leave room for panellist to add a new descriptor
	Expectation	Information given to panellists or their anticipation about the test objective/product/method influences the response	Limit information to necessary instruction to conduct the test; make samples anonymous by blind coding them and presenting them in similar container
	Habituation	Panellist tends to repeat his/her rating when stimulus varies only slightly in intensity (e.g. in quality control)	Include in the series doctored samples to disrupt monotony of the test
	Halo	Rating of several attributes may lead panellist to rate some attributes relative to others (e.g. liking score and aroma attributes)	Set up independent tests to measure these two different sets of attributes or have panellists rate one attribute at a time without possibility to change to previous answers
	Leniency	Familiarity with the company products may lead panellist to overrate them	Include company product, competitors or doctored samples in the test set
	Logic	When panellist associates in his/her mind two or more attributes (e.g. brown white wine and oxidized aroma)	Make independent tests to evaluate each sensory modality; mask visual cues when it is a concern
	Motivation	Lack of motivation leads to lack of panellist focus and decrease in efficiency	Panel leader must keep motivation high in the group, by providing feedback on panel evaluation and by valuing their contribution within the company

	Position	The first sample evaluated is often overrated on both attribute intensity or liking	Randomize the order of presentation among panellists
	Stimulus	Panellist focuses his/her attention on one irrelevant stimulus rather than responding to his/her perception	Avoid panellist accessing information regarding the study, hide samples, limit access to preparation area
	Suggestion	Panellists are easily influenced by each other	Tasting organizer should remain neutral and should not lead the discussions; evaluation takes place in individual booth to avoid communication by facial expressions and panellists are instructed not to communicate with each other
Physiological	Adaptation	Decrease of sensitivity by repeated exposure to a stimulus of same intensity	Instruct panellist to have a break between each sample evaluation, or to rinse the mouth with water, unsalted crackers or bread

rinses with water) helps reduce bitterness or astringency of red wine (Colonna *et al.*, 2004); water rinsing and eating unsalted crackers or soft bread crumbs are also efficient strategies. Other techniques to reduce sensory fatigue and remove impact of previously tasted or smelled samples include breathing fresh air, sniffing or rinsing with water between samples or resting between samples. The specific inter-stimulus protocol varies with each product.

7.2.2 Panel

An analytical sensory panel is generally formed of eight to 20 individuals, selected from within the employee pool if the company is large enough (internal sensory panel) or from the community (external panel). Both panel types have advantages and disadvantages and the final choice will depend on the resources available in the company and the type and the volume of sensory tests needed to guide the organization's activities.

To become a member of a sensory panel, candidates are screened according to their sensory acuity, availability and motivation (Issanchou *et al.*, 1995; Meilgaard *et al.*, 2007). Further, selected candidates require training to perform sensory tasks objectively and consistently (Issanchou *et al.*, 1997), unlike instruments, although both need to be calibrated and tested for reproducibility. The objectives of training are to familiarize panellists with the tasting methods and the products under study. For novice panellists, a generic training is recommended to teach them the basis of sensory physiology and psychology, good sensory practices in the booth, the data collection system and, finally, to enlarge their sensory knowledge by evaluating a large array of products exhibiting different sensory properties. Wine professionals

working in the organization should be considered for panel membership. They have usually been trained in wine appreciation (e.g. through the Wine and Spirit Education Guild) or in sensory evaluation taught at a university or college. However, this sensory training should not excuse them from recalibrating their knowledge and sensory references prior to a study. Moreover, their wine expertise could mislead them, as shown in several experiments where experts evaluated wine using cognitive strategies rather than responding to their actual sensory perceptions (Morrot *et al.*, 2001; Castriota-Scanderbeg *et al.*, 2005). Sensory specialists are encouraged to track performance of analytical panels on a regular basis to monitor any shift in the sensory acuity or ability of the panellists (Meullenet *et al.*, 2007b).

Conducting hedonic tests requires identification of typical consumers of the product category for recruitment. Winery personnel or 'experts' should not be used unless they represent the typical consumer. Therefore, it is critical to define the targeted consumers to be recruited in terms of demographics, purchase and consumption habits based on the existing knowledge of the typical consumers of a product category, or based on the consumer targeted to become users of the new product. Because of the tremendous variation in preferences, a large number of target consumers ($n > 50$) must be recruited, especially when conducting quantitative tests. Geographical and cross-cultural differences may create different responses from consumers in product liking; therefore these factors need to be considered in the screening of potential respondents and in the selection of the location of the test.

7.3 Methods

Sensory methodologies are categorized into two main types: first, the analytical tests, which answer one of the following questions: 'Is there a difference?', 'What is the difference?' and 'How large is it?'; and second, the hedonic tests assessing consumer acceptance and overall liking. Methodologies relevant to wine evaluation in research and commercial organizations are described below. Practical considerations on how to set up and analyze these tests can be found in the resources provided at the end of this chapter.

7.3.1 Discrimination tests

Difference tests

The purpose of these tests is to determine whether two different products are indeed perceived as different by human senses. Before starting any new sensory study, informal or formal tests should be conducted to determine whether perceptible differences exist between the products under study. When the products differ by differences that are too small to be described, difference tests are undertaken, the most popular being the triangle test, where three samples are presented to the

panellists; two samples contain product A and one sample contains product B. Panellists have to determine the odd sample using the sensory modality (vision, smell or taste) instructed by the panel leader. When the difference can be defined, pair tests are used to ask which sample is higher or more intense in a specified attribute.

Difference tests can be used to determine if two wines produced under two different conditions (e.g. fermented by two different yeast strains) are perceptibly different. Other examples of applications include the comparison of sensory similarities between one product and its main competitor, or to assess wine shelf life, comparing samples stored at room temperature versus refrigerator temperature (usually 4 °C).

Threshold tests

Threshold tests are performed to determine the sensitivity of panellists to a specific compound or to estimate the compound's contribution to flavour. The threshold value is the concentration of a compound at which a detectable difference in aroma or taste is found (detection threshold) or at which the characteristic odour or taste can be recognized (recognition threshold). Typically these values are determined by difference tests, with the sets presented in increasing order of compound concentration. A compilation of threshold values is available in an ASTM publication (Stahl and Stahl, 1978).

Determination of threshold values should be used to select panellists who are sensitive to specific compounds which can cause taints in the product. The distribution of individual thresholds for one given compound can range from several ppm (mg/L) to a few ppt (ng/L). Threshold determination is indeed useful in studying factors that influence individual differences, such as age, gender or disease. In most wine quality evaluation, threshold values are of only limited use for several reasons. The value applies only to the tested compound under specific testing conditions since the threshold level varies with temperature, sample media (water, wine) and the sensory methodology and the modality by which the values were determined. Guadagni *et al.* (1968) proposed the concept of odour units, whereby the concentration of a component is expressed as the concentration divided by the threshold value. However, neither the intensity nor the nature of supra-threshold concentrations of an odorant can be predicted from the threshold value and the number of odour units present. Compounds increase in intensity at different rates and can demonstrate marked differences in quality at different concentrations. For example, at low concentrations, guaiacol, a compound extracted from oak barrels, has a smoky odour which evolves to medicinal at high concentration. The contribution of individual compounds to the flavour of a wine could only be made very approximately from the threshold value and volatiles composition.

Intensity ranking tests

Ranking tests are an extension of the pair test, whereby more than two products are presented. Ranking tests are easy to set up when the objective is to compare several

products at the same time for one given attribute. Panellists are instructed to rank products according to the intensity of a given attribute. Undertaking this test requires the panellists to solicit their short-term memory and make multiple paired comparisons between samples; therefore, sensory practitioners usually recommend limiting the number of samples to seven to avoid sensory fatigue. Ties may be authorized if panellists cannot differentiate two samples or they are instructed to give their best estimate (Meilgaard *et al.*, 2007).

Intensity rating tests

To measure the size and nature of differences in wine sensory properties, the intensity of a specific attribute or attributes is rated by trained panellists. There are several different scaling procedures and types of scales. Category scales or unstructured line (graphic) scales, which are anchored at the ends by 'none' or 'low intensity' and 'high intensity', are used most frequently. Another method is magnitude estimation (ratio scaling) in which the intensity of a sensation is rated relative to the intensity of a reference. For example, if the reference is defined as an intensity of 10 and the sample is twice as intense, it is rated 20. Magnitude matching (or cross-modal matching) is a variation in which the intensity of tastes, smells or mouthfeel is rated relative to a standard sound or light (Marks *et al.*, 1988). The labelled magnitude scale (LMS), which is a combination of the ratio and category scales, was developed and proposed by Green *et al.* (1993) to measure chemosensory perceptions. The LMS is anchored by 'barely detectable' and 'strongest you can imagine'. An extension has been proposed, the general labelled magnitude scale (Bartoshuk *et al.*, 2004); this has mainly been applied to psychophysics studies where sensory properties imparted by specific compounds are studied among different individuals. A few wine studies have applied the LMS, especially to characterize the perception of astringency (e.g. Gawel *et al.*, 2001; Pickering *et al.*, 2004).

For most studies in which the sensory properties of wine are being measured, the unstructured line scales or category scales are ideal. For a normal range of intensities, the same results are obtained using ratio scaling as found with category or graphic scales, but the latter are simpler to use than magnitude estimation methods (Giovanni and Pangborn, 1983).

7.3.2 Descriptive analysis: conventional profiling methods

The technique of descriptive analysis (DA) provides a quantitative analytical characterization of appearance, aroma, taste and mouthfeel as described in detail elsewhere (Stone *et al.*, 1974; Einstein, 1991; Hootman, 1992; Heymann *et al.*, 1993; Lawless and Heymann, 1998). The first requirement for a meaningful DA is the development of a vocabulary that will describe the differences in all the sensory modalities among the samples in specific concrete terms. Several lexicons have been developed on specific products (Shortread *et al.*, 1979; Meilgaard *et al.*, 1982; Pagliarini *et al.*, 1991; Berodier *et al.*, 1997) including wine (Noble *et al.*, 1987). For each DA study, terms are derived that describe the differences in

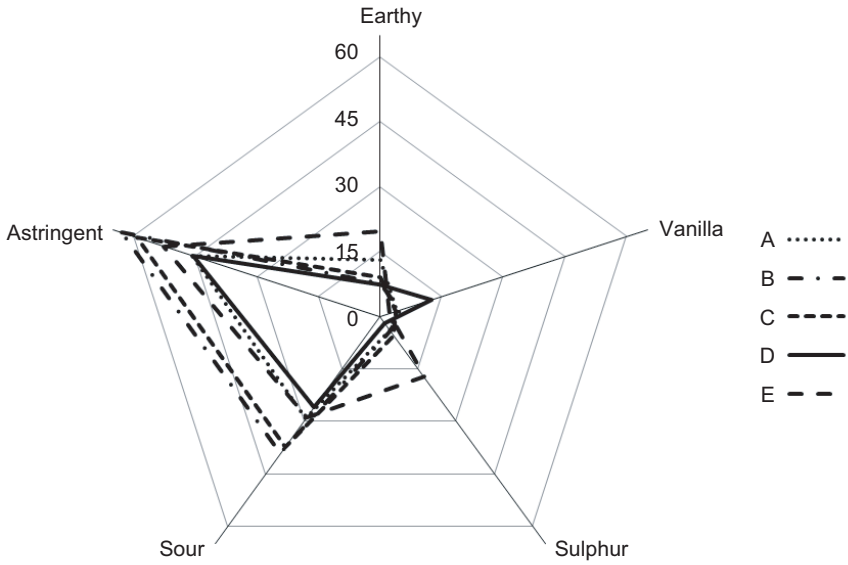

Fig. 7.1 Spider-plot representation of the flavour attributes perceived significantly different ($p < 0.05$) between five commercial Merlot wines.

sensory attributes of the experimental samples. Reference standards are provided to define each term and train judges to rate each attribute consistently (for wine see: Noble *et al.*, 1987; Gawel *et al.*, 2000; Pickering and Demiglio, 2008). Training time varies in the literature from ten (QDA® method, Tragun Corporation, Stone *et al.*, 1974) to 150 hours (Spectrum™ method, sensory spectrum described in Meilgaard *et al.*, 2007). The feedback calibration method (FCM) was proposed to accelerate the training phase and alignment of panel vocabulary (Findlay *et al.*, 2006); a comparative study of training on wine using conventional DA and DA with FCM showed that training time was reduced by 50% with the latter. Alternative methods to DA have also been proposed and will be discussed in the next section.

After the judges are trained, the intensity of each term is rated in each product according to a specific experimental design. Plotting the ratings from the descriptive analysis data reveals the sensory profiles of the samples as shown in the two following examples using two different graphical representations. Five commercial Merlot wines were profiled by a panel of eight trained panellists using 46 flavour attributes (Lesschaeve, 2003). The wines were perceived significantly different at 5% for five attributes: earthy, vanilla, sulphur, sour and astringent (Fig. 7.1). Wines B and C were more sour and astringent. Wine E was distinct by its earthy and sulphur notes while wine D was more intense in vanilla.

DA has permitted elucidation of specific viticultural terroirs based on the sensory properties expressed in the wines produced in specific areas (Fischer *et al.*, 1999; Fischer and Bauer, 2006; Willwerth *et al.*, 2008a,b). Ten Riesling vineyards located in ten different sub-appellations in the Niagara Peninsula were studied for

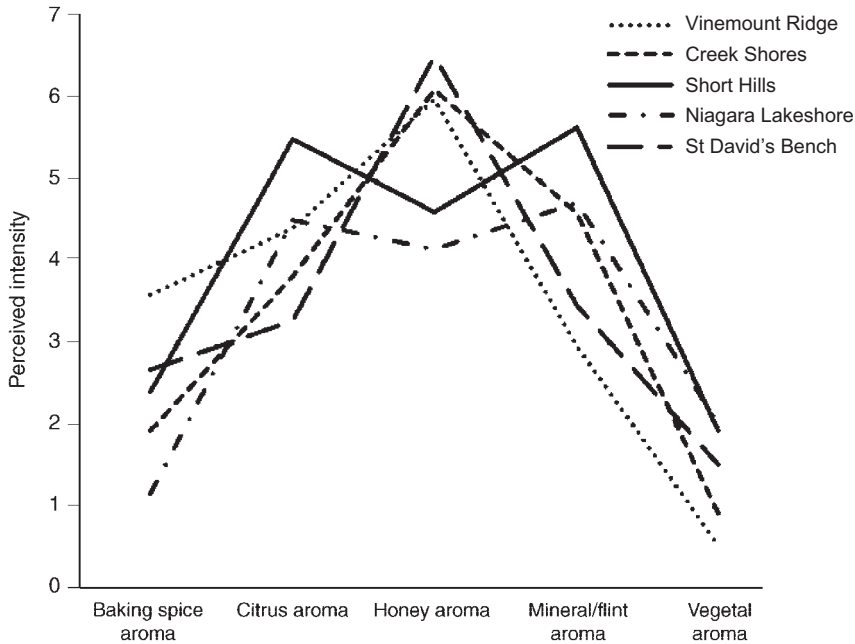

Fig. 7.2 Aroma attributes perceived significantly different ($p < 0.05$) in Riesling wines produced in five different sub-appellations.

their terroir in relation to wine quality. Descriptive analysis using a trained panel indicated that sub-appellations did indeed have an effect on wine sensory profiles (Willwerth *et al.*, 2008b). The differences perceived on aroma attributes between five of the experimental wines are displayed in Fig. 7.2. The Short Hills sub-appellation imparted more intense citrus and mineral aromas in the wine. Vinemount Ridge was characterized by baking spice aroma and the absence of vegetal notes; St David's Bench was more intense in honey and less intense in citrus than Short Hills, while Niagara Lakeshore and Short Hills sub-appellations were more intense in vegetal aroma. Ten Cabernet Franc vineyards located in the same Niagara sub-appellations were also studied for their chemical and sensory characteristics. The ten sites were found to produce wines with significantly different sensory profiles, which were interpreted relative to their proximity to the Lake Ontario (Hakimi Rezaei and Reynolds, in press). These studies therefore supported the concept of terroir in new wine producing regions.

7.3.3 Alternatives to descriptive analysis

Several methods have been proposed to characterize product perceptual differences, while reducing the training and data collection time.

Free-choice profiling

Free-choice profiling allows each panellist to use his/her own descriptors to define

product differences. The term generation therefore does not require alignment between panellists and is conducted in few sessions depending on the total number of products to profile. The nature of the data requires the use of multivariate statistical analysis and results are sometimes very difficult to interpret due to the idiosyncrasy of the descriptors (Williams and Langron, 1984; Williams and Arnold, 1985; Beal and Mottram, 1993).

Flash profiling

The flash profiling method was proposed to provide a comparative positioning of products based on their sensory properties (Dairou and Sieffermann, 2002; Delarue and Sieffermann, 2004). It combines the free-choice profiling concept and a comparative assessment of the products (all the products are presented at the same time) instead of the sequential monadic presentation traditionally utilized in DA (samples are presented one by one). The original idea was to develop a method that would provide a sensory map faster than the traditional descriptive analysis method. The authors therefore recommend using panellists trained in the description and quantification of their sensory perceptions. Data are analyzed using multivariate statistical techniques such as Procrustes analysis (Gower, 1975) or STATIS (Schlich, 1996), taking into account individual assessments to produce a consensus map describing the perceived sensory differences between the products.

Perrin *et al.* (2008) compared the sensory maps of ten wines from the Loire Valley, using three descriptive methods: conventional profiling or DA, flash profiling and Napping®. Although the results from the three methods were not identical, they led to similar representations of perceived differences between the wines. DA results were more precise; the authors suggested using Napping® in combination with flash profiling as a preliminary step in wine characterization or as a tool to screen wines before blending.

Non-verbal methods

Non-verbal techniques (e.g. sorting tasks and projective mapping) have been used to assess perceptual differences and similarities between products. These methods provide maps positioning products relative to each other, based on the overall sensory perceptions of panellists. These techniques are specially valued at a preliminary stage of a study or when a panel in highly trained DA is not available, or when the information provided is sufficient for the study objectives.

Sorting tasks

Sorting task methods have been very popular in psychology since the 1970s (e.g. Healy and Miller, 1970; Coxon, 1999) and were introduced in sensory science in the 1990s (MacRae *et al.*, 1990, 1992; Lawless *et al.*, 1995; Chrea *et al.*, 2005). It is a simple method both for the panellists to execute and for the experimenter to design and analyze. Panellists are presented with a set of samples and are asked to sort those samples based on similar perceived sensory

characteristics using one modality (either visual, smell or taste). They use their own criteria to group samples. Psychologists describe this method as a categorization task where subjects group objects according to similar features or similarity to a prototypic representation (e.g. grouping odours belonging to the Citrus family). No specific training is required for the panellists. The workload is less demanding than in a conventional descriptive analysis and usually only one session is needed to obtain a perceptual map. However, only ten to 20 samples can be reasonably sorted at once to avoid sensory fatigue or adaptation (Schifferstein, 1996). A similarity or dissimilarity matrix is drawn from the occurrence of two products being placed in a same group or in different groups. The matrix is subjected to a multivariate statistical analysis called 'multidimensional scaling' (Schiffman *et al.*, 1981); other methods input individual matrices to keep panellist contributions in the calculation of inter-product distances and the perceptual map building (e.g. generalized Procrustes analysis – Gower, 1975; Di-Statis – Abdi *et al.*, 2007).

Although this sensory technique is in essence non-verbal, some authors have added a descriptive task once the groups are formed and asked panellists to describe the sensory characteristics of each group they made. Frequencies of common descriptors are used to project the labels on the perceptual map, which are used to interpret the sensory dimensions and product positioning (Cartier *et al.*, 2006; Blancher *et al.*, 2007). Calculating frequencies of common descriptors assume that every panellist similarly labelled their perceptions, which is only the case for trained panellists.

Wine studies using sorting tasks have focused on the comparison between wine experts and naïve consumers' representation of wine quality (Chollet and Valentin 2000; Valentin *et al.*, 2003; Valentin, 2007; Ballester *et al.*, 2008). Recently, new applications of the sorting task were proposed to define the sensory dimensions of varietal typicity (Ballester *et al.*, 2005; Campo *et al.*, 2008), or of wine non-sensory properties (Chrea *et al.*, 2008). Sorting tasks could also be valuable in business operations, for example to assign wines to different brand tiers based only on sensory characteristics, or to position the company's wines relative to its competition.

Projective mapping – Napping®

Projective mapping was first introduced by Risvik *et al.* (1994) and recently revisited by Pagès (2005), who applied this method mainly to characterize sensory properties of wines from the Loire Valley. Their assumption was that projective techniques were suitable for wine professionals who do not have a lot of time to embark on a full descriptive study but who could use their tasting experience to position wines relative to each other (Perrin *et al.*, 2008). The method is again simple to implement but requires the use of multivariate statistics to analyze the data. Panellists are presented with the samples and are asked to position them in a two-dimensional space based on their perceived similarities. For each wine, both the X co-ordinate and Y co-ordinate are recorded and compiled in a data set, which is submitted to a multivariate statistical analysis, such as multiple factor analysis,

taking into account individual contributions of panellists to build the consensus map.[1] As for the sorting task, some authors requested panellists to describe the groups of products they formed to interpret the sensory dimensions of the consensus perceptual map.

7.3.4 Time–intensity measurement

Temporal procedures are used to characterize persistent sensations, such as astringency and bitterness, and to monitor perceived intensity over time as flavour is released during ingestion and mastication. As the concentration of a single compound is increased, maximum intensity and total duration increase, whereas only small differences in time to maximum intensity occur. When the structure or composition of a system is altered, the rates of release of tastants or odorants are affected, as well as the maximum intensities. Examining the rate of onset and rate of decay of intensity can be used to model perception of taste and mouthfeel (Pfeiffer *et al.*, 2000) and suggest explanations for mechanisms of perception (Linforth *et al.*, 1999).

Temporal sensations in wine can affect quality judgment. Noble (2002) showed that perceived astringency increased with repeated red wine sips, stressing the importance of rinsing thoroughly during red wine evaluation, since repeated ingestion could bias the mouthfeel assessment of a red wine. In addition, Michon and Lesschaeve (2001) showed that preferences for red wine changed when consumers rated their liking after just one sip or after four sips, and was related to temporal perceptions of astringency in particular.

A new method called temporal dominance of sensations (TDS) has been introduced by Schlich and collaborators (Pessina *et al.*, 2004; Pineau *et al.*, 2004) as a tool to record the evolution of panellists' perception of dominant sensation. This method was thought to be particularly suited for wine assessment as the sensory profile of a wine evolves in time, while being evaluated in mouth (Schlich, 2004). In TDS, terms are selected to describe flavours perceived post-ingestion of foods and beverages and panellists are trained to use the method and the descriptive terms. The questionnaire is computerized to allow the accurate tracking of the panellists' evaluations. After swallowing the sample, panellists select the dominant sensation and rate its intensity for five minutes. Whenever a different sensation predominates, it is chosen and its intensity is rated. The TDS score is calculated from the intensity and duration of each attribute (Pineau *et al.*, 2004). TDS data can also be represented by curves showing the percentage of panellists for each product who selected the attribute as dominant at a specific time. Applied on gum flavour evaluation, Labbé *et al.* (2009) indicated that results obtained with TDS captured dynamics of perception that a conventional profile could not. Studies to correlate TDS curves and wine composition (Pessina *et al.*, 2004;

[1]Multiple factorial analysis (MFA), STATIS and generalized Procrustes analysis (GPA) have in common the integration in their algorithm of the individual data to calculate inter-product distances and build a consensus map, in contrast to multidimensional scaling (MDS) which averages individual contributions before building the map.

Meillon *et al.*, 2008) demonstrated the gain of information from this sensory method compared to conventional descriptive analysis.

Rating intensity continuously (TI) or choosing the dominant sensation over time (TDS) are difficult sensory tasks that require more training than do simple rating tests. The way in which panellists move their tongues and their salivary flow rates can influence their perception of intensity and persistence of the sensation. Even with extensive training in TI, each panellist has characteristic curves with idiosyncratic, yet reproducible, patterns. Despite the difference in temporal patterns, panellists can be trained to give reproducible responses that are consistent across samples. For further considerations of statistical analyses of TI and TDS data, see Dijksterhuis and Piggott (2001), Pineau *et al.* (2004) and Labbé *et al.* (2009).

7.3.5 Quality control tests

Several sensory techniques can be used in quality control (QC) or quality assurance (QA) programs, as reviewed in detail in Muñoz *et al.* (1992), but also Yantis (1992) and Carpenter *et al.* (2000). They are adapted from the discriminative tests and intensity scaling methods described in previous sections to comply with production volume and setting requirements. The QC manual developed for evaluating corks for taint describes the procedures and guidelines for making accept or reject decisions (Butzke and Suprenant, 1998). Recently, Prescott *et al.* (2005) proposed determination of consumer rejection thresholds as a quality control method for off-flavour monitoring, such as cork taint in wine. They applied this method on 2, 4, 6-trichloroanisole (TCA) and more recently on the taint imparted by *Brettanomyces* in wines to demonstrate that the consumer was on average more tolerant and less sensitive to taint than an expert. Indeed, consumers rejected wines at a higher taint level than its detection threshold. Sometimes a 'no tolerance attitude' is not commercially viable. Knowing at what taint concentration a wine is still acceptable for consumers has significant economic impacts in the search of remedial treatments when tainted wines are detected. This does not mean that one should encourage putting faulty wines on the market! Rather it is to remind the readers that sensitivity to aroma compounds varies greatly between individuals; what an expert may reject could still be pleasant and acceptable to other consumers. This is not unique to the wine sector; many quality assurance programs rely on consumer inputs to define product specifications in terms of sensory or flavour profiles (Civille, 1991; Muñoz, 2002).

Other methods, such as the 'in/out' method, are decision-making tools for evaluating daily production, for example on the bottling line. On-line judgments are made as to whether a product is within or outside the product specifications. The disadvantage of this approach is the lack of information provided and the difficulty in defining the specification limits. 'Difference from control' tests (degree of difference tests) rate the size of the difference of a production sample from a 'control' or 'standard' one. Category scales labelled with terms describing the degree of difference (none, very slight, slight, moderate, large, extreme) or

unstructured line scales anchored by 'no difference' and 'extreme difference' can be used. Although this method is simple, the overall rating for the magnitude of difference from the standard product provides no information about the nature of the differences. The other challenge is to be able to access a control sample consistently. This is particularly important when the test objective is to determine the shelf-life of a product, which occurs when new packaging is tested or when the producer wants to preserve a fresh and fruity profile in his/her wines than can be altered with time (e.g. in rosé wines). Recommendations on how to set up a shelf-life study are available in ASTM (2004).

Assuring quality and product integrity to the end consumer is essential in any business. QA and QC operations within a winery tend to be limited to the instrumental analysis of wine components, the sensory integrity of the product being analyzed by the winemaker or wine-making team. The use of sensory techniques could, however, be useful to address customer complaints for example. If, for example, wine is shipped abroad and suffers from poor storage, comparing the sensory specifications of the shipped wine with the specification target using difference tests or difference to the control method could demonstrate that the sensory properties were significantly altered by the shipment and were not present at the winery. A guide developed by the ASTM describes sensory methods to be used in the case of claim substantiation, which could be useful to argue major customer complaint (ASTM, 2007).

7.3.6 Consumer tests

Purpose of consumer tests

Consumer tests or affective tests are conducted primarily to determine how much a product is liked by a targeted population of consumers, usually in blind tasting condition where the objective is to characterize sensory preferences independently from any marketing inputs. However, the latter does affect consumer food choice by creating expectations about the product sensory quality and other potential benefits (Lesschaeve, 2008). Consumer tests are conducted for product improvement/optimization, development of new products, assessment of market potential, product category review and support for advertising claims (Meilgaard *et al.*, 2007).

Methods

Testing alcoholic beverages using respondents recruited from the public entails liability issues that one needs to be aware of. The authorized blood alcohol content varies between jurisdictions so the test organizers need to be aware of this threshold to calculate the volume of alcohol and therefore the amount of wine consumers can drink in a test setting. This number affects the design of the whole experiment. The standard guide developed by the ASTM (ASTM, 2000) provides useful information to set up consumer tests, manage the alcohol intake and handle consumer transportation and on-site participation.

The methodologies used for consumer tests can be categorized into qualitative and quantitative affective tests. Qualitative methods are frequently used at the fuzzy front end of the new product development to test a new product concept with a small group of consumers. Focus groups are quite popular and some companies tend to use them as soon as they require consumer insights to solve an issue that may have happened on the market. Six to eight consumers usually take part in the discussion, which lasts on average 90 minutes. They interact with a neutral moderator who follows a script developed to record consumer opinions about the particular issue under investigation (Kanetkar, 2000). A setting including product tasting (blind or labelled) can be organized to elicit consumer response to the sensory or non-sensory cues presented by the samples. The information retrieved is qualitative, i.e. based on few individuals who do not represent the whole targeted population and the data collected are mainly in the form of recorded comments and not quantifiable. Focus groups can, however, be useful to identify factors that influence the consumer's preference (McNeill *et al.*, 2000), although several authors have argued about their relevance in providing objective directions for product development in terms of sensory/flavour attributes (Lawless and Heymann, 1998; Lesschaeve *et al.*, 2002). In many industries, decisions are indeed made based upon the managers' intuition while observing the focus group's 'consumer speak'.

Other techniques have been suggested to elicit consumer responses more objectively. The repertory grid methodology (RGM – Thomson and McEwan, 1988) is used to determine the attributes (e.g. flavour, colour) or features (e.g. easy opening packaging) of products that seem important to consumers. This method has been used mainly to study non-sensory factors that may affect consumers' perception of wine quality (Rocchi and Stefani, 2005; Lesschaeve, 2007a).

Sensory preference for a product is most frequently determined with pair preference tests between two samples; for several samples, products can be ranked in order of increasing preference. Liking is usually rated on hedonic category scales anchored by terms such as 'dislike extremely' to 'like extremely'. The 9-point hedonic scale developed in 1957 is still widely used by market researchers in North America (Peryam and Pilgrim, 1957). However, the category labels of the scale seem to translate inappropriately in certain languages, including Spanish and Chinese (Yeh *et al.*, 1998; Curia *et al.*, 2001). Sensory scientists tend to favour unstructured linear scales or labelled magnitude scales for their ratio properties.

It is common practice in market research to ask respondents other questions in addition to the hedonic rating, for example what they liked or disliked in the tested products. Lesschaeve (2006b) showed that using attributes generated by consumers to guide product development can be misleading. The term 'vanilla/oak' tended to be used to describe liking for wine whereas 'smoky/oak' tended to reflect an unpleasant sensory experience. For more information on consumer testing, consult Meilgaard *et al.* (2007).

Wine producers tend to rely on internal or external experts to predict consumers' likes of their wine production (Lesschaeve, 2007b). However, wine experts and naïve consumers do not characterize wines similarly (Parr *et al.*, 2002;

Valentin *et al.*, 2003; Ballester *et al.*, 2008), nor do they like the same wine profiles (Williams *et al.*, 1982; Lesschaeve, 2003, 2007b). Therefore, understanding and targeting consumer wine preference require a different set of approaches, which were introduced in the food industry in the late 1990s from the collaboration between sensory and market researchers (Lesschaeve *et al.*, 2002; Lesschaeve, 2007b). In particular, these approaches led to the increasing use of preference mapping techniques (Greenhoff *et al.*, 1994; McEwan, 1996; Meullenet *et al.*, 2007a) to determine the sensory attributes driving consumer preferences for particular wine styles; in these cases, descriptive data are collected from a trained panel and liking data from wine consumers.

Only a few wine studies have been published related to wine preferences. Among six conducted in North America, Lesschaeve (2008) examined potential underlying dimensions that could explain consumer preferences for specific wine aromas, although the objectives of these studies were different, the sensory variability among the wines was different and the consumer population were recruited from various backgrounds and geographical origins. In the case of two Chardonnay studies conducted in the USA (Yegge and Noble, 2001; Lesschaeve *et al.*, 2002), the fruity, spicy and vanilla/oak aromas were common drivers of liking, modulated across consumer segments as the optimal intensity for these attributes might differ between segments. For the two red wine studies, one conducted in the USA and one in Canada, the only common underlying dimension was vanilla/oak (Frøst and Noble, 2002; Lesschaeve, 2003). Considering the discrepancies in the experimental designs used for these studies, it is interesting to note that vanilla/oak is identified as a driver of liking for both red and white wines. As already mentioned, vanilla/oak tended to be used to describe liking for wine whereas smoky/oak tended to reflect an unpleasant sensory experience for consumers (Lesschaeve, 2006b).

7.4 Integration of sensory evaluation techniques in wine businesses

The huge potential provided by sensory evaluation techniques to wine businesses has been described and discussed through various forums (Lesschaeve, 2000, 2001, 2006a, 2007b; Noble, 2000). This section will elaborate further on the contributions that sensory and consumer data could make to major winery business decisions. The most frequent criticism voiced against this concept has argued that sensory evaluation techniques are geared towards large operations which aim at standardizing wine styles globally, reinforcing the differences in philosophy and practices existing between the traditional wine countries and the new wine-producing countries. This criticism applies especially to the use of preference mapping to identify and target consumer sensory preferences. Although these techniques provide detailed description of the wine style best liked by a consumer segment, this wine style can be interpreted differently by winemakers, depending on the tools they have at hand and without compromising their creativity. Not

every winery can compete on the mass market where such tools have proven to be efficient. However, the knowledge of which wine styles appeal to different consumer segments offers a significant competitive advantage, for example by focusing the marketing efforts on those consumers. This is valid whatever the winery size.

7.4.1 Setting up a sensory evaluation program with limited resources

When people realize the benefits of sensory evaluation, they are stopped by practical considerations: where does one find panellists when one has a three-person winery? Where does one set up the test when one cannot afford a fancy computerized tasting room? This section provides examples and suggestions for utilizing sensory techniques in small operations while preserving the identity of the winery. The key components for implementing a successful program in commercial operations are summarized in Table 7.2.

Chacon-Rodriguez *et al.* (2001) described the evaluation of experimental wines using sensory techniques where panellists were employees of the winery who received a five-day instruction course on sensory evaluation basics and were trained in discriminative and descriptive analysis. Other alternatives exist when relying on winery staff is not an option. Visitors to the winery boutique are a great and often under-estimated resource that is under-utilized by winery owners. They could participate in sensory tests when no particular training is required (e.g. difference tests or sorting tasks) or in hedonic tests where one wants to test a wine against a competitor (preference test) or seeking a hedonic rating for several wines. Setting tests in such an environment requires some creativity to minimize the psychological biases that could affect perceptions and overall liking. However, it is possible, whether a small table is set up in one quiet corner of the boutique, or in a separate room if available. A free glass of wine or entry into a draw to win a few bottles of wine could suffice as incentives and lead to collection of 20–40 responses over a weekend.

When more resources can be dedicated to sensory evaluation of wines, wineries can consider hiring sensory consultants who could advise on the implementation of sensory tests either at the winery or in a different location. Services that a sensory consultant can offer are well described by Howe (2001), and several established consulting firms have developed some expertise in the wine sector.

Another resource that should be considered is the oenology and viticulture department at the closest university or wine research institute. Sensory scientists on the staff could act as consultants or could offer to address a company's sensory challenges through a short-term research project that a student could undertake. The main advantage of the latter is its low cost compared to high consultant fees while contributing to the training of future industry members. Checking credentials of the sensory consultants is recommended: the lack of formal sensory training in North American post-secondary establishments has led to the self-proclamation of sensory experts who do not have the depth of expertise and experience that a sensory scientist trained in a renowned institution might have.

7.4.2 Making sound business decisions

Sensory data to be used for making business decisions can only be meaningful if the sensory evaluation unit (whether it is internal or outsourced) is informed about the global purpose of the test and integrated with other teams at the management level. With this integrated approach, the experiment to address a particular problem can be designed to provide the optimal information and permit the sensory group to make practical recommendations based on the test results. Below, we give two examples of the need for interpreting sensory data in an integrated context.

Significance of results

What level of significance should be used in interpreting the results? This depends on the importance of the attribute considered. A change in a wine-making practice, for example, the use of different yeasts, modifies the wine aromatic profile. Pair tests or rating tests using trained panellists show that a difference in passion fruit is perceptible at a 5% significance level. This means there is a probability of 5% ($p < 0.05$) that the results could have occurred by chance alone or conversely we are 95% confident that there is a difference. Is this difference large enough to justify the cost of purchasing the new yeast?

In contrast to this scenario, if the testing is to determine if a defect or negative characteristic is detectable, a probability of 5% indicates a difference that is of concern. If a very small number of regular consumers can detect this off-note, then the product will be rejected and the winery will lose market share. In this situation, it is important that the test has high statistical power. Power is the probability that a real difference is found or that we did not fail to find a real difference. Power is determined by three factors: the number of panellists and replications, the magnitude of the differences among the samples (effect size) and the level of confidence (or alpha) that is chosen. Power can be increased by using more panellists and conducting more replications. Trained, sensitive panellists can perceive very small differences and are more reproducible, thus increasing power over untrained panellists. Testing of power is seldom done, but it should be performed when decisions are made based on the sensory tests. For more information about power, see Cohen (1988), Lipsey (1990), Schlich (1993) and Lawless and Heymann (1998).

Relevance of analytical results to consumer perception

Usually, the very small differences that are detected by analytical panellists will not be perceived by consumers during normal consumption or use. However, if the attribute is important or relevant to the regular consumer of the product, even a small difference, for example at $p < 0.05$, may be detected by consumers under normal conditions of consumption. Paradoxically, consumers who could not detect differences between two waters demonstrated a significant preference for one of them (MacRae and Falahee, 1995). A similar result has been found using authenticity tests. Consumers who could not discriminate among milks (Wolf Frandsen *et al.*, 2003) or beers (Köster, 2009) were able to identify the brand which they usually consumed when it was presented with other samples. A sensory

Table 7.2 Key components in implementing a sensory program in commercial wine operations (Lesschaeve, 2007b); with permission to reproduce from AJEV

Component	Generic requirements[a]	Cost-effective implementation for occasional sensory activities	Optimum implementation for regular sensory activities
Tasting room	Quiet environment: free from noise, odour or visual disturbances	Conference room away from production plant	Dedicated sensory lab with preparation lab
	Individual tasting booth	Individual tables set up to minimize panellist interactions or temporary booth with odour-free cardboard dividers	Permanent booth with actual dividers and light control features
Data collection	Questionnaire featuring test instructions and scorecard to record individual assessments	Paper questionnaire	Computerized questionnaire from sensory software
Panel	Recruitment[b]	Internal panel	Preferably an external panel
	Screening	Basic screening of volunteers on basic tastes and wine faults and wine typical aroma recognition, availability and motivation (Chacon-Rodriguez *et al.*, 2001)	Two- to three-step screening of volunteers recruited outside of winery; screening based on sensory acuity, odour description ability, olfactory memory, motivation, interests and long-term availability (Issanchou *et al.*, 1995)
	Training	Short training session on the methodology (Chacon-Rodriguez *et al.*, 2000)	Comprehensive training (Meilgaard *et al.*, 1987) or a basic training (Lesschaeve, 2002)
	Control of panel performance	Occasional on some duplicated sample	Ongoing with reference identification,

		assessment	duplicate sample assessments
Methods	Sensory tests	Difference tests; ranking tests; sorting tasks; descriptive analysis on limited number of pre-defined descriptors	Difference tests; ranking tests; sorting tasks; descriptive analysis of the full sensory profile
	Analysis of sensory data	Purchase an inexpensive statistical package to perform univariate (ANOVA) and multivariate (PCA) analysis	Acquire packages to run predictive modeling or outsource sophisticated analysis or hire a statistician
Sensory personnel[b]	Laboratory technician to prepare samples. Sensory specialist to plan, conduct and analyze sensory tests	Often the same person assumes both roles. Hire students with at least two courses in sensory evaluation to conduct short-term projects or to implement the program and write standard operating procedures	Hire a consultant to implement a program and train on-site staff or hire a trained sensory specialist with a food science or an oenology and viticulture degree and a technician with a college degree in laboratory techniques

[a]Detailed information found in Lawless and Heymann (1998), Meilgaard *et al.* (1987).
[b]Detailed information in Issanchou and Lesschaeve (1993).
Source: Lesschaeve (2007b); with permission to reproduce from AJEV.

method for determining how big a difference must be for it to be meaningful (i.e. perceptible to a consumer) needs to be developed.

7.5 Conclusions and future trends

This chapter reviewed current methodologies and best practices to successfully implement sensory techniques within winery operations, whether they aim at characterizing wine sensory properties, ensuring wine production quality or developing new wine styles according to consumer flavour preferences.

Although significant progress has been made, the use of sensory evaluation remains the prerogative of large wineries. Several options have been proposed in this chapter to demonstrate how small operations could adopt good sensory practices to rely on objective data rather than personal opinions for R&D, quality control, product and market development. New sensory methodological developments are needed to assist wineries in more effectively implementing an internal sensory program. The main constraints are panel accessibility and training, and knowledge in sensory experiment designs. There are opportunities to develop simple methods and tutorials for implementing good sensory practices in a winery or to provide *ad hoc* services supplied by sensory evaluation providers. However, the easiest step to demystify sensory evaluation for small operations is to train the future generation of viticulture, oenologists and marketers to integrate good sensory practices into their trade rather than presenting sensory evaluation techniques as research tools which are somehow irrelevant to daily winery operations.

Capturing consumers' minds and attitudes towards wine will always be a challenge considering the many sensory and non-sensory factors that could affect consumer purchase behaviours. However, new wine introduction, whether it is an extension of an existing line, the development of a new wine style or the use of new packaging, should be consumer driven to be successful. Research efforts should therefore continue and focus on the identification of the key factors affecting consumer behaviours and the development of rapid methods to predict the acceptance of a new product. The conjoint development of new wine styles and marketing concept is critical to ensuring that consumer expectations created by the information on the label are not mismatched by the sensory experience of consuming the wine. The integration of sensory and market research is critical to ensuring the production of wine styles according to both consumer flavour preferences and socio-demographic segments. Particular attention is now given to Generation Y–Millenials, who are 19 to 34 years old and starting to enter the wine market, and for which new investigations techniques are required considering their high reliance on on-line information and use of social networking as main communication tools.

7.6 Sources of further information and advice

Excellent references for standard sensory methods include Carpenter *et al.* (2000), Lawless and Heymann (1998), Stone and Sidel (1985) and Meilgaard *et al.* (2007).

The sensory community shares several internet forums, including the sensory nexus (www.sensory.org). Several professional groups have formed, the more recent being the Society of Sensory Professionals (www.sensorysociety.org). The Sensory Evaluation Division of the Institute of Food Technologists (www.ift.org) and the European Sensory Network (www.esn.org) are also excellent resources to consult.

Sensory scientific journals reporting wine related studies include: *Food Quality and Preference*, *Journal of Sensory Studies*, the *American Journal of Enology and Viticulture*, *The Australian Journal of Grape and Wine Research*, the *Journal of Wine Research* and the *International Journal of Wine Business Research.*

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8

Wine authenticity, traceability and safety monitoring

I. S. Arvanitoyannis, University of Thessaly, Greece

Abstract: This chapter deals with authenticity of wine, this issue has been extensively investigated because wine is an easily adulterated product, due to its strong chemical basis (high alcohol content, low pH) and its availability throughout the world. Therefore, reliable and occasionally complicated techniques have to be applied in conjunction with multivariate analysis in order to ensure wine authenticity. Most of the classical (HPLC, GC–MS) and novel (FT–IR, NMR, AAS, DNA-based, PCR) techniques applied in wine authenticity are thoroughly reviewed in this chapter. The importance of ISO22000 is also highlighted and an application of it is presented.

Key words: authenticity, adulteration, traceability, ISO 22000:2005, ISO 22005, multivariate analysis, wine analytical methods.

8.1 Introduction to wine authenticity

The use of authenticity as a positioning device resonates with consumers of goods and services. Managing consumer perceptions of authenticity is critical because what research reveals as authentic must conform to consumers' mental image of how things 'ought to be' (Grayson and Martinec, 2004). Protecting consumers against being sold an inferior product with a false description and, in addition, defending honest traders from unfair competition are crucial issues in food quality control. In this way, wine, as all foods, must be verified as complying with its label description (Tesfaye *et al.*, 2002). Authenticity of foods and, in particular, of wine has been extensively investigated because wine is an easily adulterated product due to its strong chemical basis (high alcohol content, low pH) and its availability throughout the world (Medina, 1996).

Meticulous and continuous controls are required to maintain the quality of wine. The authenticity is guaranteed by strict guidelines laid down by the responsible national authorities (e.g. Institut National des Appéllations d' Origine in France), which include official sensory evaluation, chemical analyses, and examination of the register kept by the wine producer. Wine mobility in bulk containers within the European Community is also carefully controlled, requiring transport documents that certify authenticity as defined by the EC directive 986/89. There is currently a great range of combined techniques employing group classification to identify for wine's authenticity. The introduction of new sophisticated techniques in conjunction with great consumer demands and expectation for safer products gives a tremendous impetus to food quality assurance. Wine adulteration, mainly in terms of varieties and regions of origin (geographical), has been very widespread (Arvanitoyannis *et al.*, 1999). Therefore, apart from novel experimental techniques [gas chromatography–mass spectrometry (GC–MS), inductively-coupled plasma (ICP)–MS, ^{13}C nuclear magnetic resonance (NMR), Fourier transform infrared (FT–IR) spectroscopy and DNA among others], the need has emerged for more comprehensive statistical data analysis. Multivariate analysis comprising principal component analysis (PCA), discriminant analysis (DA), canonical analysis (CA) and cluster analysis (CLA) has, in most cases, been effectively employed in wine differentiation and classification according to geographical origin (Arvanitoyannis *et al.*, 1999; Arvanitoyannis, 2003).

8.2 Classical and novel methods for testing wine authenticity

Various methods have developed over the years aimed at detecting wine authenticity. The classification of these methods into old and novel or 'state of the art' is usually made for grouping purposes. One could classify as old methods the ones employed prior to the advent of the new generation of methods.

Older methods include: determination of total soluble solids with hydrometry and/or refractometry; high-performance liquid chromatography (HPLC) of glycerol and ethanol; determination of ethanol by ebulliometric analysis, hydrometry, GC or enzymatic analysis [employment of nicotinamide-adenine dinucleotide (NAD)]; determination of organic acid concentration by measuring the titratable acidity [titration of acids (tartaric, malic, citric, lactic, acetic) with base] or spectrophotometrically (in the UV region); determination of volatile acidity with steam distillation and titration or enzymatically (monitoring changes in NADH concentration); determination of reducing sugars by chemical (reaction of reducing sugars with copper; Joslyn, 1950) or enzymatic means (McCloskey, 1978); and determination of phenols by the Folin–Ciocalteau method (Zoecklein *et al.*, 1994). Good results have been achieved in terms of authentication of commercial samples using different analytical variables as follows: titratable acidity, total extract, ash content, glycerol, alcohol and sulphates (Guerrero *et al.*, 1994), as well as eight mineral elements (As, Ca, Cu, Fe, K, Mg, Mn and Zn) (Guerrero *et al.*, 1997).

More modern analytical techniques include the determination of the various

phenolic compounds (non-flavonoid, volatile phenols, flavonoids, catechins, leucoanthocyanidins, flavonols, tannins, anthocyanins/anthocyanidins) with HPLC in conjunction with colour determination, and the determination of minerals (Fe, Cu, Pb, Sb, Mg, Ca, Mn, Zn, etc.) with atomic absorption spectrophotometry (AAS) or ICP–MS (Zoecklein *et al.*, 1994). The employment of FT–IR spectroscopy and NMR are two more recently applied methods for determination of geographical origin and variety, respectively.

Furthermore, DNA methods have also been employed for authentication of varietal wines. In an attempt to develop a technique for the identification of grape cultivars in commercial wines, a method for the extraction of DNA from must and experimental wines was adopted, and optimal PCR conditions for the amplification of this DNA were established. DNA was analysed during the fermentation process for six cultivars. Expected profiles from these cultivars were obtained with DNA extracted from the solid parts during the fermentation process (Siret *et al.*, 2000). Capillary zone electrophoresis (CZE) was, recently, successfully applied to wines from the Canary Islands (Spain) in order to differentiate them (Pazourek *et al.*, 2000).

8.2.1 Analysis of minerals

The analysis of minerals (in particular Na, K, Ca, Mg, Mn, Li, Fe, Cu, Pb) has been extensively employed as one of the most promising methods, either on its own or in conjunction with other methods for detection of wine authenticity (variety, geographical origin) (Moret *et al.* 1994; Baxter *et al.*, 1997; Galani-Nikolakaki *et al.*, 2002; Gomez-Plaza *et al.*, 2000; Kallithraka *et al.*, 2001a; Frias *et al.*, 2003). The majority of the studies were carried out in the Mediterranean countries (Spain, France, Italy and Greece) which devote great interest to wine authenticity due to their own production.

Montilla-Moriles fino wines (50 samples) have been featured according to their concentration of Zn, P, Mn, Fe, Mg, Cu, Ca, Al, Sr, Ba, Na and K. The elements were determined using ICP atomic emission spectrometry (ICP–AES). The results obtained showed that mineral data sets were non-normally distributed and, accordingly, non-parametric statistics (median, interquartile range) were applied. The interrelation of element couples was studied through the Spearman non-parametric sample correlation, and some important element correlations were established, such as P/Mg, P/Ca, Ca/Al, Ca/Na and Al/Na (Álvarez *et al.*, 2007).

Latorre *et al.* (1994) used pattern recognition analysis (employing Li and Rb as key minerals) for successfully differentiating 41 wines from northwest Spain (between Rias-Baixas and non-Rias Baixas). Multi-element analysis of 112 Spanish and English wines by ICP–MS unequivocally identified the region of origin of Spanish wines from three different regions. Complete differentiation (100%) of English and Spanish white wines also occurred whereas red and rosé wines were distinguished with 95% accuracy (Baxter *et al.*, 1997). Frias *et al.* (2003) conducted with 100% sensitivity and specificity the classification of commercial wines from three Canary Islands (Spain) by determining their mineral concentrations with

AAS and flame emission. Pena *et al.* (1999) studied 39 red wines from Galicia (NW Spain) in terms of their trace metal composition. An acceptable level of differentiation and classification of wine samples of *Ribeira sacra* non-*Ribeira Sacra* origin was found by applying several multidimensional techniques. Employment of the key metals (Li and Fe) resulted in satisfactory level of correct classification between the two wine groups without, however, being able to exclude entirely the possibility of an incorrect classification. Employment of pattern recognition analysis with greater number of attributes on the same 39 wines led to more accurate determination of origin (Rebolo *et al.*, 2000). Kallithraka *et al.* (2001a) showed that the mineral concentration of 33 Greek red and white wines varied substantially with their origin and can be effectively employed as a reliable indicator for differentiation of wines from various regions (north Greece, south Greece and the islands).

The use of visible (VIS) and near infrared spectroscopy (NIRS) to measure the concentration of elements in Australian wines was investigated. Both white and red wine samples representing a wide range of varieties and regions were analysed by ICP–MS for the concentrations of Ca, K, Mg, P, Na, S, Fe, B and Mn. Samples were scanned in transmittance mode (1 mm path length) in a monochromator instrument (400–2500 nm). The spectra were pre-treated by second derivative and standard normal variate (SNV) prior to developing calibration models using partial least squares (PLS) regression method with cross-validation. The results showed that some macro- and microelements present in wine might be measured by VIS–NIRS spectroscopy (Cozzolino *et al.*, 2008a,b).

A comparison of concentrations of K (ppm) among wines from Greece, Spain and France showed that Greek and Spanish wines had almost similar concentrations of potassium (variation range ~ 4%) whereas the French wines contained substantially greater amounts of K (20–100% greater values). It is noteworthy that the highest K values were obtained when measurements were taken on the pressed grapes (Day *et al.*, 1994). Since K concentrations greatly depend on anthropological activity, the latter were found to gradually decrease as already shown by other researchers (Table 8.1), whereas the concentrations for other minerals may increase or decrease depending on the particular kind of treatment and equipment employed (Day *et al.*, 1994; Latorre *et al.*, 1994). The Na concentration of Greek wines was twice as high as concentrations in Spanish and French wines. The Mg concentration of Greek wines was three times higher than French wines and can be successfully employed as another promising feature for differentiation. In the case of Fe, the Greek wines equally contained greater concentrations than the other wines, but this can hardly be considered an advantage since it is well known that high concentrations of Fe and Cu can result in iron and copper casse (hazy and cloudy wines).

A multi-element graphite furnace atomic absorption spectrometry (GFAAS) method was elaborated for the simultaneous determination of As, Cd, Cu and Pb in wine samples of various sugar concentrations using the transversally heated graphite atomiser (THGA) with end-capped tubes and integrated graphite platforms (IGPs). For comparative GFAAS analyses, direct injection (i.e., dispensing

Table 8.1 Mineral concentrations (mg/L) of red and white wines of various origins

Country of origin	Samples	Minerals										Methods	References
		Na	K	Ca	Li	Mg	Mn	Fe	Cu	Cd	Zn		
France (red wine)	34	20.5	1253	76.7	–	53.3	0.62	–	–	–	–	–	Etiévant *et al.*, 1988a,b
France (white wine)	25	–	1532	65.2	–	63.3	0.44	1.6	1.06	–	0.77	AAS	Day *et al.*, 1994
Spain (white wine)	42	40 ± 3.7	810 ± 37	95 ± 8.2	24 ± 4.3	–	–	–	–	–	–	AAS	Latorre *et al.*, 1994
Italy (white wine)	59	25 ± 0.5	7400 ± 20	97 ± 21	13 ± 3	–	–	–	–	–	–	Flame emission, AAS	Moret *et al.*, 1994
France (red wine)	3	–	–	–	32.5	–	–	7768	210	–	569.8	ICP–MS	Baxter *et al.*, 1997
Hungary (white wine)	35	–	–	–	–	–	2.3	20.5	0.35	–	1.45	–	Muranyl and Papp, 1998
Spain (red wine)	30	32.5 ± 1.5	808 ± 16	58 ± 3.9	–	–	–	1.7 ± 0.1	–	–	–	AAS	Gomez-Plaza *et al.*, 2000
Spain (red wine)	39	21 ± 14	1075 ± 200	–	35 ± 11	–	2.4 ± 0.2	3.3 ± 0.2	–	–	0.27 ± 0.06	AAS	Pena *et al.*, 1999; Rebolo *et al.*, 2000
Greece (red wine)	21	63 ± 12	838 ± 20	1487 ± 35	–	174 ± 8	1.76 ± 11	16.4 ± 0.7	1.0 ± 0.1	–	1.82 ± 0.13	ICP	Kallithraka *et al.*, 2001a
Greece (white wine)	11	37.2 ± 1.5	560 ± 24	185 ± 9	–	99.6 ± 2.4	1.48 ± 0.2	4.7 ± 0.3	0.66 ± 0.1	–	1.38 ± 0.1	ICP	Kallithraka *et al.*, 2001a
Greece (red wine)	30	–	–	–	–	–	1.15	7.5	0.4	0.003	1.5	AAS	Galani-Nikolopoulou *et al.*, 2002
Spain (red wine)	45	120 ± 25	880 ± 130	82 ± 3.8	14.6 ± 1.7	87 ± 9.5	0.72 ± 0.06	3.1 ± 0.3	0.29 ± 0.02	–	0.44 ± 0.06	AAS	Frias *et al.*, 2003
Spain (red wine)	50	58.96	766.49	393.36	–	279.55	2.57	2.59	0.32	–	213.85	ICP–AES	Álvarez *et al.*, 2007
Hungary (red and white wine)	35	–	–	–	–	–	–	–	1.2	0.03	–	GFAAS	Ajtony *et al.*, 2008
Australia (red and white wine)	32 (red) 94 (white)	77.0	890.0	90.0	–	73.3	0.78	1.86	–	–	–	ICP–MS	Cozzolino *et al.*, 2008a

the sample onto the IGP) and digestion-based (i.e., adding oxidising agents, such as HNO_3 and/or H_2O_2 to the sample solutions) methods were optimised with the application of chemical modifiers (Ajtony *et al.*, 2008).

Table 8.1 summarises all the studies reported in the literature about the determination of minerals in wine, providing information regarding the method used, their concentration and country of origin.

8.2.2 Analysis with HPLC, GC (phenols, volatiles, amino acids, *trans*-resveratrol and ochratoxin)

Phenols

Epidemiological evidence shows that increased levels of fruit and vegetables in the diet reduce the risk of cancer and heart disease as well as cataracts, brain and immune dysfunction and stroke. Since phenolics are practically ubiquitous in plant material and may occur at high concentrations, it is likely that these compounds play a major role in determining the antioxidant potential of foodstuffs (Steinmetz and Potter, 1991; Block *et al.*, 1992; Hertog *et al.*, 1995; Vinson *et al.*, 1998). Phenolics are important to wine and grape juice because they contribute to colour, flavour, oxidation and other reactions. Phenolics' popularity should be mainly attributed to their antioxidant properties which could be summarised as follows: metal-ion chelating action; hydrogen donation/radical scavenging; inhibition of radical generation; anticarcinogenic action; and enzyme inhibition and specific receptor interactions (e.g. estrogen receptors) (Parr and Bolwell, 2000). Three of the principal factors affecting the phenolic concentrations of wines are: the phenolic composition of the grape; winemaking practices; and reactions that take place during ageing (Blanco *et al.*, 1998). An overview of the phenolic compounds determined is given in Table 8.2.

The most widely employed method for phenol determination is HPLC analysis. Apart from HPLC, the solid phase extraction (SPE) approach prior to capillary electrophoresis (CE) analysis was used by several investigators (Gu *et al.*, 2000) and resulted in cleaner and more concentrated samples. In addition to concentration, the procedure facilitated the identification of antioxidants by altering sample conditions and removing interfering materials. In general, the limit of detection for resveratrol determination in wine was lowered ten-fold with good recoveries (95–102%) using SPE. The CZE method was shown to separate epicatechin, catechin, quercetin, myricetin, rutin, gentistic acid, caffeic acid, gallic acid and *trans*-resveratrol (Gu *et al.*, 2000).

Goldberg *et al.* (1999) determined the concentrations of selected phenols (catechin, epicatechin, quercetin, rutin, *cis*- and *trans*-resveratrol, and *p*-coumaric acid) in white wines of various countries with the aid of a HPLC method with a diode array detector. They found that climatic factors appearing to modulate polyphenol concentrations in red wines, such as stress, fungal pressure and sunlight, do not seem to be important for white wines. The intrinsic properties of the individual cultivars or clones together with regional differences in enological practices may be the most important factors.

Table 8.2 Phenolic concentrations (mg/L) of red wines from various countries

Country	Total phenols	Catechin	Epicatechin	Myricetin	Quercetin	Caffeic acid	Ferulic acid	References
Yugoslavia	–	94.8	53.2	–	–	–	–	Kovac *et al.*, 1992
USA	2700	230	68	7.2	7.5	6.4	–	Frankel *et al.*, 1995
Spain	–	–	1.2	–	–	0.4	0.12	De la Presa-Owens and Noble, 1995
Italy	3763	160	–	3763	–	2267	38	Goldberg *et al.*, 1999
Italy	–	37	23	–	8.0	–	–	Goldberg *et al.*, 1999
France		67.3	31.3	–	3.4	–	–	Goldberg *et al.*, 1999
Australia	–	39.8	34.1	–	8.2	–	–	Goldberg *et al.*, 1999
USA	–	80	31	–	2.2	–	–	Goldberg *et al.*, 1999
Canada	–	72.2	38.8	–	2.0	5.5	1.7	Soleas *et al.*, 1997
USA	1000	32.6	40.3	–	–	–	–	Blanco *et al.*, 1998
Canada	1200	240	82.0	–	18.5	–	–	Goldberg and Soleas, 1999
Spain	–	–	45.5	–	16.0	–	–	Bonilla *et al.*, 1999
Italy	3600	–	–	–	–	2200	37	Cappiello and Famiglini, 1999
USA	–	152	–	59	7.3	–	–	Ritchey and Waterhouse, 1999
Greece	1514	16.7	7.1	6.2	25.7	62.8	3.9	Kallithraka *et al.*, 2001
Czech Republic	1600	12.0	–	–	–	0.56	0.27	Spacil *et al.*, 2008
Spain	1288	65.9	106.2	–	11.8	2.5	1.4	Heredia *et al.*, 2010
Spain	–	20.0	30.5	–	10.0	8.5	–	Alen-Ruiz *et al.*, 2009

The anthocyanin, organic acid and volatile phenol compositions of red wine obtained from Touriga Nacional grapes growing in the Dao region (Portugal) were determined by HPLC/diode array detector (DAD), HPLC/UV and GC/ flame ionisation detector (FID), respectively. By these means, nine anthocyanic compounds (malvidin-3,5-O-diglucoside, cyanidin-3-O-galactoside, cyanidin-3-O-glucoside, peonidin-3-O-glucoside, malvidin-3-O-glucoside, delphinidin, cyanidin, pelargonidin and malvidin), six organic acids (ketoglutaric, tartaric, malic, quinic, lactic and shikimic) and two volatile phenols (4-ethylguaiacol and 4-ethylphenol) were identified and quantified. Malvidin-3-O-glucoside, lactic and shikimic acids, and 4-ethylguaiacol were the compounds from each respective group (Valentão *et al.*, 2007).

Phenolic composition of 92 wine vinegars coming from different wines from southern Spain was determined with HPLC. Phenolic concentration was shown to classify and predict effectively the membership of samples according to employed treatment method or geographical origin of substrate wine (Garcia-Parilla *et al.*, 1997). Teissendre and Landrault (2000) analysed the catechins and procyanidins in an attempt to show their effectiveness *in vitro* as powerful inhibitors of low-density lipoprotein oxidation and platelet aggregation. It was found that phenolics intake is 10 times higher from consumption of red wines (400.2 mg/person/day) than for white wines (44.1 mg/person/day). Catechin monomers can represent 40% of total catechins. Moreover, it was shown that the consumption of wine by humans leads to an increase in the antioxidant capacity of plasma.

Two novel chromatographic methods both based on utilisation of sub-2-micron particle columns were developed for the analysis of phenolic compounds. An HPLC system was equipped with C_{18} silica-based analytical column (50 mm × 4.6 mm, 1.8 µm) and an ultra-performance liquid chromatography (UPLC) system with ethylene-bridged hybrid C_{18} analytical column (100 mm × 2.1 mm, 1.7 µm). In total, 34 phenolic substances were divided into groups of phenolic acids, flavonoids, catechins and coumarins and were analysed in sequence using different gradient methods. System suitability test data, including repeatability of retention time and peak area, mean values of asymmetry factor, resolution, peak capacity and the height equivalent of a theoretical plate were determined for each gradient method by 10 replicate injections. The developed methods were applied in the analysis of real wine samples (Spacil *et al.*, 2008).

HPLC–MS with capillary scale particle beam interface was used to detect 18 phenolic compounds in Italian red wine samples. This technique allowed reproducible, library searchable electron ionisation spectra at only 1 µl/min mobile phase flow-rate for a sensitive detection of the analytes in complex matrices. The method makes use of a narrow bore, reversed-phase packed capillary column for sample separation. Detection limits were in the low picogram range for most compounds. Sensitivity and response linearity were evaluated for eight phenolic acids, which are often encountered in red wines. The phenolic compound composition was outlined in two red wines obtained using different ageing processes (Cappiello and Famiglini, 1999).

Pellegrini *et al.* (2000) determined the total phenol concentration of eight

commercial Italian *vini novelli* (young red wines) from different geographical origins with regard to their antioxidant activity. The average flavanol concentration (424.7 ± 121.3 mg/L catechin equivalents) and the total antioxidant activity (16.8 ± 3.8 mmol/L Trolox equivalents) of *vini novelli* were higher than the corresponding values of 382.7 ± 174.5 mg/L catechin equivalents and 12.3±3.3 mmol/L Trolox equivalents found for aged wine. This experiment revealed that ageing and not the winemaking technique is the main factor influencing the antioxidant activity of red wines.

Volatiles

Wine is a hydroalcoholic solution containing hundreds of compounds that come from grapes or result during winemaking and storage. Several of these compounds affect wine aroma which, besides being a parameter of quality, acts as a 'fingerprint' for each wine variety. The sulphur compounds occurring in wines are classified in five different families according to their structure: thiols, sulphides, polysulphides, thioesters and heterocyclic compounds. Presence of many of them may impart an unpleasant odour to wine (Mestres *et al.*, 2000).

Fifty-two young monovarietal Spanish red wines were analysed with high-resolution gas chromatography (HRGC)–MS to obtain quantitative data on 47 odorants previously identified as potential aroma contributors by olfactometric techniques. Thirty-three odorants were present in the wines at concentrations higher than their corresponding odour thresholds. These included ethyl-octanoate, β-damascenone, ethyl hexanoate, isovaleric acid and isoamyl acetate as the most important which, together with isoamyl and β-phenylethyl alcohols, fatty acids, 2,3-butanedione and ethyl butyrate, are always found at concentrations higher than their odour thresholds. In some cases, the ethyl esters of isobutyric and isovaleric acids, β-ionone, methionol, isobutyric acid, ethyl cinnamate, ethyl dihydrocinnamate, γ-nonalactone, eugenol, *cis*-3-hexanol, geraniol, guaiacol, 3-isobutyl-2-methoxypyrazine, 4-ethylguaiacol, acetoin and t-whisky lactone were at concentrations high enough to be odour-active (Ferreira *et al.*, 2000).

Molecular approaches by means of a combined use of mass spectrometric techniques can be important in order to open new possibilities in the differentiation of typical products; in a study by Nasi *et al.* (2008), a possible approach to the analysis of varietal volatile compounds and some precursors of a non-aromatic grape variety (Falanghina cv., *Vitis vinifera* L.) was traced through a combined use of techniques based on MS–GC/MS, liquid chromatography/ electrospray ionisation (LC/ESI)–MS), matrix assisted laser desorption ionisation – time of flight (MALDI–TOF)–MS. Dominant terpene compounds (limonene, *cis*-furan-linalool oxide, geraniol, 4-carene, myrcene, linalool, α-terpineol), terpene-derivatives (bornyl acetate, menthol), terpene glycosides (glucosides, arabinosylglucosides and rhamnosylglucosides of linalool and geraniol) and norisoprenoids (β-damascenone) were identified in grapes and monovarietal wines, overcoming the analytical difficulties deriving from the low concentration of these compounds strictly related to the variety (Nasi *et al.*, 2008).

Rosillo *et al.* (1999) used a dynamic headspace analysis with GC–MS for

determining the volatiles in grapes and classifying some *Vitis vinifera* varieties. This method permits the analysis of the volatile fraction of a wine by purging with an inert gas followed by thermal desorption and gas chromatography. Application of cluster analysis to the volatiles resulted in three groups, one for white grapes, one for Monastrell, Tempranillo and Cabernet Sauvignon, and the other for Grenache. Hexyl acetate, benzyl alcohol, phenyl ethyl alcohol and benzaldehyde were the four discriminant variables for group differentiation.

Analysis of 41 volatile compounds carried out with GC–MS on 60 white Spanish wines of three varieties, different wineries and vintage years, in conjunction with PCA, revealed that some higher alcohols can help the separation of wines according to winery and vintage year (de la Presa-Owens *et al.*, 1995). In Germany, 44 odour-active compounds were quantified in Scheurebe and Gewürtztraminer wines. Calculation of odour activity of aroma compounds showed that differences in odour profiles of both varieties were caused by *cis*-rose oxide in Gewürtztraminer and by 4-mercapto-4-methylpeptan-2-one in Scheurebe. These compounds are suitable indicators for the determination of flavour differences, and can lead to wine authentication (Guth, 1997).

Stir bar sorptive extraction and liquid desorption followed by large volume injection coupled to GC–quadrupole MS (SBSE–LD/LVI-GC–qMS) has been used for the determination of volatiles in wines. The methodology showed good linearity over the concentration range tested, with correlation coefficients higher than 0.9821, a good reproducibility was attained (8.9–17.8%), and low detection limits were achieved for nine volatile compounds (0.05–9.09 μg/L), with the exception of 2-phenylethanol due to low recovery by SBSE. This methodology allowed, in a single run, the quantification of 67 wine volatiles at concentrations lower than their respective olfactory thresholds. The proposed methodology was shown to be easy to work-up, reliable, sensitive and with low sample requirement to monitor the volatile fraction of wine (Coehlo *et al.*, 2008).

Model wine solutions containing amino acids, sugar, water and yeast nutrients were fermented by *Saccharomyces cerevisiae* and the volatile composition of the fermented media was analysed with GC. Significant differences were found in the concentrations of some important volatile compounds, including ethanol, ethyl acetate, acetic acid, higher alcohols (and some of their acetates), methionol, isobutyric acid, ethyl butyrate and hexanoic and octanoic acids. The concentrations of some of the volatiles were well-correlated with the aromatic composition of wines made with grapes of the same varieties (Hernandes-Orte *et al.*, 2002).

2,6,6-Trimethylcyclohex-2-ene-1,4-dione (TMCHD, a norisoprenoid) and diacetyl (both caramel descriptors) were reported for the first time in fortified wines from the Douro demarcated area of Portugal. GC–olfactometry (GC–O) of a volatile wine extract, previously isolated with preparative GC, indicated the presence of a zone containing an intense honey descriptor. The targeted odour compound was identified by GC–MS, GC–O and Kovats index. Quantitative analysis using a selected characteristic ion (*m/z* 96) indicated that young Douro fortified wines from the 1997 vintage contained up to 4 μg/L TMCHD. The wine

Table 8.3 Analysis of volatiles of wines/distillates

Country	Number of samples/ varieties	Method	Volatiles determined	References
Spain	32/2 regions	GC (SPI)–MS	40 volatile compounds were identified and quantitatively determined	Garcia-Jares *et al.*, 1995
Spain	60/3	GC–MS	Ethyl propionate, isobutyl acetate, ethyl butyrate, isoamyl acetate, isoamyl acetate, butanol, limonene, ethyl hexanoate, hexyl acetate, isoamyl isovalerate, *cis*-3-hexen-1-ol acetate, 3-methyl-1-propanol, ethyl lactate, hexanol, 3-ethoxy-1-propanol, *cis*-3-hexen-1-ol, *trans*-2-hexen-ol, ethyl octanoate, *cis*-furan linalool oxide, octyl acetate, 2,3-butanediol, butyrolactone, ethyl decanoate, isoamyl decanoate, isoamyl octanoate, diethyl succinate, α-terpinol, isoamyl butyrate, linalool, octanol, nerol, citronellol, ethyl dodecanoate, ethyl mirystate, 2-pentanol, geraniol, benzaldehyde, hexanoic acid	De la Presa-Owens *et al.*, 1995
Spain	32/8 (grape cultivars)	GC–MS	35 free volatile compounds and 36 bound compounds released by enzyme hydrolysis and glycoside form	Lopez-Tamames *et al.*, 1997
South Korea	23 (whisky and brandy)	Capillary column GC–MS	Lactic acid, glycoloic acid, oxalic acid, malonic acid, capric acid, succinic acid, lauric acid, myristic acid, malic acid, palmitic acid, tartaric acid, stearic acid	Park *et al.*, 1999
Spain	NA/4	Dynamic headspace GC–MS	Hexanol, 3-hexen-1-ol, (*trans*) 2-hexenal, linalool, geraniol, benzyl alcohol, phenylethyl alcohol, 1-pentanol, 1-heptanol, 1-octanol, 1-octen-3-ol, ethyl hexanoate, hexyl acetate, ethyl heptanoate, ethyl decanoate, ethyl dodecanoate, isobutyric acid, hexanoic acid, heptanoic acid, octanoic acid, benzaldehyde, decanal, limonene	Rosillo *et al.*, 1999
France	9/2	GC–Olfactometry GC–MS	4-hydroxy-2.5-dimethylfuran-3(2H)-one (HDMF) and 4-hydroxy-2(or 5)-ethyl-5(or 2)-methylfuran-3(2H)-one (HEMF)	Kotseridis *et al.*, 2000
Spain	Many	Solid phase microextraction (SPME), dynamic headspace	Thiols, sulphides, polysulphides, thioesters and heterocyclic compounds	Mestres *et al*., 2000

Spain	52/4	GC–MS	Ethyl-octanoate, β-damascenone, ethyl hexanoate, isovaleric acid and isoamyl acetate as the most important, which together with isoamyl and β-phenylethyl alcohols, fatty acids, 2,3-butanedione and ethyl butyrate, ethyl esters of isobutyric and isovaleric acids, β-ionone, methionol, isobutyric acid, ethyl cinnamate, ethyl dihydrocinnamate, γ-nonalactone, eugenol, c-3-hexanol, geraniol, guaiacol, 3-isobutyl-2-methoxy-pyrazine, 4-ethylguaiacol, acetoinand t-whiskylactone	Ferreira *et al.*, 2000
Portugal	19/1	GC	2,6,6-Trimethylcyclohex-2-ene-1,4-dione, diacetyl (caramel descriptors)	Rogerson *et al.*, 2001
Spain	Six lots per must	GC	Ethanol, ethyl acetate, ethyl propionate, isobutyl acetate, ethyl butyrate, isobutanol, isoamyl acetate, acetic acid, propanoic acidisobutyric diethyl succinae, methionol, hexanoic acid	Hernandes-Orte *et al.*, 2002
Italy	93/3	GC–MS, LC–ESI –MS, MALDI–TOF–MS	Limonene, *cis*-furanlinalool oxide, geraniol, 4-carene, myrcene, linalool, a-terpineol, bornyl acetate, menthol glucosides, arabinosyl-glucosides, rhamnosylglucosides of linalool and geraniol β-damascenone	Nasi *et al.*, 2008
China	–/1	GC–MS	Isobutyl alcohol, 2-phenyl-ethanol, 1-propanol, isopentyl alcohol, β-damascenone, isobutyl acetate, 2-/3- methyl-butanols, 2-phenyl-ethanol, 2-methyl- 3-sulfanylfuran, acetic acid, 3-(methylsulfanyl) propanal, 2-/3-methylbutanoic acids, 3-sulfanylhexan-1-ol, furaneol, homofuraneol, isopentyl and β-phenylethyl alcohols, the ethyl esters of butyric, isobutyric, 2-methyl butyric, hexanoic acids, γ-nonalactone and eugenol	Li *et al.*, 2008
Portugal	–/4	SBSE–LD/LVI–GC–qMS	Guaiazulene, *E*,*E*-farnesol, β-ionone, geranylacetone, ethyl decanoate, β-citronellol, 2-phenylethanol, linalool, hexyl acetate and hexanol	Coelho *et al.*, 2008

volatile diacetyl was identified as a strong contributor to the sweet caramel aroma descriptor often associated with Port (Rogerson *et al.*, 2001).

Kotseridis *et al.* (2000) made an attempt to detect and identify the potent odorants with the caramel odour of Merlot and Cabernet Sauvignon wines with GC–O. Two odorant zones with this odour resulted in identification of 4-hydroxy-2.5-dimethylfuran-3(2H)-one (HDMF) and 4-hydroxy-2(or 5)-ethyl-5(or 2)-methylfuran-3(2H)-one (HEMF). Aroma extraction dilution analysis (AEDA) method showed a higher dilution factor (DF) for HDMF in the Merlot wine extract than in the Cabernet Sauvignon extract. Conversely, HEMF was found to produce the caramel perception in the Cabernet Sauvignon wines (as well as in the Merlot wines), but could not differentiate Merlot from Cabernet Sauvignon.

Some 69 volatile compounds of young red wines from Cabernet Sauvignon in Changli County (China), were identified by GC–MS. HS–SPME (headspace solid-phase microextraction) was used to extract and concentrate volatile and semi-volatile compounds in the wine. Higher alcohols made up about 46% of the total concentration of volatiles and this group was mainly composed of isobutyl alcohol, 2-phenyl-ethanol, 1-propanol and isopentyl alcohol. Acetates and ethyl esters made up 51% of the total volatiles, of which acetates made up 5% and ethyl esters 46%. Fatty acids made up 1.6% of the total volatiles. Among the small quantity of volatiles, there were five terpenes, one norisoprenoid (β-damascenone), seven fatty acid esters of higher alcohols, two carbonyl compounds, one volatile phenol and one sulphur compound (Li *et al.*, 2008). Table 8.3 provides an overview of the analysis of volatiles (methodology employed, compounds detected) of wines and distillates.

Amino acids

Free amino acids can characterise grape varieties when studied along with other variables in multivariate analysis (de la Presa-Owens and Noble, 1995). Etiévant *et al.* (1988b) showed that the amino acid concentration of grapes is dependent upon the fertilisation and climatic conditions and on duration of skin maceration in the must.

Amino acids were very effectively employed for separating Champagnes from sparkling wines in a study of 110 wines. Champagnes are richer than most other sparkling wines in all amino acids, except arginine, because of the second fermentation in the bottle and long contact with lees (Tusseau *et al.*, 1996).

Palma *et al.* (1995) analysed the amino acid composition (proline, hydroxyproline, arginine, ornithine, alanine, serine, glycine, valine, leucine, asparagine, threonine, isoleucine, methionine, lysine, tyrosine, phenylalanine, histidine, ethanolamine) of 34 French red wines. However, only proline, hydroxyproline and ethanolamine emerged as the descriptors leading to effective grouping.

Two biogenic amines, tryptamine and tyramine, and their precursors, tryptophan and tyrosine, were determined by a liquid chromatographic procedure. A hybrid micellar mobile phase of sodium dodecyl sulphate (SDS) and 1-propanol, a C_{18} column and electrochemical detection were used. A pH study in the range of 3–9 was performed and pH 3 was finally selected in accordance with resolution

and analysis time. The four compounds were resolved using a mobile phase of 0.15 M SDS–5% 1-propanol with an analysis time of 16 min. Repeatabilities and intermediate precision were evaluated at three different concentrations for each compound with relative size distribution (RSD) values lower than 2.6 and 4.8%, respectively. Limits of detection and quantification were also obtained within the 10–40 and 33–135 ng/mL ranges, respectively. Finally, the applicability of the procedure was tested in several types of wine and no matrix effect was observed. The possibility of direct sample introduction simplifies and greatly expedites the treatments with reduced cost, improving the accuracy of the procedures (Gil-Agusti *et al.*, 2007).

De la Presa-Owens and Noble (1995) and De la Presa-Qwens *et al.* (1995) reported the determination of free amino acids and ethanolamine for the characterisation Spanish white wines from the Penedes region. Asparagine, proline and lysine proved to be the most important compounds for distinguishing the varieties on the basis of their geographical origin.

The presence of biogenic amines in wine (see Chapter 29) has been associated with a number of undesirable physiological effects. The typical concentrations of 10 biogenic amines in Greek wines were investigated for the first time. One hundred wine samples, varying in type, colour and origin, were analysed by reversed-phase HPLC with UV detection after pre-column derivatisation with dansyl chloride. The amino acid and organic acid concentrations of these wines were also evaluated by HPLC in an attempt to explain amine presence and concentration in wines. The total amine average concentration was 4.76 mg/L. Putrescine and ethylamine were the most prevalent amines, followed by cadaverine and methylamine. Histamine was found in 54.5% of the samples, although only 5.9% of them contained more than 2 mg/L. The concentrations of histamine and tyramine that were detected are below the amounts considered to have an adverse effect on human health (Soufleros *et al.*, 2007).

In another study, Hernandez-Orte *et al.* (2002) analysed amino acid compositions reflecting the characteristic amino acid profiles of 11 different grape varieties. A multiple linear regression study produced good models for most of the odorants for which the concentration was related to the must amino acid composition. Partial least squares regression models confirmed that amino acid composition explains a high proportion of the variance in the volatile composition; the by-products of fatty acid synthesis are related to threonine and serine, the concentration of β-phenyl ethanol is closely related to phenylalanine and the concentration of methionol is linked to methionine concentration.

Polyamines and their amino acid precursors were determined in Grenache and Syrah grapes and in wines made from these grapes. The compounds analysed were the polyamines putrescine, spermidine and spermine, in addition to their precursors, ornithine, agmatine and arginine. The analytes were determined by reversed-phase HPLC with fluorescence detection using FMOC (fluorenylmethylchloroformate) as a pre-column derivatising agent. In three of the four fermentation sites concentrations of all three compounds were greater in Syrah than in Grenache wines. In both varieties, it appears that polyamine biosynthesis occurs preferentially

Table 8.4 Analysis of amino acids of wines

Country	Number of samples/ varieties	Method	Amino acids selected for classification	References
Italy	31 (73 musts)/ NA	Amino acid analyser	Glutamic acid, aspartic acid, proline, leucine, alanine, serine	Seeber *et al.*, 1991
France	34/	HPLC	Proline, hydroxyproline, ethanolamine	Palma *et al.*, 1995
Spain	60/3	HPLC with diode array UV visible after derivatisation	Asparagine, proline, lysine	De la Presa-Owens and Noble, 1995; De la Presa-Owens *et al.*, 1995
France	110/2 (Champagne/ sparkling wine)	Amino acid analyser	All except for arginine	Tusseau *et al.*, 1996
Spain	33/11	HPLC	Threonine, serine, phenylalanine, methionine	Hernandez-Orte *et al.*, 2002
Greece	42/7	HPLC with fluorescence detector	Arginine, methionine, γ-amino butyric acid	Soufleros *et al.*, 2003
Spain	–/2	HPLC and MLC	Tryptophan and tyrosine	Gil-Agusti *et al.*, 2007
Greece	47/2	HPLC	Tyramine, histamine, putrescine, cadaverine, ethylamine, methylamine, isoamylamine	Soufleros *et al.*, 2007
France	20/2	HPLC with fluorescence detector	Putrescine, spermidine, spermine, arginine, agmathine	Bauza *et al.*, 2007

from arginine via agmatine. In all cases, concentrations of polyamines found in these grapes and wines were significantly below the concentrations typically found in other fermented foods (Bauza *et al.*, 2007).

Soufleros *et al.* (2003) found that the amino acid concentrations of Greek white wines were within the range reported for other European white wines. The results indicate the influence of grape variety, geographic location and vintage on the amino acid composition of wine. The type of fermentation also had an impact on the concentration of certain amino acids. Within all wine samples tested as a group, arginine and γ-amino butyric acid were the most abundant amino acids followed by lysine, alanine, glycine, asparagine, leucine and ethanolamine (Table 8.4).

Trans-resveratrol

Although *trans*-resveratrol is a constituent of many plant species, grapes and related products comprise their most important source. The therapeutic effect (beneficial action against atherosclerosis and coronary heart disease) of *trans*-resveratrol in conjunction with the large number of publications in this area are the main reasons for presenting this compound separately from the rest of the phenolic compounds.

A rapid and sensitive method was developed for the determination of this compound in wines. *Trans*-resveratrol was determined for 29 red Greek wines with designated appellations of origin. The concentrations found varied considerably (0.550–2.534 mg/L), and it was possible to classify the wines into five groups by employing both hierarchical cluster analysis (CLA) and principal component analysis (PCA) (Kallithraka *et al.*, 2001b).

Trans-resveratrol was determined with overpressured layer chromatography (OPLC) in 25 different Hungarian wines from the same winemaking region harvested in 1998. Although CLA failed to provide clear-cut differentiation between white and red wines on the basis of their *trans*-resveratrol concentration, this was possible with PCA. Implementation of PCA led to formation of two distinct groups; perfect separation was achieved for red and white wines (Csomos *et al.*, 2002).

Concentrations of resveratrol (*trans* and *cis*) in white and red commercial wines from the five Galician Controlled Denomination of Origin (CDO Monterrei, CDO Rías Baixas, CDO Ribeira Sacra, CDO Ribeiro, CDO Valdeorras) was determined by reversed-phase HPLC with fluorimetric detection. The wines were filtered and directly injected, and also the samples were analysed after the addition of two known amounts of the two standard isomers. The results obtained showed that the concentration of *cis*-resveratrol found in 16 white and red wines ranged from 0.12–0.06 mg/L, respectively, whereas the concentration of *trans*-resveratrol was highest in the red wines (mean = 12.68 mg/L), made mainly from varieties such as Mencía, and practically zero in the white wines (mean = 2.51 mg/L), except in Godello and Albariño wines (Feijóo *et al.*, 2008).

Capillary electrophoresis has been used by several researchers to determine the concentration of *trans*- and *cis*-resveratrol in wine samples with good sensitivity,

speed and reproducibility. Most of the CE methods for measuring resveratrol in wine were able to reliably detect resveratrol at 0.2–1.0 μM concentrations. CE was shown to be effectively combined with solid phase extraction (SPE) and/or micellar electrokinetic chromatography (MEKC) for improved analysis of flavonoid compounds in wine (Gu *et al.*, 2000).

Pinto *et al.* (1999) showed that both resveratrol and its oxidised form obtained with hydroperoxidase activity are effective inhibitors of lipoxygenase activity. The fact that the maximun rate of resveratrol oxidation was obtained at a concentration of inhibitor at which dioxygenase activity was abolished suggests that the dioxygenase and hydroxyperoxidase activities of lipoxygenase are independent.

Resveratrol concentrations reported in red US wines are < 1 mg/L (Seiman and Creasy, 1992; Lamuela-Raventos and Waterhouse, 1993) and much higher in Italian, French and Spanish wines (Jeandet *et al.*, 1993; Mattivi, 1993; Lamuela-Raventos *et al.*, 1995). Goldberg *et al.* (1994) analysed more than 1000 wines, finding very little *trans*-resveratrol (typically < 0.1 mg/L) in white wines, whereas red wines had concentrations ranging from 0.1–12.0 mg/L) with lowest concentrations in wines from California, Australia and Italy and highest in wines from Oregon and Canada and from various regions of France.

Liquid chromatography and mass spectrometry conditions were developed in order to identify *trans*-resveratrol in sweet fortified Muscatel wines from Setïbal region (Portugal). Diode array, fluorescence and electrochemical detectors were used for quantitation purposes. Samples collected at one representative producer, during the winemaking process, were injected without pre-treatment and the quantitation of *trans*-resveratrol was carried out using fluorimetric detection. The *trans*-resveratrol concentration decreased slightly during the winemaking process and the concentrations ranged from 0.22 ± 0.02 to 0.16 ± 0.02 mg/L. After maturation stages, *trans*-resveratrol concentrations in wines collected at different producers were compared: values obtained ranged from 0.13 ± 0.02 to 0.38 ± 0.03 mg/L. The *trans*-resveratrol concentrations in commercially available wines from the same producers were lower (Bravo *et al.*, 2008).

Threlfall *et al.* (1999) studied the effect of grape variety, UV light exposure, enzyme addition, skin contact time and the fining agents carbon and polyvinylpolypyrrolidone (PVPP), on resveratrol concentrations of US wines. Enzyme addition increased resveratrol concentration in some wines. Skin contact time influenced the extraction of resveratrol from the skin in the red varieties, whereas carbon addition decreased the resveratrol concentration. The addition of PVPP decreased substantially the resveratrol concentration.

Ochratoxin

The control of food contaminants, like mycotoxins, has become an issue creating a strong demand for analytical methods that permit their rapid determination at the strict regulation limits established. Mycotoxins are toxic secondary metabolites produced in small amounts by various fungi growing in a wide variety of foods. Among them, Ochratoxin A (OTA) (see Chapter 14) is widespread in cereals and

derived cereal products, dried fruits, grape-based beverages, coffee, etc. According to a 2002 report on the assessment of dietary intake of OTA by Europeans, wine resulted in one of the main dietary sources (10–20%) (OIV, 2002).

Coacervates made up of reverse micelles of decanoic acid were assessed as a new strategy for the simplification of wine sample treatment in the determination of OTA. Simultaneous extraction/concentration of this contaminant was based on both hydrophobic and hydrogen bond OTA:coacervate interactions. Variables affecting extraction efficiency and concentration factors were studied. OTA recoveries from different types of wines (white, rosé and red) ranged between 85 and 100% and the actual concentration factors varied from 105 to 125 for sample volumes of 15 mL. The detection limits for OTA, after liquid chromatography/fluorimetry (LC/FL) analysis of the coacervate (20 µL), were 4.5 ng/L in white and rosé wines and 15 ng/L in red wines, values which were far below the threshold limit established for OTA by EU directives (2.0 µg/L). The approach developed was successfully applied to the determination of OTA in different wine samples from the south of Spain. The concentrations found ranged between 0.015 and 0.091 µg/L (García-Fonseca *et al.*, 2008).

The occurrence of OTA was examined in 121 special wines made using different winemaking techniques and from many European origins. The wine groups with the highest OTA concentration and occurrence, above 90%, were those where the must was fortified before fermentation (mean: 4.48 µg/L) and those made from grapes dried by means of sun exposure (mean: 2.77 µg/L). Fortified wines with long ageing in wooden casks were about 50% contaminated, with OTA concentrations below 1.00 µg/L. Wines affected by noble rot, late harvest wines and ice wines did not contain OTA. Overall, 19.8% of the wines studied contained OTA concentrations above the maximum permissible limit for the European Union (2 µg/kg) in wine (excluding liqueur wines) (Valero *et al.*, 2008).

A total of 95 wine samples including 34 white, 10 rosé and 51 red wines originating from four different Turkish areas were analysed for OTA (Var and Kabak, 2007). An analytical method based on immunoaffinity column (IAC) for clean-up and HPLC with fluorescence detection (HPLC–FD) was used to determine OTA in wines. The average OTA recoveries from spiked white wine samples varied from 79.43% to 85.07%; while the mean recoveries for rosé and red wine samples were in the range 77.48–83.96% and 76.61–83.55%, respectively. OTA was detected in 82 (86%) wine samples at concentrations of < 0.006–0.815 ng/mL, which were below the maximum allowable limit established by the European Community. The mean OTA concentration in red wines was slightly higher than in white and rosé wines. Furthermore, the data indicate that the geographic region of origin has strong influence on OTA concentration for white, rosé and red wines: wines originating from Thrace and Aegean regions of Turkey were more contaminated with OTA compared with wines originating from central and east Anatolia areas (Var and Kabak, 2007).

8.2.3 Analysis with NMR, FTIR, NIR, MS and sensory techniques

Nuclear magnetic resonance (NMR)

Proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopic methods were applied in the differentiation of 53 German white wines from the regions Rheinhessen, Rheingau and Mosel-Saar-Ruwer. The robustness of these differentiations was tested by leaving out one sample at a time and replacing it into the test as a test sample. Small variations in the base line were successfully treated with the aid of a pre-processing algorithm called partial linear fit (Vogels *et al.*, 1993).

Kosir *et al.* (2001) applied specific natural isotope fractionation–nuclear magnetic resonance (SNIF–NMR) and isotope ratio mass spectroscopy (IRMS) to 50 white wines coming from the three main wine producing regions of Slovenia. The separation of wines according to the geographical criteria and authenticity was very good when the coastal region was compared to the continental ones. In the case of enrichment of wines with beet sugar the separation is improved by application of PCA and cluster analysis.

The learning vector quantisation (LVQ) neural network is a useful tool for pattern recognition. Based on the network weights obtained from the training set, prediction can be made for the unknown objects. In this paper, discrimination of wines based on 2D NMR spectra is performed using LVQ neural networks with orthogonal signal correction (OSC). OSC has been proposed as a data preprocessing method that removes from X information not correlated to Y. Moreover, the partial least squares–discriminant analysis (PLS–DA) method has also been used to treat the same data set. It has been found that the OSC–LVQ neural networks method gives slightly better prediction results than OSC–PLS–DA (Masoum *et al.*, 2006).

1D and 2D ^{1}H and ^{13}C homo- and hetero-nuclear magnetic resonance analysis (large signal suppression methods) was employed for characterising anthocyanins and amino acids of 10 Slovenian white wines. The use of NMR signals of seven amino acids gave a good separation of wines according to vine variety and geographical origin. The reliable ^{1}H and ^{13}C NMR assignment of the signals of anthocyanins in glucoside form were used for the identification of various fractions in HPLC analysis of anthocyanins (Kosir and Kidric, 2002).

Different preparation methods of wine samples such as direct analysis, freeze-drying and nitrogen-flow concentration were compared for the characterisation of Brazilian Chardonnay wine composition by means of ^{1}H NMR spectroscopy. The direct analyses of the wine presented some limitations for the detection of 'minor' compounds whose signals were expected in the region from 3.6–4.0 and 0.8–1.3 ppm. The freeze-dried samples led to a low quantity of ethanol remaining in the lyophilisate. The freeze-dried process also revealed restrictions concerning long-time consumption and reproductibility. Diminution of the ethanol signal was observed in the ^{1}H NMR spectrum of wine samples, which were concentrated under nitrogen-flow to allow the determination of compounds such as butyleneglycol and alanine (Amaral *et al.*, 2005).

Brescia *et al.* (2002) used NMR analysis for detecting the geographical origin

Table 8.5 Use of nuclear magnetic resonance in wine analysis

Country	Number of samples/regions	Technique	Method details	References
Germany	53 white wines/3	^{1}H, ^{13}C	Pre-processing algorithm called partial linear fit	Vogels *et al.*, 1993
Slovenia	50 white wines/3	^{2}H, % $^{13}C/^{12}C$	SNIF–NMR, isotope ratio mass spectroscopy (IRMS), study of grape must	Kosir *et al.*, 2001
Slovenia	10 white wines/3	1D and 2D ^{1}H, ^{13}C	Study of anthocyanins	Kosir and Kidric, 2002
Italy	41 red wines/3	^{1}H	NMR in conjunction with inductively plasma atomic emission spectrometry	Brescia *et al.*, 2002
Brazil	18 white wines/1	^{1}H	The composition of amino acids can be used as a fingerprint for differentiation of wines according to their geographical origin, vine variety and year of production.	Amaral *et al.*, 2005
Iran	27 red wines/1	2D	Discrimination of wines based on 2D NMR spectra is performed using LVQ neural networks with orthogonal signal correction	Masoum *et al.*, 2006
South Korea	4 grape varieties	^{1}H	^{1}H NMR with multivariate statistics	Sen *et al.*, 2009

of 41 red wines supplied from various winemakers from the Apulia region (Italy). NMR spectroscopy emerged as the most advantageous technique because of the rapidity with which information can be obtained about a large number of compounds and of the smaller amount of sample required for analysis. It was suggested that the use of ^{13}C NMR, though less sensitive than ^{1}H NMR, could be a promising approach because of its minor signal overlapping and a chemical shift range 20 times larger than for protons. This allows the choice of a considerable larger number of signals for the statistical analysis, thus permitting a more efficient fingerprinting of the wines. A synoptical table of implementation of NMR in wine authentication is given in Table 8.5.

Fourier transform infrared (FT–IR) spectroscopy

Mid-infrared spectroscopy combined with appropriate software was used in an attempt to differentiate Greek red wines of different varietals origin, including the cultivars Agiorgitiko (Nemea-Peloponnesus), Xinomavro (Naousa-Central Macedonia) and Merlot from Greece. Extracts of wine phenolic components were investigated by attenuated total reflectance FT–IR (ATR–FT–IR) spectroscopy. The wine extracts were obtained by solid-phase extraction with C-18 columns and elution by methanol containing 0.01% hydrochloric acid. Libraries of spectra were created using sample from each wine variety. Spectra of unknown wine extracts were recorded and compared with those of the wine libraries, and the rate of affinity (the match value) was measured automatically using the appropriate software (OMNIC ver. 7.3) (Tarantilis *et al.*, 2008).

Roussel *et al.* (2003) showed that high-level multi-sensor fusion significantly improved the white grape must variety classification with regard to individual discrimination. The fusion procedure is not based on the combination of signals, but on the class assignments provided individually by each sensor. Although olfaction does not seem to be the best approach toward discriminating grape varieties, the adjunction of the aroma sensor identity declaration slightly improved FT–IR and UV spectral classification efficiency. The effective fusion method leads to a significant improvement in the grape variety discrimination.

Implementation of FT–IR spectroscopy (in the region between 800 and 1200 cm^{-1}) on the extracted polymeric materials of Portuguese white wines revealed that this technique can be effectively used to characterise white wine polysaccharide composition. It was possible to identify the winemaking process involved (must clarification and/or maceration) and its influence on the amount and kind of wine polysaccharides. Finally, the results showed that it is possible to use the FT–IR combined with multivariate techniques for an in-depth characterisation of white wine polymeric fractions (Coimbra *et al.*, 2002).

FT–IR spectroscopy has become a widespread technique in the agri-food industry for the quick assessment of food components, including wine. Using the region of wave numbers 1200–800 cm^{-1} of the FT–IR spectra wine polysaccharides, partial least squares regression (PLS1), independent calibration models were built for mannose quantification in complex matrices from white and in red wine extracts. With PLS1 it was not possible to build a calibration model that included

both white and red wine extracts. However, a predictive ability of the model for quantification of mannose from mannoproteins based on this FT–IR spectral region was achieved by the application of orthogonal signal correction (OSC)–PLS1 (Coimbra *et al.*, 2005).

Palma and Barroso (2002) recorded FT–IR spectra in an attempt to differentiate and classify wines and brandies during their storage as well as for classification of distilled drinks from various producing countries. Ethanol and sugars proved to have a high response in the IR spectra. Therefore, samples with different ethanol and sugar concentration are bound to have different spectral zones to be used as fingerprints in characterisation studies.

Near-infrared (NIR) spectroscopy

Many studies have reported the use of near-infrared (NIR) spectroscopy to characterise wines or to predict wine chemical composition. However, little is known about the effect of variation in temperature on the NIR spectrum of wine and the subsequent effect on the performance of calibrations used to measure chemical composition. Several parameters influence the spectra of organic molecules in the NIR region, with temperature being one of the most important factors affecting the vibration intensity and frequency of molecular bonds. Wine is a complex mixture of chemical components (e.g. water, sugars, organic acids and ethanol), and a simple ethanol and water model solution cannot be used to study the possible effects of temperature variations in the NIR spectrum of wine (Cozzolino *et al.*, 2007).

NIR spectroscopy was used to assess two physical/chemical parameters in white wines during alcoholic fermentation. NIR calibration models were developed using a set of 64 samples scanned in a rectangular quartz cuvette with a 50 mm path-length in the 700–1060 nm region, in a miniature fibre optic NIR spectrometer system working in transmission mode. Suitable wavelengths for volumic mass and reducing sugars were also proposed according to x-loading weights and regression coefficients. The results obtained suggest that the miniature fibre optic NIR spectrometer is a promising tool for monitoring the white wine fermentation process (Fernández-Novales *et al.*, 2008).

Visible (VIS) and NIR spectroscopy combined with chemometrics was used in an attempt to classify commercial Riesling wines from different countries (Australia, New Zealand, France and Germany). Commercial Riesling wines ($n = 50$) were scanned in the VIS and NIR regions (400–2500 nm) in a monochromator instrument, in transmission mode. Principal component analysis (PCA), partial least squares–discriminant analysis (PLS–DA) and stepwise linear discriminant analysis (SLDA) based on PCA scores were used to classify Riesling wines according to their country of origin. Full cross validation ('leave-one-out') was used as the validation method when classification models were developed. PLS–DA models correctly classified 97.5%, 80% and 70.5% of the Australian, New Zealand and European (France and Germany) Riesling wines, respectively. SLDA calibration models correctly classified 86%, 67%, 67% and 87.5% of the Australian, New Zealand, French and German Riesling wines, respectively. These results

demonstrated that the VIS and NIR spectra contain information that when used with chemometrics allows discrimination between wines from different countries (Liu *et al.*, 2008).

Visible and NIR (Vis/NIR) transmission spectroscopy and a hybrid chemometric method were applied to determine the soluble solids concentration (SSC) and pH of rice wines. Rice wine samples were scanned by a spectroradiometer within a wavelength region of 325–1075 nm. The calibration set was composed of 240 samples and 60 samples were used as the validation set. Two pre-processing methods were applied on the spectra prior to building PLS regression models. The correlation coefficient (r), standard error of prediction (SEP) and root mean square error of prediction (RMSEP) were 0.95, 0.16 and 0.17 for SSC, while 0.94, 0.02 and 0.02 for pH, respectively. Adequate wavelengths for the SSC and pH prediction were proposed according to the x-loading weights and regression coefficients. The results indicated that Vis/NIR spectroscopy is a promising approach for predicting the SSC and pH of the rice wine (Liu *et al.*, 2007).

High-performance liquid chromatography–mass spectrometry (HPLC–MS)

The contribution of ascorbic acid to the formation of coloured species in model white wine systems containing (+)-catechin as the oxidisable phenolic substrate was investigated. Reactions were carried out in the presence or absence of ascorbic acid in model wine systems buffered with either tartaric acid or formic acid. Either HPLC with diode array detector (HPLC–DAD) or mass spectrometry (HPLC–MS) analyses demonstrated that glyoxylic acid-derived xanthylium pigments were the main coloured species produced in all samples except those containing just (+)-catechin and formic acid (Barril *et al.*, 2008).

Grape-derived volatile compounds, including those released from odourless glycosidic precursor present in the grape, are strongly associated with the varietal aroma characteristic of non-aromatic wines. In this study, free and glycosidically bound volatile compounds of Fiano grapes were identified and quantified by means of GC–MS. The free volatile fraction was mainly characterised by the occurrence of several aliphatic alcohols, with minor amounts of the monoterpenes linalool and geraniol, and traces of the norisoprenoid β-damascenone. The volatile fraction obtained from either enzymatic or acid hydrolysis of juice glycosides was more complex, and contained compounds belonging to the chemical classes of terpenes, norisoprenoids, benzenoids and aliphatic alcohols (Ugliano and Moio, 2008).

Two different capillary electrophoresis–mass spectrometry (CE–MS) methods, namely, CE–ion-trap–MS (CE–IT–MS) and CE–time-of-flight–MS (CE–TOF–MS), applied to analyse biogenic amines in wine samples were investigated. A group of five amines was selected as case study (namely, putrescine, cadaverine, histamine, phenylethylamine and tyramine) since they are the most frequently biogenic amines found in wines. Biogenic amines were determined in three red wines and one white wine showing, as expected, a higher concentration in red wines. Moreover, CE–IT–MS and CE–TOF–MS were compared regarding their capacity to detect other biogenic amines different to the selected ones in wine

samples, showing CE–TOF–MS a much better capability (i.e., putrescine, cadaverine, histamine, phenylethylamine, tyramine, triptamine, spermidine, spermine, ethanolamine and isoamylamine were identified by CE–TOF–MS in a single analysis) (Simó *et al.*, 2008).

A screening method based on HPLC coupled on-line to a radical scavenging detection system and mass spectrometry (MS) was used to identify and characterise antioxidant compounds in two fruit wines from the family Myrtaceae, *Syzygium cumini* (variously called jambul or jamunor or jamblang) and *Cleistocalyx nervosum* var. *paniala* (Ma kiang). The active compounds were identified by comparison of retention time and mass data with the authentic standards and with the published mass spectra assisted by multidimension information from a liquid chromatography combined with electrospray ionisation tandem mass spectrometry (LC–ESI–MS/MS) and a radical scavenging detection. Major antioxidants found in *S. cumini* wine were complicated mixture of hydrolysable tannins and the fruit acids. A trace amount of an anthocyanin, malvidin -3-o-*p*-coumaroyl glucoside was also found. In *C. nervosum* var. *paniala* wine, the active compounds were identified as hydrolysable tannins and their derivative, i.e. caffeoylquinic acid, gallic acid, ellagic acid and methoxymethylgallate (Nuengchamnong and Ingkaninan, 2010).

8.2.4 Polymerase chain reaction (PCR)

A new real-time PCR (RTi–PCR)-based procedure was developed for the rapid and specific detection and quantification of *Aspergillus carbonarius* in wine grapes. The procedure includes the use of the pulsifier equipment to remove conidia from grapes which prevents releasing of PCR inhibitors, and DNA extraction with the EZNA® fungal DNA kit (Omega Bio Tek). It reduced the time for *A. carbonarius* DNA extraction from grapes to 30 min. Two specific primers (AcKS10L/AcKS10R) delimiting a 161 bp fragment and a probe were designed and directed to the β-ketosynthase domain of a polyketide synthase from *A. carbonarius*. Specificity was confirmed by testing primers towards purified DNA from 52 fungal strains, including reference and food isolates (Selma *et al.*, 2008).

Vaudano and Garcia-Moruno (2008) proposed a rapid method for *S. cerevisiae* strain identification based on multiplex PCR analysis of polymorphic microsatellite loci. Simple DNA extraction without the use of phenol, followed by a rapid PCR procedure optimised for multiplex amplification of loci SC8132X, YOR267C and SCPTSY7 and band pattern analysis of the fragments generated by agarose and polyacrylamide gel electrophoresis, has allowed us to distinguish among a panel of 30 commercial wine strains. This method was successfully performed in an ecological study where dominance between two strains was checked at two fermentation temperatures: 15 and 20 °C. The method should be useful for routine and low-budget discrimination of yeast strains, both in the wine and yeast production industries.

Zygosaccharomyces bailii is a major food and beverage spoilage organism. Existing methods for its detection involve lengthy enrichment techniques and then the result does not always differentiate between *Z. bailii* and *S. cerevisiae*. In this

work, we developed a quantitative real-time PCR assay for the rapid detection of *Z. bailii* from fruit juices and wine even in the presence of non-target DNA. Primers were designed to the gene coding for the D1/D2 loop of the 26S ribosomal RNA subunit producing a single PCR product with a melting point of 83.5 °C (Rawsthorne and Phister, 2006).

As many rice wine brewers identify the cultivar name of the material rice, authentication technology is necessary. The problems are: (i) decomposition of DNAs during the fermentation; (ii) contamination of DNAs from microorganisms; and (iii) coexistence of PCR inhibitors, such as polyphenols. The present authors improved the PCR method by: (i) lyophilising and pulverising the rice wine to concentrate DNAs; (ii) decomposition of starches and proteins so as not to inhibit DNA extraction by the use of heat-resistant amylase and proteinase K; and (iii) purification of the template DNA by the combination of CTAB method and fractional precipitation by 70% EtOH. It became possible to prepare the template DNAs for PCR from the rice wine. The sequences of the amplified DNAs by PCR were ascertained to be same as those of material rice. Mislabelling of material rice cultivars was detected by PCR using the commercial rice wine. It became possible to extract and purify the template DNAs for PCR from the rice wine and to differentiate the material rice cultivars by the PCR using the rice wine as a sample (Ohtsubo *et al.*, 2008).

8.2.5 Sensory analysis

Bakker and Arnold (1993) showed that using a panel of tasters, minimal training was needed to carry out sensory profiling when the tasters were not required to develop or agree to usage of a consensus set of terms. Analysis of the data revealed that all three transformations done with GPA, translation, rotation/reflection and scaling were highly significant. It was also found that the use of analytical colour measurements could be of considerable use in the assessment of the sensory characteristics of wines related to colour, having a good predictive value.

Le Fur *et al.* (2003) made an attempt to correlate both sensory and combined headspace GC–O analyses. Wines tested by sensory analyses and the headspace samples analysed by GC–O were described by a heterogeneous vocabulary distributed into nine overall classes of descriptors. It was found that the dynamic headspace analysis induced a distortion with respect to sensory data, which systematically affected the perception of both spicy and herbaceous characters of wines. Gawel *et al.* (2001) showed that astringency in red wine can manifest itself in many subtle yet complex forms, and that tasters can be effectively trained to reproducibly discriminate and rate the intensities of astringent subqualities elicited by young dry red wines.

Thirty-six semi-industrial fermentations were carried out in Catalunya, Spain with six different yeast strains in order to assess differences in the wines' chemical and volatile profile. Two of the tested strains (Y3 and Y6) showed the fastest fermentation rates throughout three harvests and on two grape varieties. The wines fermented by three of the tested strains stood out for their high amounts of esters,

and possessed the highest fruity character. Wines from strains producing low amounts of esters and high concentrations of medium-chain fatty acids, higher alcohols and six-carbon alcohols were the least appreciated at the sensory analysis. The data showed how yeast strain affects the final chemical and volatile composition of cava base wines and has repercussions on their sensory profile, independently of must variety and harvest year (Torrens *et al.*, 2008).

Fischer *et al.* (1999) employed descriptive analysis in order to investigate the sensory properties of commercial Riesling wines from two vintages, five wine estates and six vineyard destinations within the Rheingau viticulture region in Germany. Based on the number of significant F-ratios among ten odour and four orally perceived attributes, vintage and wine estate proved to have a similar impact as vineyard designation. Since PCA revealed substantial variations, even within the same vineyard designation, the authors claim that a classification system focusing on geographic origin would be rather confusing for consumers. The results of a study carried out by Carlucci and Monteleone (2001) disclosed that a step-by-step approach to analysing sensory data (fixed ANOVA model on raw and scaled data) can eventually be suitable for validating the sensory profile of typical regional food products. The intensity of sensory descriptors does not distinguish amongst the products. Furthermore, the results were not affected by assessor discrepancy and so can be referred to the typical sensory profile of young Aglianico red wine (Table 8.6).

The aroma of sweet Fiano wine, the most representative non-aromatic white wine variety in southern Italy, was evaluated by sensory and instrumental analysis to determine the influence of grape overripeness, drying and *Botrytis cinerea* infection. Sensory descriptive analysis was used to evaluate the sensory properties of wines. GC–MS, GC alone, and GC–O techniques were used to identify and to determine the concentration of free and bound volatile compounds and their odour impact. Sensory descriptive analysis revealed that the descriptors such as citrus jam, dried apricot, dried figs, prune, honey and coconut, occur in sweet Fiano wine more than in dry Fiano wine. Thirty-five free volatile compounds had higher concentrations in sweet Fiano wine than in dry Fiano wine; these components were mostly terpenes, β-damascenone, lactones, aldehydes and ketones. The main odour impact compounds in sweet Fiano wine were nerol, geraniol and linalool (orange flowers), vitispirane (camphor), lactones such as γ-nonalactone (coconut), δ-decalactone and γ-decalactone (apricot) and 1-octen-3-ol (mushroom) (Genovese *et al.*, 2007).

Taste expectations can influence taste evaluations. It is not known, however, whether the environmental cues that influence taste expectations – such as suggestible names and brand labels – can have an impact on the intake volume of companion foods. Adult diners who ordered a prix-fixe restaurant meal were given a complimentary glass of wine that had been re-labelled to induce either favourable ('new from California') or unfavourable ('new from North Dakota') taste expectations. An analysis of plate waste indicated that those who believed they had been drinking California wine ate 12% more of their meal than those who instead believed they drank North Dakota wine. In combination with a sensory-based lab

Table 8.6 Sensory analysis of red and white wines by trained panelists

Country	Wine/ no. of samples	Method of analysis	Number of judges	Number of attributes examined/scale	References
Portugal	39 port wines/78	Blind	7 male	9/0–9	Bakker and Arnold, 1993
Canada	60 samples/3 sites	Randomised coded samples	11–15 Agriculture Canada staff	8 attributes. Intensity recorded on a 5 cm unstructured line scoresheet	Reynolds *et al.*, 1996
France	56 Champagne wines	Two preliminary sequences of 12 and 6 sessions	18 volunteers	64 attributes reduced to 19 objective attributes Fixed choice profiling. Intensity measured on an unstructured ratio scale from 0 to 10	Vannier *et al.*, 1999
Germany	20 Riesling wines/5 estates	Reference standards prepared daily	9 enology students	10 odour attributes/4 oral attributes. Intensity ratings scored on 10 cm unstructured scale	Fischer *et al.*, 1999
France	9 wines/ 2 varieties	Two weeks training (4 sessions)/ triangular test	17 students	20 attributes reduced to 12. Intensity measured on a scale from 0–5 (no and highest perception, respectively)	Kotseridis *et al.*, 2000
Greece	21 red and 12 white/33	Randomised presentation	10	7/continuum and unstructured (from none to extreme)	Kallithraka *et al.*, 2001a
Australia	72 red wines	Panelists' ability assessment	14 tasters with 5 years' experience	24/4 classes astringency vocabulary development	Gawel *et al.*, 2001
Italy	16 young wines south Italy wines/ 4 regions	Preliminary sessions (10 + 6)	8 food science students	25 initial attributes reduced to 15 based on citation frequency. The intensity was rated on a 10 cm unstructured scale	Carlucci and Monteleone, 2001
Chile	16 samples (2 wines, 4 temp., 2 replicates)	Six preliminary training sessions	11 employees	15 sensory attributes (2 visual, 8 aroma, 5 attributes for evaluation of storage effect). Intensity scale ranging from low intensity to high intensity on a 15 cm distance	Silvertsen *et al.*, 2001
Canada	14 Riesling wines/2 regions	Initial training session	10 students and staff members	15 sensory attributes (10 aroma, 2 taste and 3 other attributes). The intensity was rated on a 10 cm unstructured scale (anchored with terms none, moderate and intense at 1, 5 and 9 cm)	Douglas *et al.*, 2001
France	6 Chardonnay wines	4 sessions/ selection of terms	14 (5 males, 9 females)	144/9 classes	Le Fur *et al.*, 2003

study, these results show that environmental cues – such as label-induced sensory expectations – can have a far-reaching impact on the food intake of companion foods (Wansink *et al.*, 2007).

Vannier *et al.* (1999) employed a fixed choice profile technique to detect sensory differences by qualitatively and quantitatively characterising gustatory and olfactory properties of over 56 Champagne wines. The original 64 attributes were reduced to a working set of 19 objective attributes showing a good range of scores, low incidence of zeros and no hedonic aspects. The trained panelist repeatability as well as their discriminative efficiency was estimated.

In Ontario, Canada, Douglas *et al.* (2001) demonstrated that Riesling wines from two Canadian terroirs were distinctly different by applying univariate and multivariate statistics. Riesling and Gewürztraminer wines are often fermented without a great deal of enological intervention. They might be expected to 'express' the effects of viticulture variation. In contrast, Chardonnay, is known to be a 'winemakers' grape' that is subject to a broad range of enological treatments. Enological treatments such as barrel fermentation, malo-lactic fermentation and barrel ageing would be expected to modify the sensory profile of the finished wine, thereby masking the detection of viticultural differences. Schlosser *et al.* (2005) examined 24 VQA Chardonnay wines from three regions ('Bench', 'Lakeshore Plain' and 'Lakeshore') of the Niagara Peninsula appellation (Ontario, Canada), plus three international Chardonnays, to investigate the effect of site using chemical and sensory analyses. Differences between proposed sub-appellations were found for five aroma terms, three flavour terms, one mouthfeel term, and colour. Wines originating from the 'Bench' were characterised as being highest in apple, melon, and citrus aromas, melon and citrus flavour and acidity, and lowest in grassy and earthy aromas. 'Lakeshore' and 'Lakeshore Plain' regions were characterised by grassy aroma and flavour. With the exception of melon aroma and colour, no attributes between the 'Lakeshore' and 'Lakeshore Plain' regions were different. In the case of red wines, chemical and sensory analysis were performed on 41 commercially available red Niagara Peninsula Bordeaux-style wines to determine differences that might support the designation of three aforementioned sub-appellations (Kontkanen *et al.*, 2005). As with the Riesling and Chardonnay studies, there were clear effects of the sub-appellations in terms of several aroma, flavour and colour attributes.

In Table 8.6, a summary is given of the sensory analysis methods (i.e.blind test, attributes, intensity scale) employed on red and white wines of various countries by trained panelists.

8.3 Multivariate analysis

8.3.1 Principal component analysis (PCA)

PCA implementation of all instrumental and sensory data of a collection of Greek wines did not show any major differences among either red or white wine categories, despite their different geographical origins. On the other hand, the PCA

of anthocyanins and sensory analysis resulted in effective classification of red wines into two groups: north Greek and south Greek (Peloponnese and islands) wines. Therefore, anthocyanins emerged as the crucial factor in terms of red wine classification, whereas minerals (ions) and phenols did not allow any valid clustering (Kallithraka *et al.*, 2001a).

Several lots of Chardonnay and Grenache blanc grapes were treated with pomace contact and hyperoxidation prior to classification. Variations in the chemical and sensory properties were examined by PCA and factor analyses. PCA was performed on the compositional and browning capacity data to illustrate the relationships among the analytical variables and the wines. Three PCs were shown to be the most significant ones accounting for 87% of the total variance. The PCA of the sensory data showed that pomace contact was beneficial to Chardonnay wines and detrimental to Grenache wines (Cheynier *et al.*, 1989).

Lozano *et al.* (2008) reported a novel application of an electronic nose (e-nose) for recognition and detection of wine ageing. Two different measurements were performed with the following samples: first, in an experimental cellar the same wine was aged in different types of oak barrel (French and American oak) and during different times (0, 3, 6 and 12 months); and second, several wines were made with the same grape variety and from different wine cellars aged in French and American oak. This identification has a great importance for origin denominations for control of frauds. The e-nose is home-developed and home-fabricated for this purpose: a tin oxide multisensor prepared with RF (radio frequency) sputtering onto an alumina substrate and doped with chromium and indium is used. The sampling method employed is static headspace followed by a dynamic injection. Linear techniques like PCA and non-linear ones like probabilistic neural networks (PNN) are used for pattern recognition. A classification success rate (correct predicted number over total number of measurements) of 97% and 84% was achieved in detection of the different ageing treatments among the wines tested.

Changes in phenolic compounds during accelerated browning in white wines from two Spanish varieties were evaluated using PCA. Two variables produced the best differentiation between the wines of the two varieties studied (Mayen *et al.*, 1997).

Cozzolino *et al.* (2008) investigated the potential use of a direct headspace – MS–e-nose combined with chemometrics as a rapid, objective and low-cost technique to measure aroma properties in Australian Riesling wines. Commercial Riesling wines were analysed using an MS–e-nose instrument and by a sensory panel. The MS–e-nose data were analysed using PCA and PLS1 regression using full cross-validation ('leave one out' method).

8.3.2 Cluster analysis

Attempts to differentiate vinegars were based on the kind of raw material employed or on the process involved (Guerrero *et al.*, 1994). Seven significant factors were used for PCA and, with these factors, 76% of total variance was explained. Cluster analysis was applied for searching natural grouping among the samples.

Thus, the data matrix was subjected to a hierarchical agglomerative cluster analysis of cases. A dendrogram (tree diagram) was obtained, taking the Euclidean distance as metric and the Ward method as an amalgamation rule. These two methods have assumed knowledge of the number of classes. Some variables were selected for the classification according to manufacture and some others for the classification according to geographical origin with the use of LDA.

Inductively coupled plasma–optical emission (ICP–OES), in combination with different chemometric approaches, was used to verify the origin of different red wine samples from Utiel-Requena, Jumilla, Yecla and Valencia protected designation of origin (PDO). The ability of multivariate analysis methods, such as hierarchical cluster analysis (HCA), principal component analysis (PCA), classification and regression trees (CARTs) and discriminant analysis (DA), to achieve wine classification from their elemental concentrations has been investigated. The calculations were performed using 38 variables (concentrations of Al, Ba, Be, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Gd, Ho, K, La, Li, Lu, Mg, Mn, Mo, Na, Nd, Ni, Pb, Pr, Sc, Se, Sm, Sr, Tb, Ti, Tm, V, Y, Yb and Zn, at mg/L concentration, determined by ICP–OES) (Gonzálvez *et al.*, 2009).

An HPLC method with photodiode array and MS detection was applied to study the changes in non-anthocyanin phenolic compounds from 47 red wines as a result of different technological treatments. The procedures used included: malo-lactic fermentation in barrels or in stainless steel containers, the ageing of wines or not in the presence of lees, including periodic stirring of the lees, racking, clarification, cold stabilisation and filtration. Wine samples were taken before and after malo-lactic fermentation and during the three months of the experiment until they had been aged for 14 months in oak barrels. Cluster analysis, analysis of variance and stepwise discriminant analysis were applied to the wine data (Hernández *et al.*, 2006).

8.3.3 Discriminant analysis

Marsala is a popular Sicilian fortified aged wine with ancient tradition. Marsala is exported all over the world and is considered one of the most important dessert wines. The aim of this study was to determine the concentration of carbohydrates, polyphenols and heavy metals in different types of Marsala wines and to achieve statistical classifications by stepwise forward canonical discriminant analysis (CDA). CDA, performed using heavy metals as independent variables, showed that Superiore Ambra Secco and Vergine Marsalas were not discriminated, whereas a good separation among Fine Oro Dolce, Superiore Riserva and Fine Ambra Secco wines was obtained. Finally, an overall statistical model showed that the variables with the highest discriminant power were: tyrosol, caffeic acid, procyanidin B1, catechin, quercetin, kaempferol, lactose, rhamnose, zinc, copper and lead (La Torre *et al.*, 2008).

The presence of phenolic compounds has been extensively studied in Sherry and Balsamic vinegars due to their impact on quality, but little work has been done on red wine vinegars. Phenolic compounds were monitored during the

acetification of red wine vinegars produced by surface culture in different wood barrels (oak, chestnut, acacia and cherry). A total of 166 samples were analysed for phenolic compounds using HPLC–DAD, the total phenol index and the total monomeric anthocyanins. Twelve phenolic compounds were identified corresponding to phenolic acids, flavanols and stilbens. Most phenolic acids did not significantly change their concentrations in the different acetifications. (+)-Catechin and resveratrol glycoside underwent significant decreases during acetification while gallic acid and gallic ethyl ester increased substantially for those vinegars produced in chestnut wood. The concentrations of phenolic compounds were used to build the functions for discriminant analysis (Cerezo *et al.*, 2008).

The voltammetric responses on selected white wines of different vintages and origins have been systematically collected by three different modified electrodes, in order to check their effectiveness in performing blind analysis of similar matrices. The electrode modifiers consist of a conducting polymer, namely poly (3,4-ethylenedioxythiophene) (PEDOT) and of composite materials of Au and Pt nanoparticles embedded in a PEDOT layer. Wine samples were tested, without any prior treatments, with differential pulse voltammetry technique. The subsequent chemometric analysis was carried out both separately on the signals of each sensor, and on the signals of two or even three sensors as a unique set of data, in order to check the possible complementarity of the information brought by the different electrodes. After a preliminary inspection by PCA, classification models were built and validated by PLS–DA. The discriminant capability was evaluated in terms of sensitivity and specificity of classification; and in all cases quite good results were obtained (Pigani *et al.*, 2008).

8.4 Wine traceability

Traceability means the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be, incorporated into a food or feed, through all stages of production, processing and distribution. This definition is from the Regulation (EC) No. 178/2002 of the European parliament and the council laying down the general principles and requirements of food law.

Consumer perceptions regarding traceability were investigated by means-end-chain laddering. Consumers in four European countries were questioned about the benefits they associate with traceability-related attributes. The benefits consumers associate with traceability are in terms of health, quality, safety and control, of which the latter was associated with trust and confidence. These benefits were similarly important in the countries investigated. Importantly, both quality and safety were shown to be related to traceability in the consumers' minds with quality implying safety. The results show that traceability may contribute to improving consumer confidence (van Rijswijk *et al.*, 2008).

Traceability is becoming a method of providing safer food supplies and of connecting producers and consumers. Recent diseases such as bovine spongiform

encephalitis and the questions concerning genetically modified organisms mean systems that enable control of each link in the food chain have become particularly relevant. Possible technical resources were clarified by analysing assessment criteria obtained from studies of alphanumerical codes, bar codes, and radio frequency identification (RFID) (Regattieri *et al.*, 2007).

Traceability systems are constructions which enable traceability. There are six essential elements of traceability constituting an integrated agricultural and food supply chain traceability system (Opara, 2003). These elements are: (i) Product traceability: defines the physical location of a product at any stage in the supply chain; (ii) Process traceability: ascertains the type of activities that have affected the product during the growing and post harvest operations (what, where and when); (iii) Genetic traceability: determines the genetic composition of the product and includes information on the type and origin (source, supplier); (iv) Inputs traceability: determines type and origin (source, supplier) of inputs, e.g. fertilisers, additives used for preservation or transformation of the raw materials into processed products; (v) Disease and pest traceability: traces the epidemiology of microbiological hazards and pests which may contaminate food products; (vi) Measurement traceability: relates individual measurement results through calibrations to reference standards and assures the quality of measurements by observing various factors which may have impact on results (such as environmental factors, operator, etc.).

Liquid food production often involves continuous processing. This leads to problems in traceability systems due to mixing zones and therefore indistinct batch identities causing difficulties with regard to withdrawals or recalls. Skoglund and Dejmek (2007) outlined the possible use of the concept of dynamic simulation to improve the handling of batch identities in continuous production of liquid food.

An RFID tracking system was developed after wine companies from the Bordeaux region in France expressed concerns over the difficulty of ensuring that the quality of their wines was preserved during handling, transportation and distribution. Maintaining temperatures in a reasonable range during shipment and storage plays an important part in preserving the quality of fine wine and spirits. However, there is often no record of variations of temperatures after the wine has left the wine producer's cellars. RFID bottle tag provides secure traceability and simplifies inventory management. Assuring provenance builds brand value and prevents counterfeiting. The direct connection to the consumer allows the producer to build a more intimate relationship and market more effectively (Launois, 2008).

Reliable methods for DNA traceability in grapevines and wines are in great demand for protecting areas of declared origin and detecting potential transgenic events. Currently, real-time PCR is one of the most promising tools for conducting plant and microorganism DNA assays and detecting genetically modified material. However, in grape, quantitative analysis based on PCR is lagging behind. Moreover, in musts and wines, efficient DNA extraction and amplification need to be developed. In the present research, we compared several DNA extraction proce-

dures on various grape tissues, musts and wines of the Trentino-Südtirol Region (Savazzini and Martinelli, 2008).

An easily automatable flow-injection method for the determination of total catechins is reported. The method is based on the reaction of vanillin in acid medium to yield a coloured product with maximum absorption at 500 nm. After optimisation by the univariate and multivariate approaches as required, the linear range was established (between 10 and 90 mg/L and 10 and 250 mg/L for white and red wines, respectively). Then, the assessment of the proposed versus the reference method was studied in terms of repeatability (2.57 mg/L), reproducibility (3.56 mg/L) (no differences were found), detection and quantification limits (not far from those of the reference method and acceptable for the determination of catechins in any type of wine), traceability (excellent correlation under all conditions) and sample throughput (23 samples per hour for the proposed method versus three samples per hour for the reference method) (González-Rodríguez *et al.*, 2002).

In the European Union, EU Directive 178/2002 sets out a general framework for food safety, including traceability (see appendix to this chapter). The International Organization for Standardization (ISO) introduced in the beginning of 2006 two new standards that define the requirements for a traceability system within a food safety management system and the data that need to be retained (ISO 22000:2005). ISO's technical committee 34 (food products) has also established a standard ISO/FDIS 22005:2007 Traceability in the feed and food chain – general principles and basic requirements for system design and implementation (http://www.iso.org).

ISO 22005:2007; Traceability in the feed and food chain – general principles and basic requirements for system design and implementation, establishes the principles and requirements for the design and implementation of a feed and food traceability system. This standard allows organisations operating at any step of the food chain to: (i) trace the flow of materials (feed, food, their ingredients and packaging); (ii) identify necessary documentation and tracking for each stage of production; (iii) ensure adequate coordination between the different actors involved; and (iv) require that each party be informed of at least his direct suppliers and clients (http://www.intracen.org/tdc/Export%20Quality%20Bulletins/EQM85eng.pdf).

8.5 HACCP systems for wine

Traceability can be seen as one aspect of a wider system of food safety management. ISO includes traceability in the Hazard Analysis and Critical Control Point (HACCP) food safety management system set out in ISO 22000:2005. This system involves identifying hazards during production and the key points (known as critical control points or CCPs) at which they can be prevented or eliminated (Kourtis and Arvanitoyannis, 2001). The main stages for wine production are schematically presented in Fig. 8.1.

Grape harvesting is a critical control point (CCP) comprising both physical and

chemical hazards. Physically, the grapes should be sound without rotten parts; otherwise oxidative and microbial contamination can rapidly develop. Therefore, harvesting should be conducted with the greatest possible care, and an efficient disease management system should be applied (Dibble and Steinke, 1992; Ellison *et al.*, 1998).

The next CCP is destemming. Destemming includes the removal of stems, leaves and grape stalks before crushing. This procedure has several advantages because the total volume of processed product drops by 30%, thus resulting in smaller tanks and eventually increasing the product's alcoholic concentration (Tsakiris, 1996). Crushing typically immediately follows stemming, since some crushing of the fruit occurs during stemming. The released juice is highly susceptible to oxidative browning and microbial contamination. It is very important to avoid crushing the seeds to preclude contaminating the must with seed oils, the oxidation of which could produce rancid odours and constitute an undesirable source of bitter tannins (Zoecklein *et al.*, 1994).

Maceration is the breakdown of grape solids after crushing of grapes and represents the next CCP. While maceration is always involved in the initial stage of red wine fermentation, the long-standing trend has been to limit maceration in white wine production. Temperature and duration of maceration depend on grape and wine variety. Usually for white and rosé wines the maceration time is less than 24 h, red destined for early consumption is macerated for three to five days and red for ageing is macerated from five days to three weeks. Fermentation usually occurs during this or at the end of maceration. The amount of the antimicrobial to be used, usually added to white musts that are most sensitive to oxidation, depends on the crop health and maceration temperature. Sulphur dioxide has a distinct advantage over other antimicrobial agents, because of the relative insensitivity of the wine yeasts to its action. However, it is also toxic, or inhibitory, to most bacteria and yeasts (i.e., *Candida*, *Pichia*, *Hansenula*) at low concentrations (Farkas, 1984) and has a rather low retention capability after the clarification step (Gnaegi *et al.*, 1983).

Pressing represents the next CCP. The must is allowed to remain in the press for several minutes, during which juice runs out under its own weight. Depending on the press type (horizontal, pneumatic, continuous screw presses), the produced juice and wine fractions vary in terms of their physicochemical properties. Combining different wine fractions, the winemaker can influence the character of the wine. However, a potential hazard might be the occurrence of oxidation reactions if there is a delay in the process (Lichine, 1985).

The next important CCP is fermentation itself. Alcoholic fermentation is usually carried out by strains of *S. cerevisiae* because this species is remarkably tolerant to high sugar, ethanol and sulphur dioxide concentrations and also grows at low pH values typical for grape must (pH 3.2–3.4). The culture of *S. cerevisiae* is either part of the indigenous microflora or may be partially added to achieve a population of about 10^5–10^6 cells/mL in the must (Constanti *et al.*, 1997). Possible contamination of must with killer yeasts (a property mainly present in wild strains of *Saccharomyces* but also in other yeast genera such as *Candida*, *Debaryomyces*,

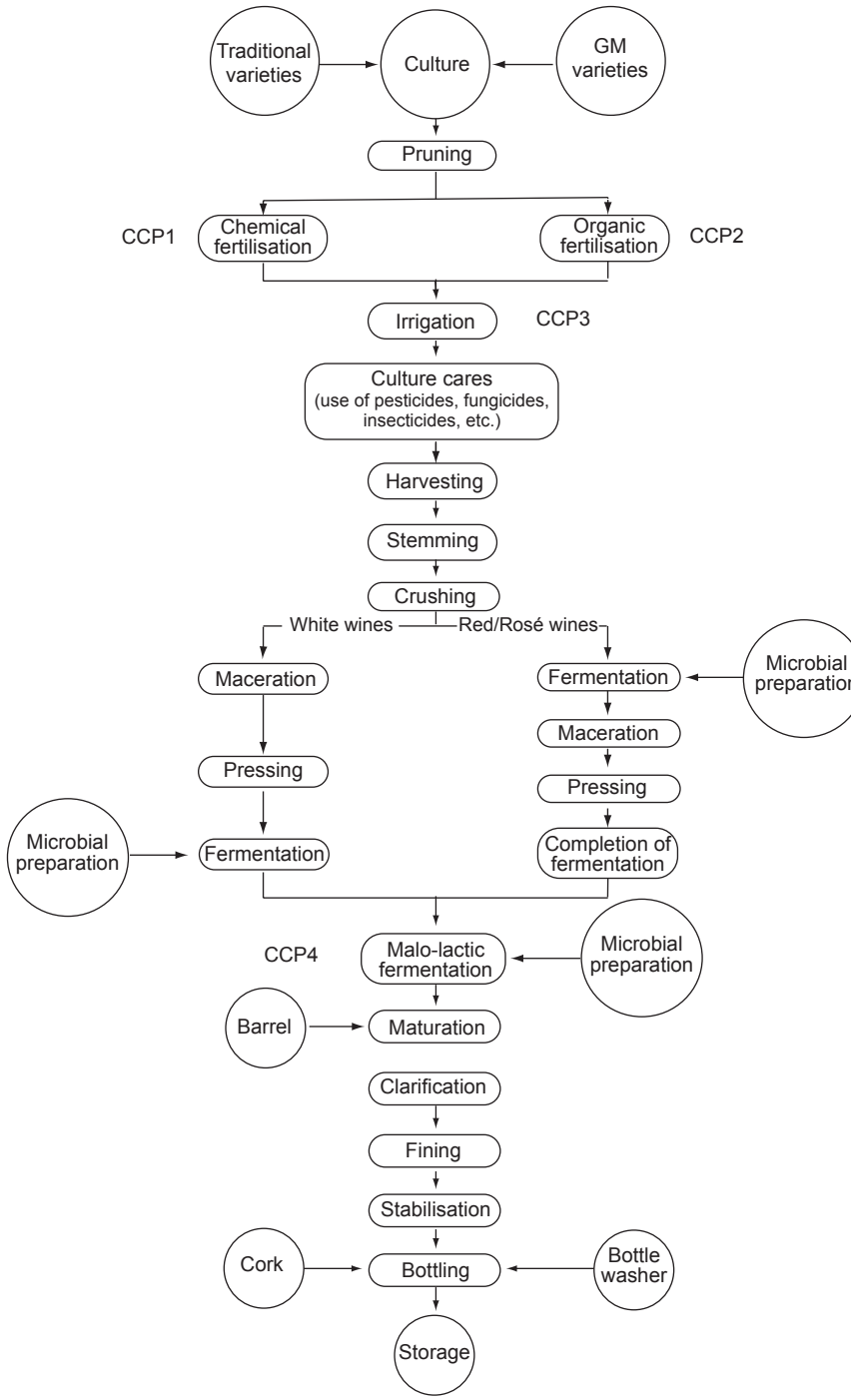

Fig. 8.1 Flow diagram of wine production

Table 8.7 Summary of hazards, CCPs, CLs, monitoring, corrective actions and responsible personnel for wine production

Process step	Hazards (P, C, M)	Preventive measures	CCPs	Critical limits	Monitoring procedures	Corrective actions	Responsible personnel
Culture	P	Cultivation of non-GM varieties	Allergies, change in the immunity system	The cultivation of GM products is forbidden in Greece	Existence of a company certificate	Farmers' training	Farmer
Pruning	M	Clear pruning hooks	Presence of pathogens	Absence of pathogens in tools	Microbiological analysis	Farmers' training	Farmer
Chemical fertilisation	C	Balanced fertilisation	Residues of nitrates	$[NO_3^-]$ = 50 mg/L According to Directive 2000/60/EC	Soil chemical analysis	Farmers' training	Farmer
Organic fertilisation	M	Manure from certified suppliers	Presence of pathogens (*E. coli*)	Absence of pathogens in grapes	Microbiological analysis	Change supplier and instruct farmers	Farmer
Irrigation	C	Improper water quality Inadequate quantity of water	Plant drying	Control of permanent withering point	Water chemical analysis	Control of water quality and quantity	Farmer
Culture cares	C	Use of pesticides and fungicides at the correct time	Residues of pesticides and fungicides	Per pesticide according to Codex Alimentarius	Specific chemical analysis	Delay of harvesting date	Farmer
Harvesting	P	Careful handling	Sound fruit without rotten parts	Reduced to acceptable level	Inspection during harvesting	Instruct personnel	Trained personnel
	C	Specify the last day of applying pesticides	Pesticides residues	Per pesticide according to Codex Alimentarius	Specific chemical analysis	Delay of harvesting date	Quality control manager

Table 8.7 *cont.*

Process step	Hazards (P, C, M)	Preventive measures	CCPs	Critical limits	Monitoring procedures	Corrective actions	Responsible personnel
Fermentation	C	Material without heavy metals, corrosion checks	Heavy metals presence	As < 0.2, Cd < 0.01,Cu < 1, Pb < 0.3 (mg/L)	Specific chemical analysis	Rejection of specific batch, demetallisation	Quality control manager
		Certified suppliers, control of the product	Pesticide residues	Per pesticide according to Codex Alimentarius		Rejection of specific batch	
		Careful maintenance of equipment, use of non-toxic glucose	Residues of ethylene glycose and detergents	Absence		Rejection of specific batch, dilution with	
		GMP	Methanol content	300 mg/L (red), 150 mg/L (white and rose)		large quantities, machinery modification	
		Avoid intensive fertilisation Avoid high temperatures Use proper yeast cultures Employ urease	Ethyl carbamate formation	< 15 (30) and < 60 (100) ppb for table and desert wines in USA (Canada), respectively	Gas chromatography	Rejection of specific batch, dilution with large quantities	
Malolactic fermentation	M	Certified suppliers, strictly following instructions	Microbiological contamination	100% clean	Microbiological analysis	Change supplier or method preparation	Quality control manager
Maturation	M	Certified suppliers, proper barrel decontamination	Nicrobiological contamination	Absence of yeasts, moulds and lactic acid bacteria	Microbiological analysis	Rewash the barrel	Quality control manager

Stabilisation	C	GMP, materials without heavy metals, calculation of ferrocyonide needed according to Fe presence	Heavy metals presence Residual ferrocyonide	As < 0.2, Cd < 0.01,Cu < 1, Pb < 0.3 (mg/L) Fe: 5 mg/L	Specific chemical analysis	Rejection of specific batch, demetallisation Filtration or dilution with larger quantities	Quality control manager
Bottling	C	GMP, materials without heavy metals	Heavy metals presence	As < 0.2, Cd < 0.01,Cu < 1, Pb < 0.3 (mg/L)	Specific chemical analysis	Rejection of specific batch, demetallisation	Quality control manager
		Certified suppliers, control of the product	Pesticide residues	Per pesticide according to Codex Alimentarius		Rejection of specific batch	
		GMP, avoidance of high doses	Detergent and SO_2 residues	None 175 mg/L (red), 225 mg/L (white and rosé)		Modification of the CIP, rejection of batch	

Table 8.8 Determination of critical control points for wine production

STEP	HAZARD	Q1: Do control measure(s) exist?	Q2: Is the step specifically designed to eliminate or reduce the likely occurrence of a hazard to an acceptable level?	Q3: Could contamination with identified hazard(s) occur in excess of acceptable level(s) or could these increase to acceptable levels?	Q4: Will a subsequent step eliminate identified hazards or reduce likely occurrence to an acceptable level?	CCP
Culture	M:	YES	NO	NO	–	CP
	C:	YES	NO	NO	–	CP
	P:	YES	NO	NO	–	CP
Pruning	M:	YES	NO	NO	–	CP
	C:	YES	NO	NO	–	CP
	P:	YES	NO	NO	–	CP
Chemical fertilisation	M:	YES	NO	YES	NO	**CPP1**
	C:	YES	NO	YES	NO	**CPP1**
	P:	YES	NO	YES	NO	**CPP1**
Organic fertilisation	M:	YES	NO	YES	NO	**CCP2**
	C	YES	NO	YES	NO	**CPP2**
	P:	YES	NO	YES	NO	**CCP2**
Irrigation	M:	YES	NO	YES	NO	**CPP3**
	C:	YES	NO	YES	NO	**CPP3**
	P:	YES	NO	YES	NO	**CPP3**
Cultural practices	M:	YES	NO	NO	–	cp
	C:	YES	NO	NO	–	cp
	P:	YES	NO	NO	–	cp
Harvesting	M:	YES	NO	NO	–	cp
	C:	YES	NO	NO	–	cp
	P:	YES	NO	NO	–	cp
Stemming	M:	YES	NO	NO	–	cp
	C:	YES	NO	NO	–	cp
	P:	YES	NO	NO	–	cp
Crushing	M:	YES	NO	NO	–	cp

	C:	YES	NO	NO	–	cp
	P:	YES	NO	NO	–	cp
Maceration	M:	YES	NO	NO	–	cp
	C:	YES	NO	NO	–	cp
	P:	YES	NO	NO	–	cp
Pressing	M:	YES	NO	NO	–	cp
	C:	YES	NO	NO	–	cp
	P:	YES	NO	NO	–	cp
Fermentation	M:	YES	NO	NO	–	cp
	C:	YES	NO	NO	–	cp
	P:	YES	NO	NO	–	cp
Malolactic fermentation	M:	YES	NO	YES	NO	**CPP4**
	C:	YES	NO	YES	NO	**CPP4**
	P:	YES	NO	YES	NO	**CPP4**
Maturation	M:	YES	NO	NO	–	cp
	C:	YES	NO	NO	–	cp
	P:	YES	NO	NO	–	cp
Clarification	M:	YES	NO	NO	–	cp
	C:	YES	NO	NO	–	cp
	P:	YES	NO	NO	–	cp
Fining	M:	YES	NO	NO	–	cp
	C:	YES	NO	NO	–	cp
	P:	YES	NO	NO	–	cp
Stabilisation	M:	YES	NO	YES	NO	**CPP5**
	C:	YES	NO	YES	NO	**CPP5**
	P:	YES	NO	YES	NO	**CPP5**
Bottling	M:	YES	NO	NO	–	cp
	C:	YES	NO	NO	–	cp
	P:	YES	NO	NO	–	cp
Storage	M:	YES	NO	NO	–	cp
	C:	YES	NO	NO	–	cp
	P:	YES	NO	NO	–	cp

Table 8.9 ISO 22000 analysis worksheet for the determination of prerequisite programs for wine production

Processing step	Are the technical infrastructure and the preventative maintenance program adequate?	Is it feasible to evaluate them?	Do they contribute in the control of recognisable food safety hazards?	Does the effectiveness of the remaining control measures depend on them?	Is it a prerequisite program?
Culture	YES	YES	NO	YES	YES
Pruning	YES	YES	NO	YES	YES
Chemical fertilisation	YES	YES	NO	NO	NO
Organic fertilisation	YES	YES	NO	NO	NO
Irrigation	YES	YES	NO	NO	NO
Culture cares	YES	YES	NO	YES	YES
Harvesting	YES	YES	NO	YES	YES
Stemming	YES	YES	NO	YES	YES
Crushing	YES	YES	NO	YES	YES
Maceration	YES	YES	NO	YES	YES
Pressing	YES	YES	NO	YES	YES
Fermentation	YES	YES	NO	YES	YES
Malolactic fermentation	YES	YES	NO	NO	NO
Maturation	YES	YES	NO	YES	YES
Clarification	YES	YES	NO	YES	YES
Fining	YES	YES	NO	YES	YES
Stabilisation	YES	YES	NO	NO	NO
Bottling	YES	YES	NO	YES	YES
Storage	YES	YES	NO	YES	YES

Hansenula, *Kluyveromyces*, *Pichia*, *Torulopsis* and *Cryptococcus*) may result in stuck fermentation (van Vuuren and Jacobs, 1992).

A crucial CCP subsequent to fermentation is the malo-lactic fermentation. Early onset and completion of malo-lactic fermentation allows the prompt addition of sulphur dioxide, storage at cool temperatures and clarification. It is conducted by lactic acid bacteria (*Oenococcus oenos*), which directly decarboxylate L-malic acid (dicarboxylic acid) to L-lactic acid (monocarboxylic acid). This metabolism results in acidity reduction and pH increase, which are in turn related to an increased smoothness and drinkability of red wines but might also generate a flat taste (Davis *et al.*, 1985; Guzzo *et al.*, 1998). The initial pH, the sulphite concentration (Vaillant *et al.*, 1995), the phenolics and the anthocyanin concentration (Vivas *et al.*, 1997) of juice/wine strongly affect whether, when, and how (with what species) malo-lactic fermentation will occur. Bacterial viruses (phages) can severely disrupt malo-lactic fermentation by attacking the *O. oenos* cells, thus causing microbial destabilisation of wine (Gnaegi and Sozzi, 1983).

The maturation step is a CCP that often lasts six to 24 months and normally takes place in oak barrels. During maturation, a range of physical and chemical interactions occurs among the barrel, the surrounding atmosphere and the maturing wine, leading to transformation of flavour and composition of wine (Martinez *et al.*, 1996). The CCP in this case is the oak barrel, which should be fault-free and should have undergone a decontamination treatment. The wood must also be free of pronounced or undesirable odours, which could taint the wine (Mosedale and Puech, 1998). During the maturation period, several components of the wood (most of them phenolics) are extracted to the wine tannin (Viriot *et al.*, 1993; Towey and Waterhouse, 1996).

Clarification of wine may be a CCP depending on the nature of the clarity problem. Clarification involves only physical means of removing suspended particulate matter. Juice clarification by racking, centrifugation or filtration often improves the flavour development in white wine and helps the prevention of microbial spoilage. If sufficient time is provided, racking and fining can produce stable, crystal clear wines but, now that early bottling in a few weeks or months after fermentation is employed, centrifugation and filtration are used to obtain the required clarity level (Ribéreau-Gayon *et al.*, 1998). Microbial contamination of wine during the above mentioned procedures constitutes a potential problem for its stability (Ubeda and Briones, 1999). Racking is also effective on pesticide residue reduction of wine (Gennari *et al.*, 1992).

A subsequent CCP involves stabilisation procedures in wines. The reason for stabilisation is production of a permanently clear and flavour fault-free wine. The most important procedures include: (i) tartrate stabilisation by chilling the wine to near its freezing point and then filtering or centrifuging to remove the crystals; (ii) protein stabilisation with absorption, denaturation, or neutralisation by fining agents (bentonite) (Blade and Boulton, 1988); (iii) polysaccharide removal with pectinases that hydrolyse the polymer, disturbing its protective colloidal action and filter plugging properties (Ribéreau-Gayon *et al.*, 1998); and (iv) metal casse (Fe, Cu) stabilisation.

Table 8.10 Comparative presentation of CCPs of HACCP and ISO 22000 in conjunction with PRP for wine production

Process step	HACCP CCPs	Prerequisite program according to ISO 22000	ISO 22000 CCPs
Culture	–	YES	–
Pruning	–	YES	–
Chemical fertilisation	1	NO	1
Organic fertilisation	2	YES	–
Irrigation	3	YES	–
Culture cares	–	YES	–
Harvesting	–	YES	–
Stemming	–	YES	–
Crushing	–	YES	–
Maceration	–	YES	–
Pressing	–	YES	–
Fermentation	–	YES	–
Malolactic fermentation	4	NO	2
Maturation	–	YES	–
Clarification	–	YES	–
Fining	–	YES	–
Stabilisation	5	YES	–
Bottling	–	YES	–
Storage	–	YES	–

Wine is bottled in glass bottles sealed with cork, and it is the cork itself that constitutes the CCP. The bottles must pass a decontaminating step and an inspection control to assure the absence of any defects and the stability of the product until its consumption (Cooke and Berg, 1984). The cork should be correctly sized, 6–7 mm bigger than the inner neck diameter, to avoid any possible leaks. In bottling, all three hazards may be encountered. In particular, cork microflora, residues of heavy metals, SO_2, pesticides and detergents and absence of cracks, scratches and rifts in the lute represent microbiological, chemical and physical hazards. Although cork is noted for its chemical inertness in contact with wine, it might cause off-flavours when contaminated (Simpson *et al.*, 1986; Simpson, 1990) or when the producers are not applying effective quality control (Neel, 1993).

Shipping and storage of wines at elevated temperatures can initiate rapid changes in colour and flavour of wine. Direct exposure to sunlight corresponds to the effect of warm storage temperatures. Temperature affects reaction rates involved in the maturation, such as the acceleration of hydrolysis of aromatic esters and the loss of terpene fragrances (De la Presa-Owens and Noble, 1995). Temperature can also affect the wine volume and eventually loosen the cork seal, leading to leakage, oxidation and possibly microbial formation resulting in spoilage of bottled wine.

The hazards, CCPs, critical limits, monitoring, corrective actions and personnel responsible for wine production are summarised in Table 8.7. The determination of CCP and ISO 22000 analysis worksheet for the determination of prerequisite

programs for wine production are given in Tables 8.8 and 8.9, respectively. Finally, the comparative presentation of CCPs of HACCP and ISO 22000 in conjunction with PRP for wine production is shown in Table 8.10.

8.6 Conclusions

The food authenticity issue, in particular in foods of added value (wine, olive oil, etc.), has been one of the most important problems confronting scientists. Several instrumental detection methods such as HPLC, GC–MS, AAS, ELISA, RT–PCR and DNA Comet Assay have been put forward and, in conjunction with multivariate analysis, managed to improve considerably the adulteration detection in wine. Furthermore, the introduction of ISO 22000:2005 in conjunction with ISO 22005:2007 (related to food traceability) can act as an extra supportive tool for effectively ensuring the wine authenticity.

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Appendix: EU Directive 178/2002

Regulation (EC) No.178/2002 (entry into force 1/1/2005) has three main axes: 1. Provides the basis for the assurance of a high level of protection of human health and consumers' interest in relation to food, taking into account the diversity in the supply of food, including traditional products, while ensuring the effective functioning of the internal market. It establishes common principles and responsibilities, the means to provide a strong science base, efficient organizational arrangements and procedures to underpin decision-making in matters of food and feed safety. 2. Lays down the general principles governing food and feed at Community and

national level. It establishes the European Food Safety Authority (EFSA). 3. It shall apply to all stages of production, processing and distribution of food and feed. It shall not apply to primary production for private domestic use or to the domestic preparation, handling or storage of food for private domestic consumption.

The food law shall aim at the prevention of: 1. fraudulent or deceptive practices; 2. the adulteration of food and; 3. any other practices which may mislead the consumer. The requirements of food safety are: 1. Food shall not be placed on the market if it is unsafe; 2. Food shall be deemed to be unsafe if it is considered to be: (a) injurious to health and (b) unfit for human consumption. The requirements of feed safety are summarized as follows: 1. Feed shall not be placed on the market or fed to any food-producing animal if it is unsafe. 2. Feed shall be deemed to be unsafe for its intended use if it is considered to: (a) have an adverse effect on human or animal health and (b) make the food derived from food-producing animals unsafe for human consumption. The EFSA comprises: (a) a Management Board, (b) an Executive Director and his staff, (c) an Advisory Forum and (d) a Scientific Committee and Scientific Panels. Its task shall be: (a) to provide the Community institutions and the Member States with the best possible scientific opinions, (b) to promote and coordinate the development of uniform risk assessment methodologies, (c) to provide scientific and technical support to the Commission, (d) to commission scientific studies, (e) to search for, collect, collate, analyze and summarize scientific and technical data, (f) to undertake action to identify and characterize emerging risks, (g) to establish a system of networks of organizations operating and be responsible for their operation, (h) to provide scientific and technical assistance in the crisis management procedures implemented by the Commission with regard to the safety of food and feed, (i) to provide scientific and technical assistance, (j) to ensure that the public and interested parties receive rapid, reliable, objective and comprehensible information, (k) to express independently its own conclusions and orientations and (l) to undertake any other task assigned to it by the Commission. A rapid alert system for the notification of a direct or indirect risk to human health deriving from food or feed is hereby established as a network. The emergency measures taken in the case of food or feed imported from a third country are: (i) suspension of imports of the food or feed in question from all or part of the third country concerned and, where applicable, from the third country of transit, (ii) laying down special conditions for the food or feed in question from all or part of the third country concerned and (iii) any other appropriate interim measure (Arvanitoyannis *et al.*, 2005).

9

Terroir: the effect of the physical environment on vine growth, grape ripening and wine sensory attributes

C. van Leeuwen, ENITA – Université de Bordeaux, France

Abstract: Terroir can be defined as an interactive cultivated ecosystem, in a given place, including climate, soil and the vine. Wine sensory attributes are influenced by terroir. The first condition for high terroir expression is that the precocity of the grapevine variety should be suited to the local climatic conditions in such a way that full fruit ripeness is reached by the end of the growing season. The second condition is that vines should experience a factor limiting yield and vigour, e.g. water deficit stress or low nitrogen availability. Because terroir involves many factors, related to both the plant and the physical environment, terroir must be studied by a pluri-disciplinairy approach.

Key words: terroir, vineyard soils, climatic zones for vine growing, vine water status, viticultural zoning.

9.1 Introduction

9.1.1 Definition of terroir

Vine development, grape ripening and wine sensory attributes are highly influenced by the physical environment in which the vines grow. In viticulture, the interactions between the physical environment and the vines are referred to as the 'terroir effect'. Many factors are involved in terroir expression, making this concept difficult to study on a scientific basis: geology, geomorphology, soil, climate, biology of the vine (Seguin, 1986). Human factors should also be

included, because terroir deals with cultivated vines (van Leeuwen and Seguin, 2006). 'Terroir' has long been acknowledged as an important factor in wine quality and style, especially in European vineyards (Falcetti, 1994), but more and more so also in New World wine-producing countries. In the 1990s, Jackson and Lombard wrote in a review about environmental and management practices affecting grape composition and wine quality: 'Despite its complexity, the concept of terroir is a valuable one. The reductionist might find difficulty in appreciating its value for scientific analysis, but the fact that appellations have maintained their status over many years in French districts suggests the effects are real' (Jackson and Lombard, 1993). Terroir can be defined as an ecosystem, in a given place, including many factors, like climatic conditions, cultivar and rootstock, geography and topography, as well as soil characteristics like mineral nutrition and water supply (Seguin, 1986, 1988). A definition proposed by the Organisation Internationale de la Vigne et du Vin (OIV) refers to the originality of the wine produced in a given terroir, and hence the added value: 'A terroir is a unique and delimited geographic area for which there is a collective knowledge of the interaction between the physical and biological environment and applied viticultural practices. The interaction provides unique characteristics and creates a recognition for goods originating from that area. Terroir includes specific landscape characteristics and territory values.' (OIV, 2008).

9.1.2 The importance of interactions among terroir factors

To understand the terroir effect in viticulture, it is essential to take into account the interactions among the factors that contribute to terroir. While very high-quality wines are grown in various climates, it is impossible to define the ideal climate for fine wines in terms of temperature, rainfall (amount and distribution) or solar radiation. High-quality wines are grown on a great diversity of soils; hence, it is impossible to define the best possible soil for growing high-quality wines in terms of pebble, clay or lime content, soil depth or mineral content. Many authors have published on terroir taking into account only one single discipline, generally their own, e.g., climatology (Tonietto and Carbonneau, 2004), geology (Wilson, 1998), geomorphology (Rouvelac, 2006), pedology (Van Leeuwen *et al.*, 1989; Lévèque *et al.*, 2006) or soil microbiology (Bourguignon, 1995). Although it is useful to highlight one single factor of terroir, this sort of approach remains descriptive and does not lead to a greater understanding of how terroir influences wine quality and style. Particularly in terroir studies, '(researchers are) victims of their own discipline' (Moran, 2001) and often fail to take into account other diciplines and interactions between various terroir factors.

9.1.3 Interactions between climate and the grapevine variety

The role of climate in terroir cannot be understood without considering the grapevine variety. The timing of grape ripening is crucial in wine production. Grapes that ripen too late in the season and are therefore harvested before a desired

maturity result in the production of green, acidic wines. However, when grapes reach maturity early in the warmest part of the summer, fruit composition is also unbalanced. Sugar may be high and acidity low, but grapes generally lack aromatic expression. In order to obtain high terroir expression in wine production, grapes should ideally ripen in cool conditions at the end of the growing season (van Leeuwen and Seguin, 2006; van Leeuwen *et al.*, 2007). Timing of fruit ripening in a given situation is related to (i) local climatic conditions and (ii) phenological precocity of the cultivar. The latter is a genetically determined characteristic that is highly variable from one grapevine cultivar to another. In traditional wine-growing regions of Europe, growers have empirically used variability in phenology to adapt cultivars to local climatic conditions in order to maximize terroir expression. At high latitudes, where the limiting factor for producing high-quality wines is the level of ripeness of the grapes, early-ripening varieties have been planted. At low latitudes, where the climate is warmer, late-ripening varieties have been planted to avoid quick ripening of the grapes in the hottest part of the summer. As a result, in Europe, grape picking generally takes place between the 10th of September and the 10th of October, despite huge climatic differences between, for example, the Mosel in Germany, Bordeaux in France and Alicante in Spain. In New World countries in the Southern Hemisphere, greatest terroir expression is obtained with varieties that ripen in March.

9.1.4 Interactions between the soil and the grapevine

Great wines are produced worldwide on a wide range of soils, with a pebble content from 0 to over 50% and a clay content from a few per cent to 60% in the best *Cru* of Pomerol (Bordeaux, France) (Seguin, 1986). It is impossible to define a high-quality potential vineyard soil in terms of soil texture, soil type or soil minerals (Seguin, 1983). Vineyard soils have to be studied with respect to their potential impact on vine development and fruit ripening. Good vineyard soils for the production of red table wines limit yield and vine vigour, either by limited water supply to the vines or by limited nitrogen availability (van Leeuwen *et al.*, 2007). A limitation in water uptake reduces vine vigour, berry weight and yield and increases berry anthocyanin and tannin concentration (Duteau *et al.*, 1981; Matthews and Anderson, 1988, 1989; van Leeuwen and Seguin, 1994). Most vineyards producing high-quality red wines receive little or no nitrogen fertilization. Hence, vine nitrogen uptake depends to a large extend on the amount of nitrogen the soil supplies to the vines, through mineralization of soil organic matter. A limitation in nitrogen uptake reduces vine vigour, berry weight and yield and increases berry sugar, anthocyanin and tannin concentration (Choné *et al.*, 2001a; Hilbert *et al.*, 2003). These effects are beneficial to grape quality potential for red wine making.

9.1.5 Human factor in terroir

The human factor should be taken into account in terroir studies, because wine production involves the intervention of man. Man selects vineyard sites. From a

historical perspective, this selection was often a negative one. Fertile soils were used for cereals and cattle and poor soils, that could not be used for other purposes, were planted with vines, because of their low agronomic needs (van Leeuwen *et al.*, 2007). Geography also plays an important role in the establishment of new viticultural areas. Until the development of railway systems in the 19th century and trucks in the 20th century, wine was difficult to transport over land. Therefore, vineyards used to develop in contexts where geographic factors were favourable, either close to consumption areas or close to harbours from where the wine could be shipped. During crises (phylloxera at the end of the 19th century in Europe, economic crises) winegrowing regions that happened to be in an environment favourable to wine quality (e.g. Bordeaux and Burgundy in France) managed to survive, while regions that were in a situation where it was more difficult to produce high-quality wine disappeared (e.g. regions around La Rochelle or Orléans in France; van Leeuwen and Seguin, 2006).

Technical knowledge and experience is also needed to make high-quality wines expressing terroir. Phylloxera reached Bordeaux (France) before it reached La Rioja (Spain). When Bordeaux was hit by phylloxera, many winemakers emigrated to La Rioja and that region benefited from the technical knowledge they brought with them. The application of viticultural and enological techniques that enhance terroir expression generally increases production costs. Hence, these techniques can only be implemented when the wine is produced in a favourable economic environment. Wine quality and terroir expression increased dramatically in the Haut-Médoc (Bordeaux, France) when the market was favourable due to export to England (17th and 18th centuries). At that stage, wines from Pomerol (Bordeaux, France) remained completely ignored by the international market. It was only when powerful negociants settled in Libourne (a town close to Pomerol) in the early 20th century that the high-quality potential of the terroir of Pomerol was discovered. The increase in selling prices allowed the owners to make the investments necessary to reveal their terroir (e.g. yield control, canopy management, barrel ageing, etc.; van Leeuwen and Seguin, 2006).

Similar trends were seen in developing New World vineyards. Although transport of wine over land is no longer a major problem, most famous New World winegrowing regions have developed in the vicinity of prosperous urban areas (Adelaide and Melbourne in Australia, San Fransisco in the USA). The economic power of California undoubtly enhanced the development of a dynamic wine industry in this American state. The role of viticultural practices in the optimization of wine quality in relation to the natural environmental factors is extensively reviewed by Jackson and Lombard (1993). Other aspects of the role of the human factor in terroir are reviewed by Deloire *et al.* (2008).

9.1.6 Scale issues

Terroir is difficult to study because many factors are involved, and these interact. Scale issues make terroir studies even more difficult. The terroir effect can be considered at various scales: country, region, commune, estate, vineyard block or

part of a vineyard block. Depending on the scale, the hierarchy of the effect of terroir factors might vary. On a regional scale, or even more so at the scale of a whole country, climate, in interaction with the grapevine variety, is likely to be the most dominant factor. Inside a region or a commune, geology, geomorphology and related topo-climatic effects might be the driving factors that are able to explain differences in vine development and grape composition. Variability at the scale of an estate or a block might be related to variations in soil type or soil depth. Hence, scale issues are highly important in terroir studies. Some authors state that terroir can only be satisfactorily studied for small areas mapped at a large scale (White, 2003; Bramley and Hamilton, 2007; White *et al.*, 2007), which is certainly true for soil effects, but not necessarily for the effects of other terroir-related factors (climate, topography, geology). Perreira and co-workers managed to classify 134 grape samples from four sub-appellation of the Bordeaux area (France) by the analysis of 17 physico-chemical variables (Perreira *et al.*, 2005a). Environmental variables were not controlled in this study and the separation might have been the result of differences in soil, climate and/or cultivar. Scale issues in terroir studies have been reviewed by several authors (Vaudour, 2002, 2003; Deloire *et al.*, 2005).

9.1.7 Main factors involved in terroir expression

In the following sections, the effects of the main factors involved in terroir expression are discussed (climate, geology, geomorphology, soil, water relations), as well as their interaction with the vine.

9.2 The climate component of terroir

9.2.1 The effect of climate in terroir expresion

Climate is considered as an important factor in terroir expression (van Leeuwen *et al.*, 2004). While very high-quality wines are grown in various climates, it is impossible to define the ideal climate for fine wines in terms of temperature, rainfall (amount and distribution) or solar radiation. Each of the factors of the natural environment contributing to terroir expression, including climate, has to be considered in terms of its interaction with the vine. The climate influences vine physiology through temperatures, rainfall, vapour pressure deficit (VPD), reference evapotranspiration (ET_0), sunshine hours and wind. Agro-climatic indices are useful to account for the influence of climate on vine development and grape ripening. Vine phenology can be modelled by means of the sum of active temperatures (Winkler *et al.*, 1974; Huglin and Schneider, 1998). Evolution of vine water deficit stress over the season can be monitored by a water balance model (Lebon *et al.*, 2003), which takes into account the effects of climatic variables like rainfall, ET_0 and active temperatures. Some authors also consider the effect of minimal temperatures during the ripening period as a critical climatic variable (Tonietto and Carbonneau, 2004).

Climate varies in time and space. Yearly variations in climatic conditions are well known in viticulture as the vintage effect. Variations in vine behaviour and grape ripening from one year to the other on a given plot reflect the effect of climate alone, because soil type and plant material can be considered constant (van Leeuwen *et al.*, 2004). Climate varies in space at various scales. At the macroclimate level (climatic variations from one region to another), temperatures, solar irradiance, humidity and rainfall are variable depending on the geographical coordinates of latitude and longitude. With the same coordinates, local variations of other geographical factors such as altitude, slope, exposure, and of physical factors, such as dominant wind, distance from mountains or big water masses, affect the convective exchanges at the lower layer of the atmosphere and induce topo-climatic conditions which are thus landscape related. This topo-climate (or mesoclimate) has a large influence on vine growth and grape quality, especially in regions with a complex geomorphology, which is the case in several renowned European wine-producing zones (Novello and de Palma, 2007). Climatic conditions of most of the winegrowing regions of Australia, and some other winegrowing regions in other parts of the world, are compiled by Gladstones (1992). Microclimate is the variation of climatic variables at the plot level or inside the canopy. Microclimate can be manipulated by canopy management (Smart and Robinson, 1991).

Great changes in climate are predicted by climatologists during the 21st century due to greenhouse effect induced by human activities (IPCC, 2001). Adaptation to these changes is a major challenge for viticulturists worldwide over the next decades.

9.2.2 Air temperature

Winter minimum temperature is a limiting factor in vine cultivation and has a major impact on the distribution of viticultural areas over the world. Most cultivated grapevine varieties belong to the species *Vitis vinifera*. This species is resistant to minimum winter temperatures as low as –15 °C, although considerable variation exists among cultivars. When fully acclimatized, some cultivars can resist to temperatures down to –25 °C. In regions where minimum winter temperatures regularly descend below –15 °C, hybrids between *Vitis vinifera* and other more cold-resistant vine species are often cultivated, but wine quality is generally inferior. After budbeak, vine vegetation does not resist temperatures below –2.5 °C. In situations where spring frost is a common phenomenon, frost protection is a necessity for economically viable viticulture.

Air temperature is a critical variable in vine development and grape ripening. Vine photosynthesis is maximized at 25 °C (Alleweldt *et al.*, 1982). Effects of temperature on grape composition were reviewed by Coombe (1987). Grape potassium and proline concentrations increase linearly with temperatures, while malate concentration decreases with temperature. The relation between temperature and grape sugar concentration is curvilinear, with a maximum for temperatures between 25 °C and 30 °C. The optimum range for anthocyanin accumulation is

17–26 °C (Pirie, 1977). Low temperatures, and particularly low night temperatures, enhance coloration in red grapes (Kliewer and Torres, 1972). Cool temperatures are considered favourable to the aromatic expression of wines. Among the few references published on this important subject, Ewart (1985) showed that cool climatic conditions enhance aromas in Riesling grapes.

Temperatures decrease with latitude. Temperature differences among seasons increase with continentality. Temperatures are also very much influenced by geomorphology. In mountainous regions, temperature decreases by 0.65 °C for 100 m in altitude (Guyot, 1997). However, temperature inversions can occur, particularly for minimum temperatures, because cool air can flow down slopes and accumulate in lower areas (Dumas *et al.*, 1997; Guyot, 1997). Hence, vines located in valleys can be more subject to spring frost damage than vines located on slopes. Some contradictory data have been published on the effect of small differences in altitude on temperature. In the Loire Valley (France), Jacquet and Morlat (1997) observed a decrease in temperature with altitude even for differences in altitude of 60 m, while Duteau *et al.* (1981) did not, in a comparable environment in Bordeaux. Water masses have a mitigating effect on temperatures by decreasing maximum temperatures and increasing minimum temperatures. This effect was shown in the Bordeaux region by Bois *et al.* (2008a). Because the increase of minimum temperatures is greater than the decrease of maximum temperatures, average temperatures are higher in vineyards in the vicinity of the Garonne and Dordogne rivers and the Gironde estuary. The occurrence of sea breezes in the Cape Town region (South Africa) reduces air saturation deficit and air temperature, resulting in a longer optimal period for photosynthesis (Carey and Bonnadot, 2004).

9.2.3 Rainfall

Rainfall is highly variable among winegrowing regions. Most renowned winegrowing regions have an annual rainfall between 300 and 1000 mm a year. Rainfall increases disease pressure and particularly downy mildew and grey rot (*Botrytis cinerea*). Although some rain is beneficial for grape development, the production of high-quality wines expressing terroir requires at least moderate water deficit stress in a part of the season. Moreover, it is easier to add water to the vines in dry climates through supplementary irrigation than to withhold water in excessively rainy climates. Water uptake conditions of the vines are determined by climatic variables (rainfall, potential evapotranspiration) and soil water holding capacity. They can be calculated with a water balance model (Lebon *et al.*, 2003). Effects of vine water status on vine development, yield and grape ripening are discussed in Section 9.5.

9.2.4 Solar radiation

Vine photosynthesis increases with solar irradiance until approximately one third of the maximum light intensity on a sunny day (Kriedeman and Smart, 1971).

Some contradictory data have been published about the effect of light intensity on grape composition, probably because the effects of sunlight and temperature are difficult to separate. Grape anthocyanins increase with light intensity, while they decrease with temperature (Spayd *et al.*, 2002).

Spatial variability of solar radiation can be mapped with satellite data and a digital elevation model (DEM). In Bordeaux, solar radiation is dependent on slope and aspect (Bois *et al.*, 2008b). Spatial distribution of solar radiation in an alpine environment was described by Failla *et al.* (2004).

9.2.5 Reference evapotranspiration (ET_0)

Little data are published about the effect of potential reference evapotranspiration (ET_0) on vine development. ET_0 is not measured in most weather stations, because several variables are required for its calculation (temperature, VPD, solar radiation, wind speed; Allen *et al.*, 1998). However, ET_0 is an important climatic variables because of its role in the water balance (vine transpiration increases with ET_0). ET_0 can be estimated with remotely sensed solar radiation (Bois *et al.*, 2008c). The implementation of this method makes it possible to map water balance at a regional scale.

9.2.6 Agro-climatic indices

The effect of climate, and particularly temperature, on vine development can be modelled with agro-climatic indices. The most commonly used agro-climatic indices in viticulture are sums of active temperatures. Winkler introduced the concept of growing degree days (GDD, Winkler, 1938; Winkler and Williams, 1939; Winkler *et al.*, 1974). These are temperature sums with a base of 10 °C from 1 April through 31 October (in the Northern Hemisphere). Using the concept of GDD, Winkler divided the winegrowing regions of California into climatic zones. Huglin published an index (Huglin's Index), based on the half sum of daily maximum and daily average temperatures from 1 April through 30 September (in the Northern Hemisphere; Huglin, 1978; Huglin and Schneider, 1998). Huglin calculated the index for the main winegrowing regions of France, and some other winegrowing regions of the world (Huglin and Schneider, 1998). He also established the heat requirements for a wide range of grapevine varieties. Although this classification of the precocity of grapevine varieties remains rather rough, it opens an interesting perspective of adjusting the precocity of varieties to local climatic conditions. This is an important aspect in viticulture, because terroir expression is enhanced when grapes reach maturity at the end of the growing season and thus ripen in cool conditions (van Leeuwen and Seguin, 2006). More information about heat requirements for grapevine varieties is provided by van Leeuwen *et al.* (2008a).

Daily average temperatures do not provide specific information on minimum and maximum temperatures. Hence, climatic indices based on daily average temperature sums remain rather rough. Much more precise information is provided

by hourly temperature data. Based on the observation that high temperatures during the last stages of grape ripening negatively affect flavour in grapes, Happ (1999) created an index that sums the hourly temperatures over 22 °C during the last 28 days prior to harvest. Most famous wine-growing regions in the world have an hourly heat load base of 22 °C < (500 °C. hour) during the last four weeks before maturity (Happ, 2000). Heat load is better correlated to daily maximum temperatures than to daily average temperatures. At high latitudes, hourly heat loads drop quickly when the season advances. Even in locations with high summer temperatures, low hourly heat loads during grape ripening can be obtained by growing late ripening varieties. Maritime locations have lower hourly heat loads than their average temperature would suggest: Albany in Western Australia has a similar hourly heat load in the four weeks before ripeness (270 °C. hour) compared to Dijon, despite markedly higher average temperatures (19.2 °C versus 17 °C; Happ, 2000).

A multicriteria climatic classification system for grape-growing regions was proposed by Tonietto and Carbonneau (2004). This climate classification system is based on a temperature sum (Huglin's Index), a simplified water balance model and the average minimum temperature of September (March in the Southern Hemisphere). This classification system allows accurate characterization of the climate in most of the world's grapegrowing regions.

9.3 The effect of geology and geomorphology in terroir expression

9.3.1 Geology

No consensus exists regarding the direct influence of geology in terroir expression. Some authors have suggested that minerals derived by the vines from weathering parent material confer distinctive characters to the wine (Wilson, 1998). Others think that a lot of what is written about geology in wine books is at best misguided and at worst utterly wrong. For instance, Chablis wines are often associated with 'flinty' characters, but it is difficult to imagine how a material as insoluble in groundwater as flint could contribute to the flavour of any wine, let alone what the flavour of anything so hard and insoluble could be (Huggett, 2006). The same author advances that only rarely, as in the Coonawarra (Australia) and the Douro (Portugal), the geological bedrock is an important factor in wine quality (Hancock and Huggett, 2004; Huggett, 2006).

High-quality wines can be produced on a diversity of geological outcrops: schists (Porto, Mosel), chalk, limestone, marl or sandstone containing different amounts of active calcium carbonate (Champagne, Bourgueil, Chinon, Chablis, Saint-Emilion, Burgundy, Jerez, Rioja, Barolo, Barbaresco, Chianti, Marsala, Rheingau etc.); clay (certain *crus* of Pomerol, Sauternes); sand (Nebbiolo d'Alba); schist, granite and porphyry (Beaujolais) (Seguin, 1986). This author forwards the hypothesis that geology does not influence overall wine quality, but might influence wine typicity. Certain cultivars apparently thrive best in certain parent

material. For example Nebbiolo and Chardonnay are at their best on marl; Gamay produces its best wines on the schist, granite and porphyry of the Beaujolais, but a very ordinary one on the marly limestone of Burgundy (Seguin, 1986).

Champagnol (1997) also published empirical observations about the effect of soil and geology on wine style. Red wines have bigger structure when produced on clayey soils compared to sandy soils, but might lack *finesse*. Wines produced on sandy soils are lighter, but can be nicely fruity. Results might differ depending on the grapevine variety. In the Languedoc (France), wines produced with Carignane on schisteous soils are much appreciated, because they are rounder and have more flavour and *finesse* than those produced on high lime clays. Wines produced from Grenache on schisteous soils, however, might lack freshness and acidity. In the Douro (Portugal) only schisteous soils are used for port production. In this region, soils developed on granite are used for the production of red or white table wine because port quality is disappointing on these soils (Champagnol, 1997).

For Huggett (2006), the role of geology is indirect: it influences the soil type, it permits penetration of vine roots to varying degrees depending on the nature of the rock, it controls geomorphology (slope) and it assists or hinders drainage of rain water (slope). In Chablis, the role of geology on wine quality is believed pre-eminent, because the best Chablis wines are produced on Kimmeridgian limestone, while the lesser Petit Chablis is produced on Portlandian limestone. However, it seems obvious that this effect of geology is indirect: all the south-facing slopes are located on Kimmeridgian limestone, while the Portlandian limestone bears cool and windy plateaux with less favourable exposure (Wilson, 1998; Huggett, 2006).

Excellent descriptions of grape growing regions worldwide by their geology and geomorphology are available in Fanet (2001). The geology of French wine-growing regions is presented in Wilson (1998) and Pomerol (1978).

9.3.2 Geomorphology

Geomorpology of viticultural landscapes is related to geological substrate. In sedimentary basins, hard and soft rock layers alternate. Hard rock layers are resistant to erosion and therefore end up in dominant position in the landscape (plateau). Vineyards generally develop on the slopes sculpted by erosion in the softer layers around the plateau. This is the case in Chablis (Burgundy), Corton (Burgundy) and Champagne. In Saint-Emilion, vineyards (Fig. 9.1) cover both the limestone plateau and the surrounding slopes (van Leeuwen *et al.*, 1989). On primary schists, rivers sculpt steep slopes on which vineyards are established (Douro, Portugal; Côte-Rotie, France). On Quaternary alluvium, slopes are generally not very steep and the landscape can even be completely flat. Because the deposits containing a high amount of gravel are more resistant to erosion than sandy deposits, mounds of gravel can end up in dominant position. In the Médoc (Bordeaux), these are called 'croupes' Médoc (Fanet, 2008). Geology and geomorphology of the Cape Town wine growing region (South Africa) is presented in Fig. 9.2.

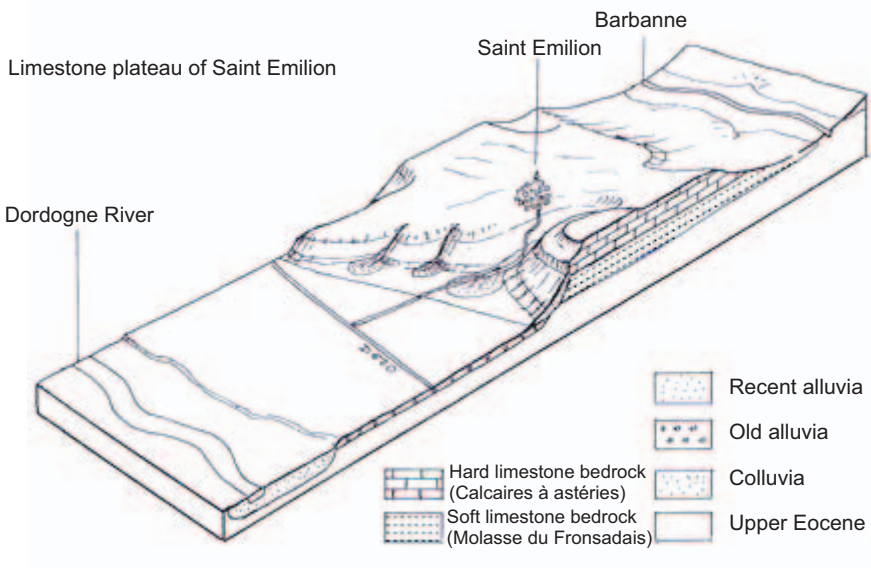

Fig. 9.1 Geology and geomorphology of the Saint-Emilion region (France). Adapted from Fanet (2001).

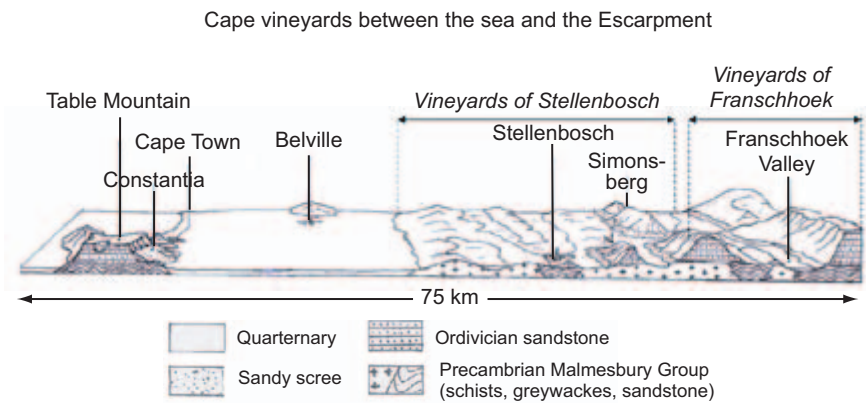

Fig. 9.2 Geology and geomorphology of the Stellenbosch region (South Africa). Adapted from Fanet (2001).

The effect of geomorphology in terroir is indirect, through altitude, slope and exposure. The latter influence the radiation balance and, hence, air temperatures (Novello and de Palma, 2007). Climatic variability induced by topography is called topo-climate (Dumas *et al.*, 1997). Slope also enhances erosion. Severe erosion can destroy soils, but some erosion may keep the soil depth limited in top hill position, while sediments accumulate in downhill position. Grape quality potential is generally higher on shallow soils in top hill position, where grape berries are smaller and contain more sugar and anthocyanin (Nadal *et al.*, 2008). This aspect is further discussed in Section 9.6.1 on soil depth.

9.4 The soil effect in viticulture

9.4.1 The soil factor in terroir

Most authors agree on the fact that soil has a strong influence on vine development and grape composition. van Leeuwen *et al.* (2008b) looked for a possible relation between soil type and the destination of the produced wine (first, second or third quality) in seven famous Bordeaux estates covering a total of 400 hectares of vines. Average wine quality was high on gravelly soils, sandy soils and heavy clay soils. Wine quality was low on soils with permanent water logging, on leached soils and on deep soils developed on down hill colluviums. Although soil influences wine quality and style, great wines are produced worldwide on a wide range of soils, with a pebble content from 0 to over 50% and a clay content from a few per cent to 60% in the best *Cru* of Pomerol (Seguin, 1983, 1986). Champagnol (1984, 1997) explained the effect of the soil by its fertility. Soil fertility can be divided into physical fertility (possibilities for the roots to explore the soil over great depth), hydric fertility and chemical fertility. When no limiting factor is present in the soil, vine growth is vigorous and shoot growth cessation occurs late in the season. Vine hormonal equilibrium remains in favour of auxins, cytokinins and gibberellins. These hormones are favourable to protein synthesis. When vine water uptake becomes limiting before *véraison*, roots synthesize abscisic acid (ABA). ABA is favourable to anthocyanin and tannin synthesis, while cytokinins depress anthocyanin and tannin synthesis (Pirie and Mullins, 1976). Costantini *et al.* (2006) also found that soil variables impact vine physiology in accordance with the degrees of limitation to the vine that they induce. For example, some of these limitations were considered positive for the production of high-quality wine, because they limited excessive vigour, in this case, in Sangiovese vines. Löhnertz *et al.* (2008) found great differences in the wine style of Riesling wines produced on various soils. Wines produced on limestone soils contained more calcium, but this did not increase wine pH. On quatzitic soils, wines showed a typical minerally flavour. However, vine water status had a stronger influence on wine style and quality than soil mineral composition. Wines from soils with fine texture (clay) and wines produced from vines that experienced water deficit stress during the season had more body.

Other authors challenge a strong soil effect on wine quality. Bader and Wahl (1996) excavated soils from various winegrowing regions in Germany and reconstructed the soils in one location in order to compare the soil effect without interference from differences in climatic variables. Soil influence on wine quality turned out to be very small. These authors conclude that, in cool climate, climate is a more important factor than soil in wine sensory attributes. Rankine *et al.* (1971) also find that soil influence (in particular mineral composition) on grape composition and wine quality is small.

The soil is a complex medium. Its role cannot be explained without breaking it down in several sub-effects: texture, mineral composition, water supply to the vines, temperature in the root zone, and many other factors.

9.4.2 Soil texture

The effect of three different soil textures on vine development and grape ripening was investigated in the Bordeaux area over five vintages (van Leeuwen *et al.*, 2004). Berries contained high sugar, anthocyanin and total phenolic concentrations on a heavy clay soil. Shoot growth stopped early in the season on a gravelly soil, where berry size was small, sugar and total acidity low, but anthocyanin concentration high. Shoot growth cessation was delayed on sandy soil with a water table within the reach of the roots, berries were large and contained low concentrations of sugar and anthocyanin but high concentrations of malic acid. These effects were shown to be mediated through vine water status. Zamboni *et al.* (2008) showed a strong correlation between soil clay content and anthocyanin concentration in Sangiovese grapes from Emilia Romagna (Italy). However, vine water status was not controlled in this study. Reynolds *et al.* (2007) collected data over three vintages on 215 georeferenced Riesling vines in a 4 ha vineyard block. Vine size and soil texture affected berry composition and must components, including monoterpenes, but no consistent effects were observed among the growing seasons. Mineral aroma in the wine was related to high clay content, but other sensory attributes were not consistently affected by soil texture or vine vigour.

Wines produced on soils with a high clay content are less subject to oxidation (Noble, 1979). Trought *et al.* (2006) opined that vine development is related to soil texture. Vine vigour is greater on gravelly soils compared to silty loam soils and intermediate on silt over gravel. On the gravelly soils, flowering, *véraison* and maturity are reached earlier and grapes contain more sugar and have lower titratable acidity. Grapes contain more isobutylmethoxypyrazine (the characteristic aroma of green bell pepper that can negatively affect wine aroma) on sandy-silt soils compared to gravelly soils (Roujou de Boubée *et al.*, 2000).

It is likely that the effect of soil texture in viticulture is indirect. Soil texture (pebble, sand, silt and clay content) influences soil water holding capacity, cation exchange capacity, root penetration, temperature in the root zone and susceptibility of soils to water logging.

9.4.3 Soil mineral composition

In popular wine books, the terroir effect is often attributed to vine roots going down in the soil for metres to pick up specific minerals that confer typicity to the wine. In some cases, the allusion is even more direct, e.g. wines produced on soils developed on flint should have flinty aromas. However, nobody has yet been able to demonstrate the processes by which elements of the soil are transformed to the flavours, colours or other qualities of wines (Moran, 2001). Other studies showed that it is impossible to establish any correlation between the quality of the wine and the soil content of any nutritive element, be it potassium, phosphorus or any other oligoelement (Seguin, 1986). This statement was confirmed by Fregoni (1997) and van Leeuwen *et al.* (2004). Duteau and Seguin (1973) studied soil profiles in *Premiers Grands Crus Classés* of the Haut-Médoc (Bordeaux, France) and in other, less famous *Grands Crus Classés* located in the same region. The soils of the

Premiers Grands Crus Classés contained more pebbles, more clay, more organic matter, more potassium and more phosphorus. However, the authors of this study do not conclude that high clay, organic matter, potassium and phosphorus concentration in the soil leads to a better wine quality. The *Premiers Grands Crus Classés* sell their wine more expensively and have more regularly amended their soils. An historical survey showed that even the higher clay content was the result of human intervention: in the 18th and 19th centuries the addition of clay to the poor gravelly soils of the Haut-Médoc before plantation was a regular practice for those who could afford this expensive intervention. Only the higher pebble content in the soils of the *Premiers Grand Crus Classés* was not due to the intervention of men. If wine quality was related to specific minerals (potassium, phosphorus, iron, oligoelements), then wine quality could be improved by addition of these elements. Viticultural practice shows that, except for the correction of severe deficiencies or the application of excessive fertilization, wine quality is not easily manipulated in either way by fertilization practices.

Although soil mineral composition does not seem to be, in general, a decisive terroir factor, three elements the vine picks up from the soil need further attention: nitrogen, potassium and calcium. Nitrogen and potassium influence vine development and grape composition. Calcium is an important factor in improving soil structure.

Soil nitrogen

Among elements the vine picks up from the soil, nitrogen is undoubtedly the one that most impacts on vine growth, vigour and grape composition. Much data on this subject is available from fertilization trials with nitrogen (Kliewer, 1971; Bell *et al.*, 1979; Delas *et al.*, 1991; Spayd *et al.*, 1993, 1994). However, even in soils that are not much, or not at all, supplied with nitrogen fertilizers (which is the case in many high-quality wine producing estates), great differences in nitrogen uptake by the vines do exist. The nitrogen availability to the vine is related to the soil type and the soil depth. Nitrogen availability increases with soil organic matter content and soil organic matter turnover. The latter is high when C/N ratio of soil organic matter is low, pH is high, soil temperature is high and soil moisture content is close to field capacity, resulting in high soil microbiological activity. Soil aeration also stimulates organic matter turnover. In this way, the nitrogen availability to the vine is soil type-related and can thus be considered as a terroir characteristic (van Leeuwen *et al.*, 2000). Grape quality potential for red wine production is correlated to vine nitrogen status, particularly so when water status is not limiting. Low vine nitrogen status reduces shoot growth, vigour (Figs. 9.3 and 9.4), berry size and yield, and increases grape sugar (Fig. 9.5), tannin and anthocyanin concentration (Choné *et al.*, 2001a; Trégoat *et al.*, 2002; van Leeuwen *et al.*, 2007). In this way, grape quality potential for red wine production is increased by a limited soil nitrogen availability to the vines.

In white wine production, low nitrogen availability to the vines is not favourable to wine quality. It has been shown to reduce aromatic potential in Sauvignon blanc grapes (Choné *et al.*, 2006). Low vine nitrogen status reduces aroma

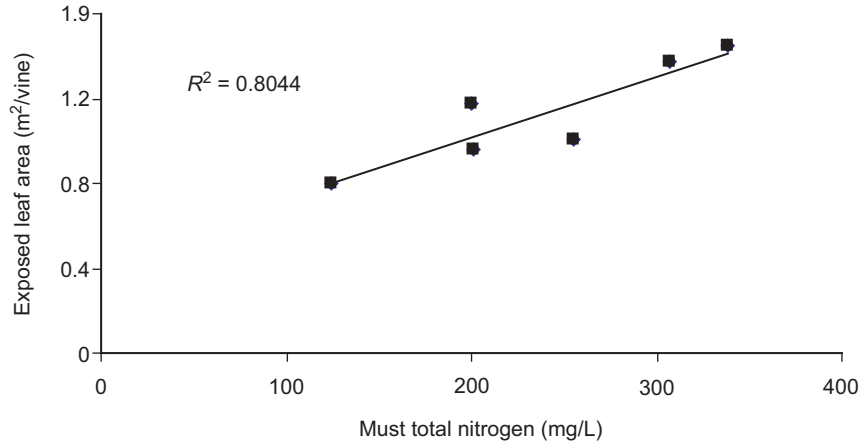

Fig. 9.3 Impact of vine nitrogen status, assessed by measurement of must total nitrogen concentration, on exposed leaf area (Choné *et al.*, 2001a).

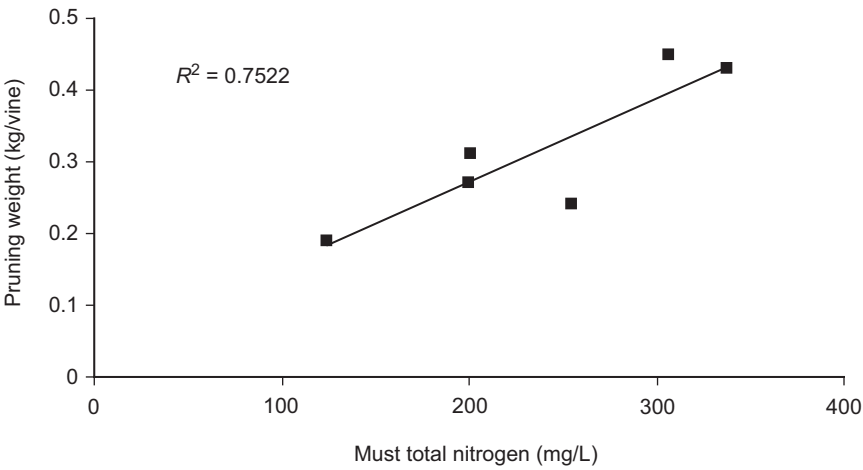

Fig. 9.4 Impact of vine nitrogen status, assessed by measurement of must total nitrogen concentration, on pruning weight (Choné *et al.*, 2001a).

precursor concentration in grapes and enhances grape tannin concentration. Tannins are favourable for quality in red wine grapes, but not for grapes used for white wine making. During juice extraction processes, tannins can be oxidized to quinones that are highly reactive with volatile thiols, the aromas of Sauvignon blanc and some other high-quality white grape varieties (Peyrot des Gachons *et al.*, 2005). Moreover, grapes produced from low-nitrogen grapes contain less glutathione, which limits the ageing potential of the wine (Choné *et al.*, 2006). Even though vine nitrogen status for white wine production should be at least moderate, excessive nitrogen uptake by the vines is not desirable, because it promotes leaf

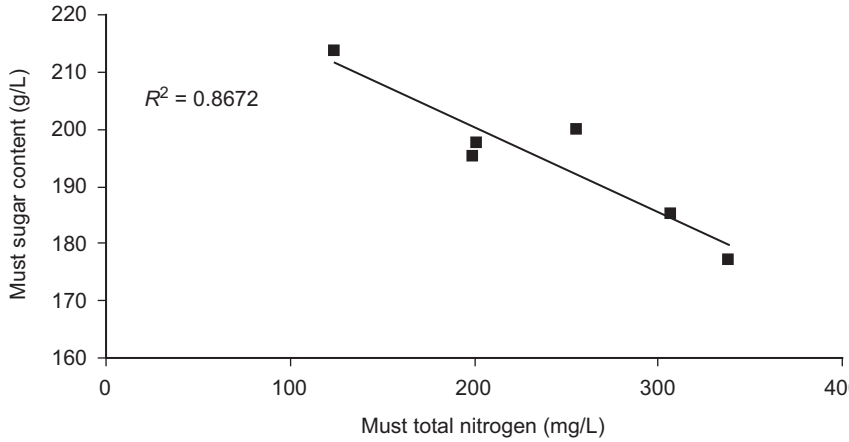

Fig. 9.5 Impact of vine nitrogen status, assessed by measurement of must total nitrogen concentration, on must sugar concentration (Choné *et al.*, 2001a).

crowding and susceptibility to diseases, and particularly *Botrytis cinerea* (Gysi, 1984).

Soil nitrogen availability is also related to irrigation practices. One of the reasons for the success of regulated deficit irrigation on fertile soils is the moderation of nitrogen uptake, especially between fruitset and *véraison*. This effect can occur directly through decreased nitrogen demand by the vine due to moderate stress, and indirectly through a decrease in nitrogen mineralization rate in the soil that is kept drier than under unrestricted irrigation (White *et al.*, 2007).

Because of the important role of nitrogen on grape quality attributes, assessment of vine nitrogen uptake conditions is highly important in terroir studies. Vine nitrogen status can be monitored by soil analysis or analysis on vine organs. Soil analyses are difficult to interpret, because of high spatial variability of soil composition. Moreover, they provide values for total soil nitrogen and not mineral nitrogen. Leaf blade, petiole, must total nitrogen and must yeast available nitrogen are accurate and practical indicators of vine nitrogen status (van Leeuwen *et al.*, 2000, 2007).

Soil potassium

Geology is a major factor in the abundance of potassium in soils; it is present chiefly in potassium feldspar, mica and illite. Potassium is also abundant in soils formed on volcanic rocks (e.g. Madeira and the Kaiserstühl in Southern Germany), slate (e.g. Mosel and Porto) and shale (e.g. Porto) (Huggett, 2006). Excess potassium can increase must and wine pH (Morris *et al.*, 1983; Soyer and Molot, 1993; Cahurel, 2007). However, except in situations of clear deficiency or obvious excess, no evidence has been reported about the possible effects of potassium on grape quality potential (van Leeuwen *et al.*, 2004). Dundon *et al.* (1984) reported that the effect of soil potassium supply to the vines on grape and wine potassium concentration is quite small, unless excessive

amounts are applied. Noble (1979) did not find a correlation between soil and wine potassium concentrations.

Soil calcium

Active calcium carbonate is often associated with wine quality. Many high-quality wine-producing vineyards are located on parent material that contains calcium carbonate (Burgundy, Champagne, Loire Valley, Saint-Emilion in France, Rioja Alavesa in Spain, Coonawarra in Australia). However, its presence in the soil is not indispensable, since some high-quality vineyards are located on acidic soils with very low calcium content (e.g. most of the *Grands Crus Classés* of the Haut-Médoc, Bordeaux, France) (Seguin, 1986). The positive effect of calcium might not be direct. The presence of calcium improves soil structure (Seguin, 1986) and this enhances root penetration and internal soil drainage as well as increasing soil temperature. Active calcium carbonate also reduces soil organic matter turnover, limiting soil nitrogen on offer to the vines. Active calcium carbonate can induce chlorosis (leaf yellowing) due to a difficulty of iron absorption. This problem can be overcome by using adapted rootstocks (e.g. 41B, Fercal, 140 Ruggeri).

9.4.4 Soil colour

The effect of soil colour on grape composition is poorly documented in scientific literature. Soil surface colour modifies microclimate in the bunch zone by two mechanisms: temperature and spectral composition of reflected radiation. A white soil reflects more sunlight and induces a higher amount of skin phenolics in red grapes compared to a black soil (Stoll *et al.*, 2008). Dark coloured soils absorb more heat and warm up more quickly during the day (Fregoni, 1977).

9.4.5 Soil biological activity

Soils host a wide range of macro- and microorganisms. Earth worms play a role in creating soil macro-porosity and in mixing organic and mineral matter in the soil. Microorganisms participate in the transformation of raw organic matter into humus. Most of the nitrogen present in the soil is incorporated into humus. In this form, the nitrogen is not available for plants. Microorganisms mineralize the humus (1–2% of the total humus content of the soil is mineralized each year, depending on the soil type and the climatic conditions), which makes the nitrogen return into a mineral form that can be absorbed by the vine (NO_3^-). Hence, microorganisms are essential in providing nitrogen to the vines. The development of microorganisms in the soil is enhanced by the presence of humus, low C/N ratios of the humus, soil aeration, pH close to neutrality and humidity close to field capacity (Chaussod *et al.*, 1996). Copper, which is used in sprays against downy mildew, has a strong depressive effect on soil microorganisms (Courde *et al.*, 1998). For Bourguignon (1995), wine quality and terroir expression are related to microbial activity of vineyard soils. Although a healthy soil should have at least an adequate level of microorganisms to ensure mineralization of humus, no scientific

evidence exists about a direct influence of soil microorganisms on wine quality or terroir expression. Because high soil nitrogen availability is not desirable for the production of high-quality wines, it is rather likely that a very active microflora in the soil (in relation to high soil organic matter content and low organic matter C/N ratios) is *not* desirable in vineyard soils.

9.4.6 Soil temperature

Soil temperature, which is related to energy balance, can be 18 °C higher during a sunny day and 2 °C lower during the night compared to air temperature (Verbrugghe *et al.*, 1991). The proportion of sunlight reflected on the soil is called *albedo* and depends on the colour of the soil. Light coloured soils reflect more sunlight than dark coloured soils. Elevation of soil temperature is related to soil water content, because water has a high specific caloric capacity: wet soils warm up more slowly than dry soils (Tesic *et al.*, 2002a). Verbrugghe *et al.* (1991) compared soil surface temperatures on three soils in Chateauneuf-du-Pape (France). Soil surface temperature was higher on stony soils during the night. Vegetative growth is positively correlated with root temperature. High root temperature increases pH in berries and enhances soluble solids accumulation, while berry malic acid concentration is decreased (Zelleke and Kliewer, 1979). Vine development and dry matter shoot/root ratio increase with root temperature (Woodham and Alexander, 1966). High temperature in the root zone during initial phases of the annual cycle increase precocity. In cool climate zones, like the Loire Valley (France), this effect is critical for grape quality (Morlat, 1989; Barbeau *et al.*, 1998). However, it is difficult to separate the effect of temperature in the root zones from the effect of water deficit stress. Low soil water content and/or shallow rooting increase temperature in the root zone, but these factors are also likely to induce water deficit stress.

Air temperature in and around the canopy is also influenced by the soil type, in relation to soil water content, soil thermal conductivity and soil albedo. Air temperature measured at 2 m in altitude is higher in the morning on a soil with a fine texture, because of a good thermal conductivity (Fig. 9.6a). In the afternoon, air temperature is higher above a stony soil (Fig. 9.6b 1FON), because this soil type warms quicker during the day (Jacquet and Morlat, 1997). Grape temperature was measured with infrared thermometry on three soils in Chateauneuf-du-Pape (Verbrugghe *et al.*, 1991). On stony soils, grape temperature was higher (up to 3 °C) compared to grape temperature measured on soils with fine texture and no stones. During the day, this is the result of a high albedo on stony soils and, hence, more reflected radiation to the grapes. Higher grape temperature during the night can be explained by infrared radiation to the grapes on the stony soils.

9.4.7 Soil water

Soil water is stored in the porosity of the soil. The state of the water in the soil depends on the size of the pores. In large pores (> 10 μm in diameter), water cannot

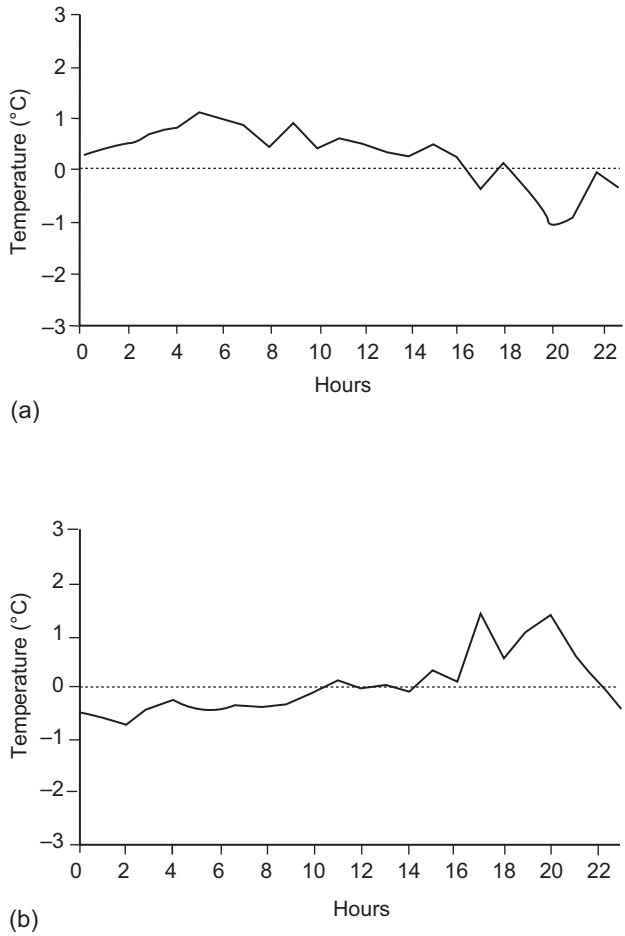

Fig. 9.6 (a) Difference in air temperature during a sunny day, measured at 2 m in altitude, in a plot with a fine textured soil compared to a reference station; (b) difference in air temperature during a sunny day, measured at 2 m in altitude, in a plot with a stony soil compared to a reference station (Jacquet and Morlat, 1997).

be held in the soil and drains out of the soil by gravity, unless an impermeable layer provokes water logging. In very small pores (< 0.2 μm in diameter), the water is so firmly held by the soil that plants are not able to extract it. In pores between 0.2 μm and 10 μm in diameter, water is held by the soil but can be extracted by plant roots. Sand holds little water, but a large proportion of the water is readily available for plant use. Clay holds a lot of water, but the majority of it is held in very small pores and cannot be extracted by plant roots. Silt holds a relatively large amount of water and the majority of this water can be used by the plants. Hence, plant available water varies with soil texture, but also with the proportion of stones and rooting depth. Soil water holding capacity is highly variable among vineyard soils and

varies from 50 to over 350 mm. Water logging occurs in soils with poor internal drainage and makes free water readily available to the vines. More details about water in vineyard soils can be found in White (2003).

9.5 Effect of vine water status in terroir expression

9.5.1 Impact of climate and soil on vine water status

The highly important role of vine water status in terroir expression was first shown in the 1960s by Seguin (1969), who studied vine water uptake condition in the Bordeaux area with a neutron moisture probe, and it has been confirmed ever since. Vine water status is influenced by climatic variables (rainfall, reference evapotranspiration), soil-related variables (water holding capacity) and viticultural management (training system, plant material, irrigation). The assessment of vine water status is an integrative approach in terroir studies, that combines climate, soil and plant material effects. Vine water status is equally impacted by climate and soil (van Leeuwen *et al.*, 2004).

9.5.2 Effect of water status on vine growth and grape composition

The effects of water status on vine development, growth, vigour, grape composition and wine quality, in relation to terroir expression, have been extensively studied. Seguin (1975) showed that grape composition in *Grands Crus Classés*, located on gravelly soils in the Haut-Médoc (Bordeaux, France) was positively influenced by a regulation of the water uptake condition of the vines. Duteau *et al.* (1981) measured, by means of a neutron moisture probe, water uptake conditions

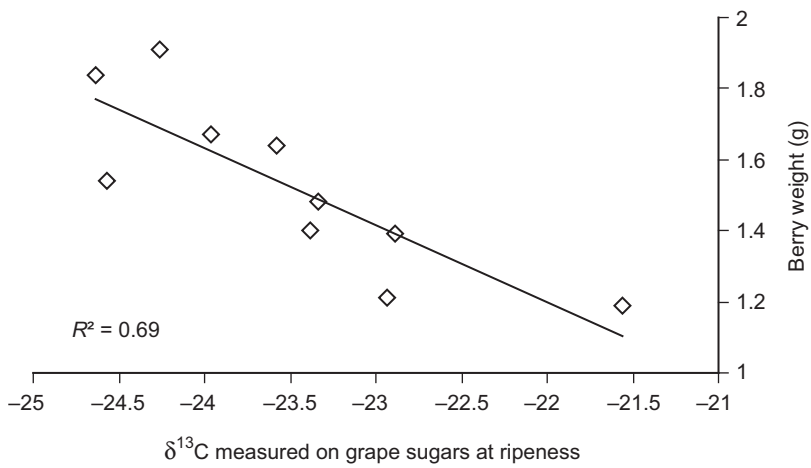

Fig. 9.7 The impact of vine water deficit stress, assessed by carbon isotope discrimination measured on grape sugar ($\delta^{13}C$), on berry weight. In Figs 9.7 to 9.10, the more negative the $\delta^{13}C$ values, the greater the water deficit stress between *véraison* and harvest (Trégoat *et al.*, 2002).

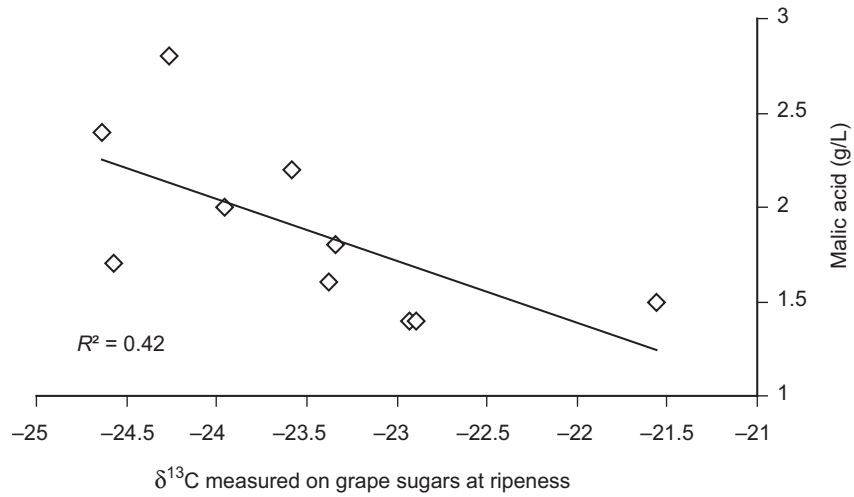

Fig. 9.8 The impact of vine water deficit stress, assessed by carbon isotope discrimination measured on grape sugar ($\delta^{13}C$), on berry malic acid concentration.

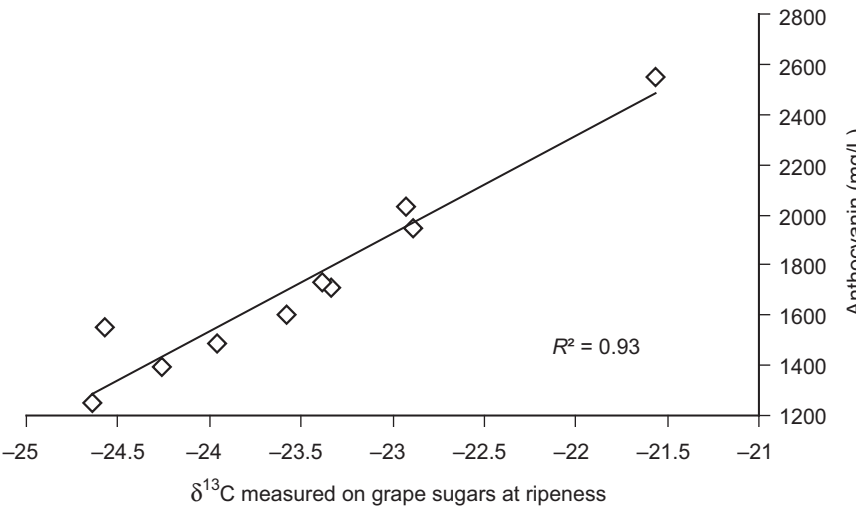

Fig. 9.9 The impact of vine water deficit stress, assessed by carbon isotope discrimination measured on grape sugar ($\delta^{13}C$), on grapeskin anthocyanin concentration.

in several soil types in Saint-Emilion and Pomerol (Bordeaux, France). Reduced water supply to the vines limited berry malic acid concentration and increased berry anthocyanin and tannin concentration. Berry sugar concentration was increased by mild water deficit stress, but reduced by severe water deficit stress. In one shallow soil, on a hard limestone bedrock not explored by roots, the bedrock yielded by capillarity up to 40% of the water consumed by the vines in a dry summer (Duteau, 1987). This effect participated in the regulation of vine water

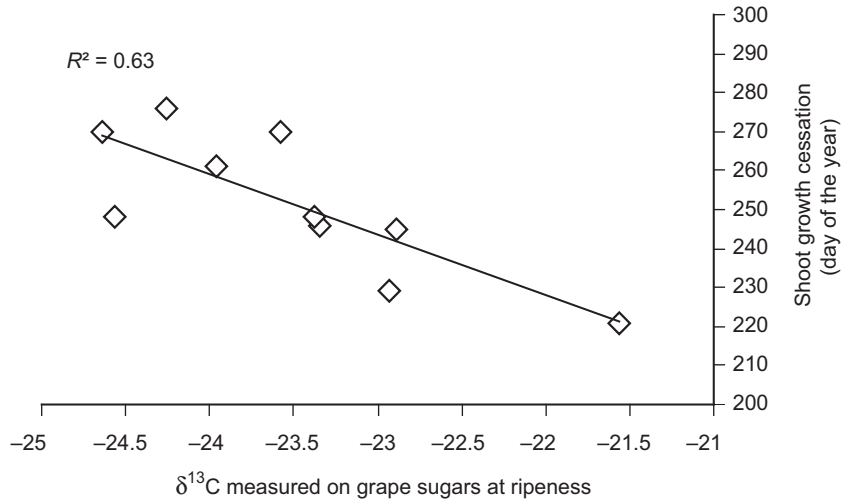

Fig. 9.10 The impact of vine water deficit stress, assessed by carbon isotope discrimination measured on grape sugar ($\delta^{13}C$), shoot growth cessation.

uptake conditions. Water deficits reduced berry size and enhanced shoot growth cessation, which increased the proportion of carbohydrates available for fruit ripening (van Leeuwen and Seguin, 1994). Berry anthocyanin and total phenols concentrations were increased and berry malic acid concentration was decreased.

Trégoat *et al.* (2002) assessed vine water status on 10 blocks planted with Merlot in Bordeaux, during the dry 2000 vintage, by means of stem water potential measurements and carbon isotope discrimination measured on grape sugars at ripeness. Vine water status varied with soil water holding capacity, depending on soil texture and vine rooting depth. Vine water deficit reduced berry weight (Fig. 9.7) and berry malic acid concentration (Fig. 9.8) and increased grapeskin anthocyanins (Fig. 9.9). Shoot growth cessation was enhanced on plots where vines experienced water deficit stress (Fig. 9.10). In one plot, shoot growth stopped early in the season, berries were small, high in anthocyanin concentration and low in malic acid concentration despite unrestricted water uptake conditions. The limiting factor in this plot was low vine nitrogen status.

In a very complete terroir study in Nemea (Peloponesus, Greece) on unirrigated vines, most viticultural and enological variables were correlated to vine water status, that was assessed by pre-dawn leaf water potential measurements (Koundouras *et al.*, 2006). Pre-dawn leaf water potential values at harvest ranged from –0.1 to –0.9 MPa depending on the soil water holding capacity of the plot and the climate of the vintage (rainfall and reference evapotranspiration). Water deficit accelerated sugar accumulation and malic acid breakdown and enhanced earliness of phenological stages and shoot growth cessation. Early water deficit had beneficial effects on the concentration of anthocyanins and total phenolics in berry skins and wines. Limited water availability increased glycoconjugates of the main aromatic components of grapes. Wine quality, assessed by a tasting panel, was

highly correlated to the level of water deficit experienced by the vines during the growing season.

Similar results were found in Tuscany (Italy) by Storchi *et al.* (2005). A large proportion of the variance of yield and berry composition variables was determined by soil type and soil water holding capacity. High water availability from *véraison* to harvest induced more vegetative growth and reduced sugar, colour and phenol concentrations in the berries. In Catalunya (Spain), the effect of soil type on Grenache wine sensory attributes was strong and consistent over two following vintages with contrasting climatic condition (de Andrés-de Prado *et al.*, 2007). The soil with low organic matter content and low water holding capacity produced wines with more colour and total phenolics. Vintage quality in Bordeaux is related to the water balance: the drier the year, the better the overall quality of the wine produced (van Leeuwen *et al.*, 2003, 2009). Similar positive effects of moderate water deficits on grape quality potential have been obtained in irrigation trials (Matthews and Anderson 1988, 1989; Ojeda *et al.*, 2002 and many other references).

9.5.3 Terroir expression and irrigation

Many studies have shown that terroir expression is closely related to the occurrence of water deficits. Hence, terroir expression is depressed, if not completely eliminated, by full irrigation. In many regions in the world, an economically sustainable viticulture is not possible without irrigation, because dry farming in dry climates strongly depresses yield. In those situations, terroir-driven wines can be produced with finely-tuned deficit irrigation. In Europe, most of the renowned terroir-based wines are produced in dry farmed vineyards, even in dry areas like Priorat (Spain). In New World countries, where irrigation is a common practice, some of the most famous *boutique* wines are produced without irrigation.

9.5.4 Assessment of vine water uptake conditions in terroir studies

Because of the important role of vine water status in terroir expression, its assessment is of critical importance in terroir studies. Vine water uptake conditions can be assessed through (i) soil water monitoring by means of tensiometers (Nadal and Arola, 1995), watermark probes (Hanson *et al.*, 2000), neutron moisture probes (Seguin, 1986) or time domain reflectometry (Koundouras *et al.*, 1999); (ii) water balance modelling (Lebon *et al.*, 2003); or (iii) the use of physiological indicators (van Leeuwen *et al.*, 2001a; Jones, 2004; Cifre *et al.*, 2005). Among physiological indicators, three are particularly accurate and useful in terroir studies: pre-dawn leaf water potential (van Leeuwen and Seguin, 1994), stem water potential (Choné *et al.*, 2001b) and the $^{13}C/^{12}C$ ratio ($\delta^{13}C$ or carbon isotope discrimination) measured on grape sugar at ripeness (van Leeuwen *et al.*, 2001b; Gaudillère *et al.*, 2002). These methods are discussed in Deloire *et al.* (2005) and van Leeuwen *et al.* (2007, 2009).

Pre-dawn leaf water potential and stem water potential allow a precise monitoring of vine water status during the season. However, these methods are

time-consuming. Stem water potential can be used in soils with heterogeneous soil humidity (irrigated vineyards, measurement of residual water deficits after summer rain), while pre-dawn leaf water potential cannot (Améglio *et al.*, 1999). Carbon isotope discrimination measured on grape sugars is an integrative measurement of average vine water deficit during the period *véraison*–harvest. No field measurements are necessary other than grape sampling at ripeness. Many measurements can be carried out, which makes this method useful for water status spatialization purposes (van Leeuwen *et al.*, 2006, 2009). Trégoat *et al.* (2002) related vine water status assessed by carbon isotope discrimination to shoot growth, berry size and grape composition. Pre-dawn leaf water potential, stem water potential, carbon isotope discrimination and water balance modelling were shown to be accurate to assess vine water status in terroir studies in Switzerland (Zufferey and Murisier, 2007). Costantini *et al.* (2008) used carbon isotope discrimination to show a highly significant negative correlation between $\delta^{13}C$ values and grape phenolics in Sangiovese grapes in Tuscany, Italy (the more water deficit stress, the more total phenolics).

9.6 Global indicators in terroir assessment

9.6.1 Soil depth

Much confusion exists about terroir expression in relation to soil depth. To soil scientists, soil depth is the depth to which the parent material is altered by pedological processes. Viticulturists consider rooting depth as soil depth. In general, shallow soils allow only shallow rooting, while deep soils allow deep rooting. However, if the parent material is not hard rock, roots may extend beyond the layers altered by pedological processes. This might increase the amount of water available to the vines. The first comprehensive terroir studies were carried out by Seguin (1969, 1975, 1983, 1986) in gravelly soils in the Haut-Médoc (Bordeaux, France). This author showed that in these poor, free draining soils, rooting depth is a quality factor in red wine production, because it regulates vine water uptake conditions. These observations have been incorrectly extended to other soil types, and in many popular wine books, the terroir effect is presented to be mediated trough vine roots exploring deep soil layers in search for minerals. In reality, on most substrates *shallow* soils enhance terroir expression.

This aspect was extensively studied by Bodin and Morlat (2003, 2006) in the Loire Valley (France), who developed a field model based on soil depth and average clay content, in relation to the level of weathering of the parent rock. Sites were characterized as weakly weathered rock (WWR), moderately weathered rock (MWR) and strongly weathered rock (SWR). Vine vigour was high and phenology was delayed on SWR (deep soils); vigour was low and phenological stages were reached earlier on WWR (shallow soils). This effect was largely mediated through vine water status, as is shown by the authors through pre-dawn leaf water potential measurements and carbon isotope discrimination ($\delta^{13}C$) measurements on grape sugar at ripeness (Fig. 9.11). On WWR, grape berries were smaller, richer in sugars

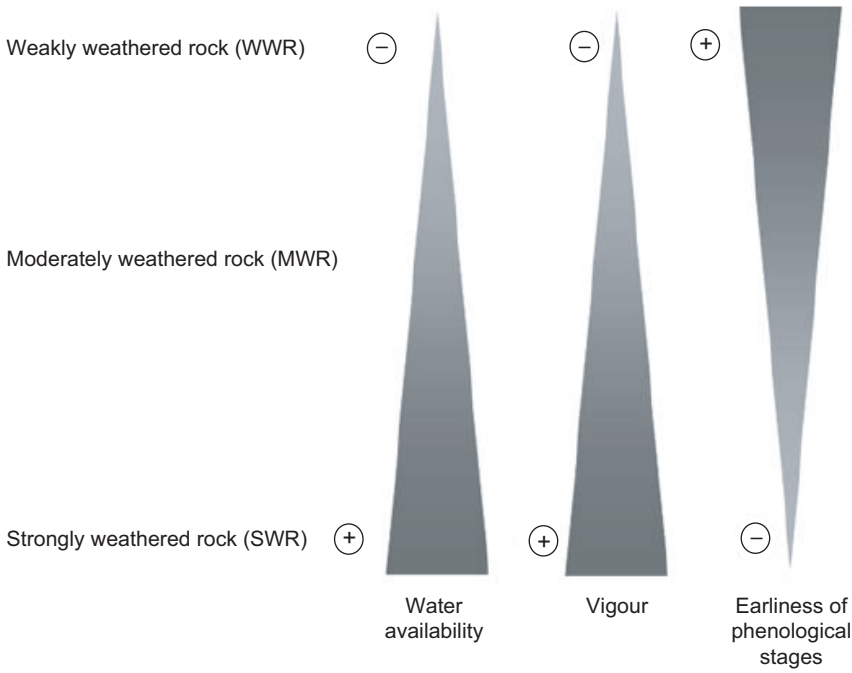

Fig. 9.11 The impact of the level of weathering of the rock, and related soil depth, on soil water availability, vine vigour and earliness of phenological stages of the vines (Bodin and Morlat, 2006).

and anthocyanin and had a higher total phenolics than those of the vines cultivated in SWR (Morlat and Bodin, 2006). On WWR, grapes contained less malic acid, resulting in a lower titratable acidity. Thus, shallow soils have higher grape quality potential for red wine production because their low water holding capacity is more likely to induce water deficit stress compared to deep soils. Vine nitrogen status was not controlled in this study. However, it is likely that soil nitrogen offer is also lower on shallow, weakly weathered soils and that this reinforces the devigorating effect of low water supply on these soils. Similar results were published by Coipel *et al.* (2006) in the very different environment of the Rhône Valley (France). Even in a very dry vintage, vine vigour was lower and grape quality potential higher on shallow soils compared to deep soils, because water and nitrogen supply were restricted on shallow soils. Soil depth can thus be an integrative variable in terroir studies. This approach might suffer some exceptions in alluvial soils.

Several soil variables influence vine rooting. The quantity of roots and their vertical distribution is positively correlated to water supply, and negatively to penetrometer soil strength, bulk density and water logging (Morlat and Jacquet, 1993).

Limitation in soil depth reduces the soil water holding capacity, which can be a quality enhancing factor. However, the first 20 cm of the soil are relatively rich, because they contain most of the organic matter and it is not desirable to have most

of the roots in this part of the soil. Weed destruction by ploughing, or the use of cover crop, prevents roots from colonizing the surface layer of the soil, while the use of herbicides promotes them to colonize this layer (Soyer *et al.*, 1984).

9.6.2 Precocity

In cool climate viticulture, grapes attain ripeness in cool conditions at the end of the season (September or October in the Northern Hemisphere, March or April in the Southern Hemisphere). In warm climate viticulture, grapes ripen in the warmest part of the summer (July or August in the Northern Hemisphere, January or February in the Southern Hemisphere). Viticultural regions belonging to the first category are called 'alpha zones' by Jackson and Lombard (1993), while regions belonging to the second category are called 'beta zones'. Terroir expression, it is suggested, is obtained in cool ripening conditions and is accordingly high in alpha zones. In beta zones, it is similarly suggested that terroir expression is weak or non-existent. In alpha zones, attaining full ripeness for the grapes growing in those regions might be challenging, particularly so when the ripening period of the local grapevine variety (or varieties) is late compared to the local climatic conditions. In those situations, precocity can be an important quality factor. Conversely, precocity is not important in beta zones.

In the Loire Valley, grape quality potential is closely related to precocity (Barbeau *et al.*, 1998). In this region, high anthocyanin concentrations appeared related to early grape ripening (Brossaud *et al.*, 1999). Precocity can be explained by advantagous exposition or high soil temperatures. Precocity is also important in the cool climate of Hawke's Bay, New Zealand (Tesic *et al.*, 2002b). It is positively correlated to grape sugar concentration and wine quality score and negatively correlated to berry malic acid concentration (Tesic *et al.*, 2002a). Precocity is enhanced by low vigour. Mild water deficit enhances precocity, because grapes ripen faster when shoot growth stops before *véraison* (van Leeuwen *et al.*, 2003, 2009).

With global warming, alpha zones might become beta zones and precocity will no longer be an advantage. Today, the most highly rated sub-regions in the Bordeaux area (Pauillac, Saint-Emilion, Pomerol, Pessac-Léognan, Sauternes) accumulate more growing degree days over the season than other parts of Bordeaux (Bois *et al.*, 2008a). In the hot 2003 vintage, wines from Pomerol appeared to be disappointing, while wines from cooler sub-regions (northern part of the Médoc) were surpringly good in that vintage. This might be an indicator of what will happen over the next decades as a result of global warming.

9.6.3 Vigour

Vine vigour is related to genetic and environmental conditions. When similar plant material is compared (same rootstock and cultivar), vine vigour expresses the impact of climatic conditions and soil fertility. It can thus be used as an integrative indicator in terroir studies. Vigour was mapped by means of remote sensing, and

grape composition varied with vigour zones (Strever, 2007). Vine size was used by Reynolds *et al.* (2007) to map vine vigour at intra-block scale. The relationships among grapevine (*Vitis vinifera* Pinot noir) vigour variation and resulting fruit composition, wine chemical analyses, and sensory attributes were investigated by Cortell *et al.* (2008).

9.7 Terroir zoning

9.7.1 The need for terroir zoning

Environmental conditions are highly variable in space. Spatial variability of terroir is essential information in viticulture: (i) to select production zones according to their quality potential; and (ii) to adapt viticultural management for optimizing quality in relation to the potential offered by the local environmental conditions. In Europe, and particularly so in France, wine is sold by its origin and winegrowing regions have since long been demarcated in the *Appellation d'Origine Contrôlée (AOC)* system. However, the AOCs were initially only created to protect the name of a commune or winegrowing region, and their delimitation was based on administrative boundaries rather than extensive studies of the natural environment. Progressively, some environmental variables have been included in delimitation practises (geology, soil type, susceptibility of soils to water logging) but the AOC concept is also based on human factors ('*usages loyaux, locaux et contants*', which can be translated as 'loyal, local and consistent practices') (Flutet *et al.*, 2008).

Moreover, the scale of terroir effect is great (homogeneous terroirs are very small, generally a few hectares only) and the scale of *Appellations* is small (hundreds or thousand of hectares). One *Appellation* comprises in general several terroirs. Although for political reasons it is not very likely that boundaries of production zones will be dramatically changed, increasing knowledge about how environmental variables inpact on grape quality potential, as well as the development of new technologies, make it possible today to proceed to viticultural zoning on a scientific basis.

9.7.2 Climate-based zoning methods

Climatic zoning can be carried out by means of agroclimatic indices. Tonietto and Carbonneau (2004) developped a multicriteria climatic zoning sytem (MCC system), based on Huglin index (temperature sum), a water balance model and a cool night index (average minimum temperature in September in the Northern Hemisphere and March in the Southern Hemisphere). This system is very accurate for classifying the climate of winegrowing regions worldwide. An interactive database has been created to compile climatic MCC data of wine growing regions worldwide (Bello Fialho and Tonietto, 2008). Climatic zoning at the meso-scale level requires a high density of automatic weather stations and long series of data records. Precision of climatic zoning at a meso-scale level can be increased with the use of digital elevation models, satellite-sensed solar radiation data and land

cover databases. Point information obtained in weather stations can be interpolated with kriging techniques to produce maps (Bois, 2007; Bois *et al.*, 2008a,b,c).

9.7.3 Soil-based zoning methods

Soil zoning can be based on various disciplines: geology, geomorphology or pedology. Geology and geomorphology allow small-scale zoning (1:25 000 to 1:250 000). However, geology and geomorphology do not explain vine development and grape ripening. Pedology (soil type zoning) allows zoning at greater scale (1:3000 to 1:100 000) and some information provided by soil maps is useful for vine management (soil lime content, soil pH, susceptibility to water logging). High-resolution soil mapping (i.e. 1:25 000 scale; van Leeuwen *et al.*, 1989) over large areas is very costly and for budget reasons often low-resolution maps are established (1:100 000 or 1:250 000). To increase the precision of low-resolution soil maps, 'reference areas' (small areas inside the mapped regions where soils are highly variable) can be mapped at a much higher resolution (i.e. 1:5000 or 1:10 000). In these reference areas, the main soil types of the region are described and rules for their spatial distribution (mainly with respect to geology and geomorphology) are established. These rules of soil distribution are then applied to the whole region. This approach was successfully applied to the Cognac region in France (Cam *et al.*, 2003).

9.7.4 Integrated zoning methods

Morlat (1989, 2001) developed in the Loire Valley (France) the concept of *Basic Terroir Units* (BTU). A BTU is a zone in which geology, soil type and mesoclimate is homogeneous enough to produce specific response in vine behaviour and grape composition as well as a characteristic type of wine. Major parts of the Loire Valley were mapped by Morlat and co-workers with this concept. However, a BTU can be very small and it is therefore not always easy to produce a specific wine on a single BTU. Moreover, mapping of BTUs is time-consuming and thus very expensive. To overcome these difficulties, Bodin and Morlat (2006) developed a field model based on soil depth and average clay content, in relation to the level of weathering of the parent rock (see Section 9.6.1 on soil depth).

Astruc *et al.* (1980) developped a viticultural zoning method in the Aude (Languedoc, France) that combined climatic zoning (rainfall, temperatures), inventory of the spontaneous vegetation, vine phenology records and soil mapping. The use of spontaneous vegetation as a viticultural zoning criterion is of particular interest, because spontaneous vegetation react to all environmental variables (climate, soil type).

A site index was published for Hawke's Bay in New Zealand that was negatively correlated with final shoot length and grape malic acid concentration and positively correlated with total soluble solids, grape skin anthocyanin and wine sensory scores (Tesic *et al.*, 2002b). This site index increased with air temperature in January and October (in the Southern Hemisphere) and gravel percentage in the

soil; it decreased with the amount of seasonal rainfall (October to April in the Southern Hemisphere), soil clay to silt ratio and rooting depth. However, this index, based on observations in a limited number of sites (six) and vintages (two), is not likely to be of general interest. Its implementation is not very practical, because several variables (particularly rooting depth) are difficult to assess.

9.7.5 Zoning based on physiological indicators

Terroir effect is largely mediated through the impact of environmental variables on vine water status and vine nitrogen supply (van Leeuwen *et al.*, 2004, 2007; Bodin and Morlat, 2006). Both can conveniently be assessed with plant-based indicators. Vine water status can be mapped with carbon isotope discrimination ($^{13}C/^{12}C$ ratios or $\delta^{13}C$) at various scales (van Leeuwen *et al.*, 2007, 2009). In non-irrigated vines, $\delta^{13}C$ gives a good insight in the ability of the natural environment (soil and climate) to supply water to the vines. Vine nitrogen status can be assessed with yeast available nitrogen (YAN) measurements in grape juice at ripeness. Because YAN is easy to establish, many measurements can be taken for spatialization purposes, at various scales. When nitrogen fertilization is low or non-existent, YAN measurements allow an assessment of the soil nitrogen availability to the vines.

9.7.6 GIS-based zoning methods

Traditional winegrowing regions in Europe have had hundreds of years to define, develop and understand their best terroirs. For winegrowing regions in the New World, modelling viticultural landscapes can be a way to reduce this period of trial and error. Geographic information systems (GIS) are powerful tools to combine spatial information about altitude slope, aspect, climatic variables and soil types into a map of suitability for viticulture. Data about altitude, slope and aspect can be obtained at a very narrow grid from a digital elevation model. Viticultural suitability analysis by means of a GIS was successfully applied to the Umpqua Valley in Oregon (USA) by Jones *et al.* (2004).

The main vine cultivation areas (VCA) of the province of Siena (Tuscany, Italy) were studied by means of a GIS database system. The thematic layers created included climate, morphology, land use, geology and soil type. Although Montepulciano was slightly dryer and Montalcino slightly warmer, a greater variability was found inside VCAs than between VCAs. With respect to geology, Montepulciano and Chianti Colli Senesi are rather uniform (dominated by Pliocene marine sand), while Chianti Classico is quite heterogeneous (marly limestone, marls and turbidities, marine sand, sandstone). The vineyards of Chianti Classico and Montalcino have significantly stonier soils than the other VCAs, as a consequence of the large quantity of hard rocks inside the soil parent material (Costantini *et al.*, 2006).

9.7.7 Zoning based on the perception of growers

Growers correctly perceive the main characteristics of their own terroirs, in terms

of soil depth, soil water holding capacity, air temperature, precocity, vigour potential and vine water uptake conditions (Thélier-Huché and Morlat, 2000; Bodin and Morlat, 2003). A survey conducted among growers gave results similar to the terrain model based on soil depth developed by the same authors (see Section 9.6.1 on soil depth).

9.7.8 The use of new technologies for terroir studies and zoning

The development of new technologies offers new perspectives for terroir studies and viticultural zoning. Geomorphology can be easily studied with a digital elevation model (Jones *et al.*, 2004). Plant-related variables (vigour, water stress) can be mapped by means of remote sensing (Hall *et al.*, 2002; Dobrowski *et al.*, 2003; Strever, 2007; Costa-Ferreira *et al.*, 2007). Point measurements can be transformed into maps by the use of geostatistics (Bois, 2007). Soil resistivity measurements can yield very high-resolution maps of soil variability. They can also give an insight into how and where vines pick up water from the soil (Goulet and Barbeau, 2006). However, this technique is not precise enough to derive water balance maps from successive measurements carried out during the season. Although these new technologies are rather associated with precision viticulture, they are also useful in terroir studies, particularly when resolution is high (Bramley and Hamilton, 2007).

9.7.9 Terroir studies at the intra-block scale (precision viticulture approach)

Soil and topography are key drivers of vineyard variability and, as a consequence, intra-block patterns of yield, vigour and quality are stable in time (Bramley and Hamilton, 2004, 2007; Bramley, 2005) (Chapter 12). For Tisseyre *et al.* (2008), intra-block patterns of pruning weight, yield and canopy size were stable over the years, but patterns for grape sugar, pH and titratable acidity were not. van Leeuwen *et al.* (2006) showed that intra-block variations in vine water status can be mapped with stem water potential measurement or carbon isotope discriminaton measured on grape sugars. Vine water status varies from one vintage to another depending on rainfall and reference evapotranspiration of the vintage, but intra-block patterns are consistent over the years. Reynolds *et al.* (2007) collected data over three vintages on 215 georeferenced Riesling vines in a 4 ha vineyard block. Specific areas of the vineyard that produced high yields and highest concentrations of monoterpenes appeared to be transient, and their spatial distribution varied temporally. The precision viticulture approach enables a better insight into *how* terroir influences vine development and grape composition, specifically soil-related aspects of terroir because most other factors are constant in such a small area as a vineyard block (cultivar, rootstock, vine age, mesoclimate, management practices). However, it raises the question of at what scale terroir is operating (see also Section 9.1.6 on scale issues).

9.8 Hierarchy of terroir factors

9.8.1 Hierarchy among soil, climate and cultivar and human factors

The main factors involved in terroir expression are soil, climate and cultivar, and these factors interact. Other factors are involved, like topography, origin and human factors. It is critical to know if some of these factors are more important than others. This question also has strategic implications in wine production. If the cultivar is more important than the soil and the climate, wine should be sold by the name of the cultivar. If soil and climate impact more on wine quality, then the wine should be sold by origin. If the human factor is descisive, it makes sense to market wines by means of strong brands. It is not easy to hierarchize terroir factors, because the weight of each terroir factor might vary depending on their interactions. However, fragmentary data are published on the subject and they are, not surprisingly, sometimes contradictory.

Rankine *et al.* (1971) studied, simultaneously, the effect of soil, climate and cultivar in Australian vineyards. These authors found a dominating effect of the cultivar. However, the soils they studied were situated in different climatic zones, making it difficult to separate effects of soil and climate. Moreover, these authors compared white (Riesling and Clare Riesling) and red (Shiraz) varieties.

In Bordeaux, a similar study was carried out (van Leeuwen *et al.*, 2004). Three soils (gravel, sand with water table and heavy clay) and three grapevine varieties (Merlot, Cabernet franc, Cabernet-Sauvignon) were compared during five vintages. Differences in climatic conditions from one vintage to another were

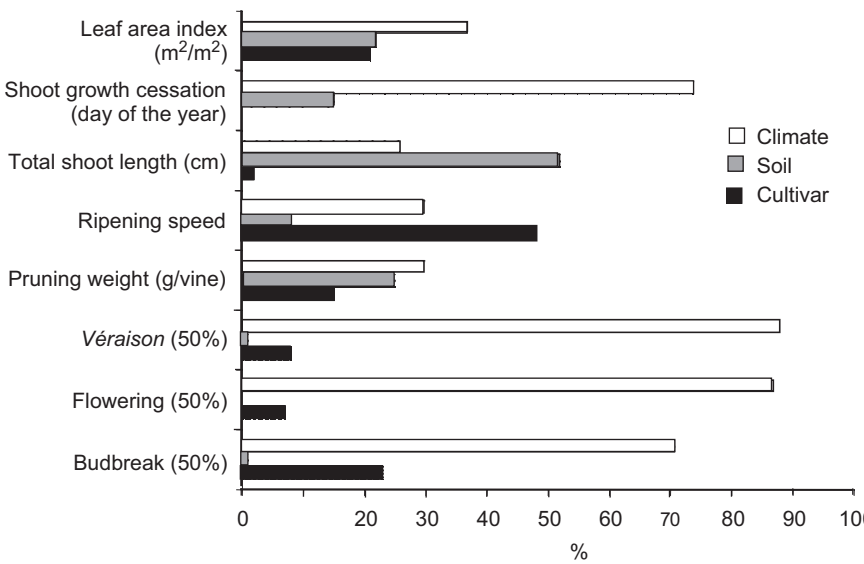

Fig. 9.12 Precociousness and vigour: percentage of variance attributable to climate (vintage effect), soil and cultivar.

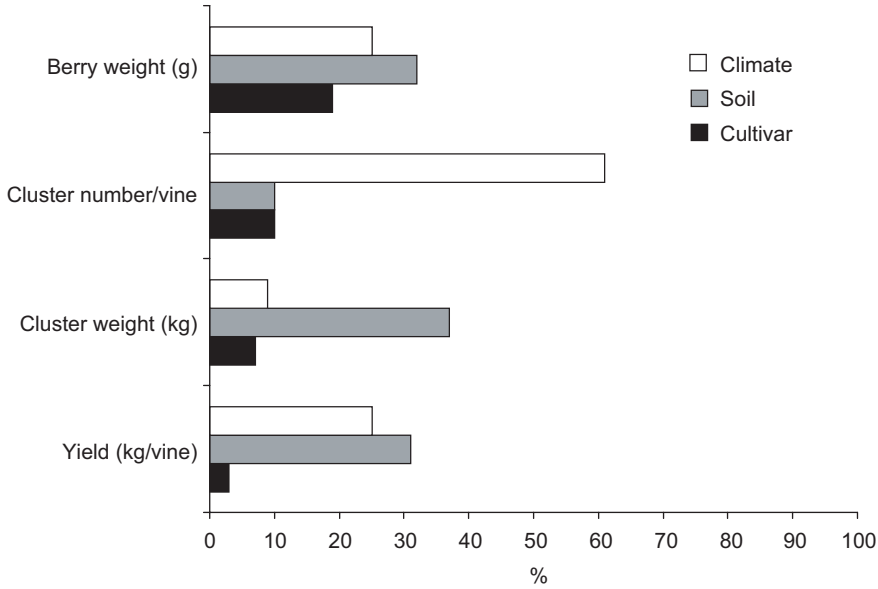

Fig. 9.13 Yield components: percentage of variance attributable to climate (vintage effect), soil and cultivar.

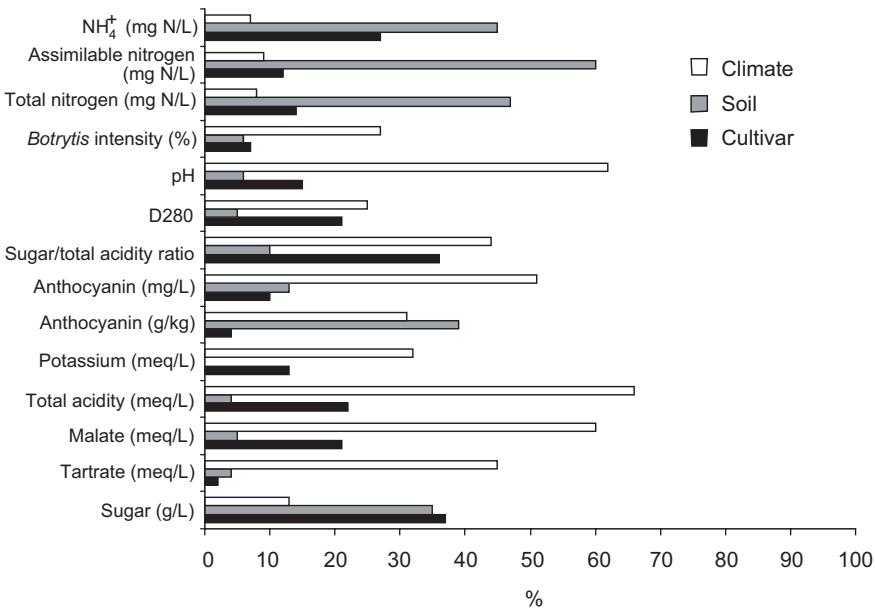

Fig. 9.14 Berry composition at ripeness: percentage of variance attributable to climate (vintage effect), soil and cultivar.

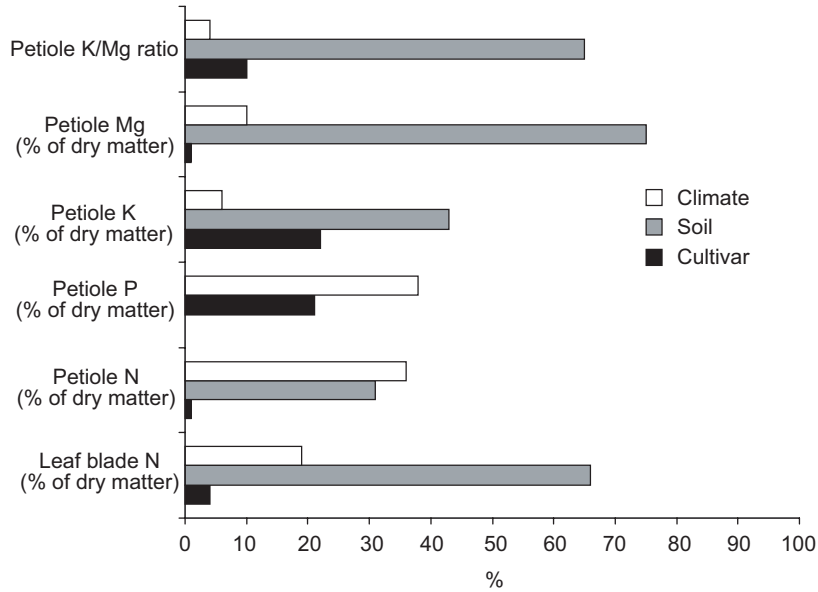

Fig. 9.15 Vine mineral status: percentage of variance attributable to climate (vintage effect), soil and cultivar.

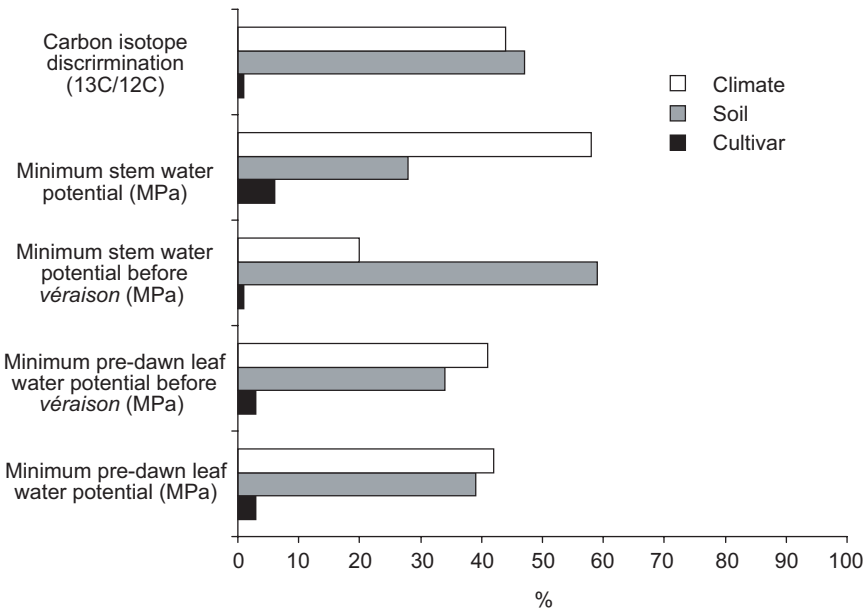

Fig. 9.16 Vine water status: percentage of variance attributable to climate (vintage effect), soil and cultivar.

considered as the climatic factor. Precocity of phenological stages and shoot growth cessation as well as leaf area index were mainly determined by the climatic conditions of the vintage. Total shoot length at shoot growth cessation was determined by the soil type, followed by the climatic conditions of the vintage (Fig. 9.12). Cluster number per vine was mainly determined by the climate, while cluster weight was determined by the soil type. Berry weight and yield were equally impacted by soil and climate (Fig. 9.13). All variables related to acidity (pH, titratable acidity, malic acid, tartaric acid), *Botrytis* and berry anthocyanin concentration were most strongly influenced by the climatic conditions of the vintage. Berry nitrogen concentration (total nitrogen, YAN and NH_4^+) were mostly influenced by soil type. Berry sugar concentration was mainly influenced by the cultivar and the soil type, while sugar/acid ratio was determined by the vintage and the cultivar (Fig. 9.14). Mineral status was mainly determined by the soil type, except for petiole phosphorus and nitrogen content, which were equally influenced by soil type and the vintage (Fig. 9.15). Overall, the climatic conditions of the vintage had the strongest effect on most variables, followed by soil type and cultivar. The effects of climate and soil type were shown to be mediated through vine water status, which itself depended equally on the weather of the vintage (rainfall amount and distribution, reference evapotranspiration) and the soil type (Fig. 9.16). This is consistent with Ubalde *et al.* (2007), who found that variability in yield and grape quality attributes were more determined by the weather of the vintage than by the soil type.

Metabolic profiling by ^{1}H-NMR (nuclear magnetic resonance) spectra of wines can be used to compare environmental and genetic effects on wine variability (Perreira *et al.*, 2007). Metabolic profiles of Bordeaux wines were most influenced by the climate of the vintage, followed by the genotype (cultivar) and soil type. Inside a given vintage, the soil effect was much clearer in a dry vintage (2003), compared to a wet vintage (2002). In a wet vintage, vine water status is not very much differentiated among blocks. In dry vintages, differences among blocks in vine water status appear to be dependent on soil water holding capacity. Hence, these results support the hypothesis that the soil effect on grape and wine composition is largely mediated through vine water status. Metabolic profiling by ^{1}H-NMR spectra of grape pulp and skin extracts enabled separation of samples according to the vintage but not according to the soil type (Perreira *et al.*, 2005b).

Effect of soil type on Grenache wine sensory attributes was strong and consistent over two consecutive vintages with contrasting weather conditions (de Andrés-de Prado *et al.*, 2007). In this experiment, the soil with low organic matter content and low water holding capacity produced wines with more colour and total phenols. Roujou de Boubée *et al.* (2000) found that grape isobutylmethoxypyrazine varied in similar proportions with the soil type and the vintage. In a study of Riesling wines of the Rheingau region (Germany), sensory properties were equally influenced by vineyard designation (origin), vintage and wine estate (Fischer *et al.*, 1999).

9.8.2 Value of landscapes

Many winegrowing regions have beautiful landscapes and are located in regions

with high cultural value. Landscapes are added value for the wine: sensory perception of wine is improved when the wine is associated with attractive landscapes (Tomasi *et al.*, 2006). However, landscapes are endangered by urbanization, road construction and railway contruction. The protection of viticultural landscapes is essential and has to be organized with the politics of communes and regions (Assemat *et al.*, 2006).

9.9 Conclusions

Terroir is an ecosystem, in a given place, including many factors, like climatic conditions, cultivar and rootstock, geography and topography, as well as soil characteristics like mineral nutrition and water supply. The human factor also plays an important role in terroir. Terroir is difficult to study on a scientific basis, because many factors are involved, and because these factors interact. To obtain terroir expression, the precocity of the cultivar should be matched to the local climatic conditions in such a way that the grapes reach full ripeness at the end of the season (September or October in the Northern Hemisphere, March or April in the Southern Hemisphere). In this way, they ripen in relatively cool conditions, which enhance aroma and anthocyanin accumulation in grapes. A second condition for terroir expression is that the environment should provide one or several limiting factors that reduce vine vigour and yield and provoke shoot growth cessation before or around *véraison*. In many high-quality terroir for red wine production, water supply to the vines is limited and well regulated. Nitrogen supply is also often restricted. Reduced nitrogen and water supply enhance phenolic compound synthesis, which is an important quality factor in red wine production. Severe environmental stress is not good for white wine quality, because a high grape phenols concentration is undesirable. Moreover, severe stress (water or nitrogen) reduces aroma potential in Sauvignon blanc grapes.

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10

Genetics and genomic approaches to improve grape quality for winemaking

P. R. Young and M. A. Vivier, Stellenbosch University, South Africa

Abstract: Wine has a long association with humans and is an integral part of many of the world's diverse cultures, traditions and religions. Most probably due to our long association with wine, winemaking is traditionally regarded more as an art than a science. Descriptors for quality are generally subjective and very difficult to translate into objective measurements. Unlike most modern beverages, wine quality is more dependent on a subtle array of sensations than on a single consistent flavour and/or aroma. These factors make research into grape and wine quality particularly difficult. The sequencing of the grapevine genome (twice) has propelled grapevine research into the genomics era. The whole genome sequences, DNA molecular markers, the ability to analyze the transcriptome, proteome and metabolome, the large germplasm collections, a transformation system for generating transgenic grapevine, and an internationally organized grapevine research community gives grapevine researchers the ability to analyze the molecular basis for quality in grapes. This review provides an overview of the genetic and genomic resources currently available for grapevine research and discusses the application of molecular tools to research into grape quality.

Key words: *Vitis vinifera*, grape quality, genetic resources, genomic resources, plant improvement, molecular breeding.

10.1 Introduction

Grapevine is the most widely cultivated fruit crop in the world and makes a significant contribution to the economies of a number of countries in both the Northern and Southern Hemispheres. According to the Food and Agriculture

Organization (FAO, 2007 statistics: http://www.fao.org), grapes are cultivated on 7.5 million ha of the world's surface area. Of the approximately 66 million tonnes produced worldwide, 71% of grape production is used for wine, 27% as fresh fruit and 2% as dried fruit. The area for grapevine cultivation is increasing by approximately 1–2% per year. In the last 6000–10 000 years, grapevine and wine have formed an integral part of many of the world's diverse religions, traditions and cultures. Today wine is still an integral part of our world cultures and is consumed for reasons that can range from health benefits to pure enjoyment.

Scottish author R. L. Stevenson (1850–1894) described a wine maker's quest for perfection as 'bottled poetry'. This, and many other similar analogies in literature, illustrates the fact that wine has traditionally been considered more of an art than a science. For many consumers of wine, history and tradition are an important part of the wine drinking experience. It is these indefinable, and usually unquantifiable, factors that make research into quality aspects particularly challenging.

The age-old traditions and practices of winemaking have shown that a good wine is made in the vineyard. This is reflected in the often stated adage 'a bad wine can be made from good grapes, but a good wine cannot be made from bad grapes'. The concepts of 'good' and 'bad' used in this sense refer to the largely subjective assessment of grape and wine quality. Viticultural and oenological practices exist because they influence the grape, and subsequently the wine quality. Here quality refers to the largely subjective assessment of flavour, aroma, colour, etc. Viticultural strategies typically attempt to obtain uniform/homogenous optimal grape maturity throughout the bunch, vine and vineyard. These strategies can include anything from clone selection, irrigation and canopy management to cropping levels and harvest date. Practical experience and, more recently, quantitative scientific research has identified a number of viticultural and oenological practices that affect the grape, and consequently the wine quality. Viticulturists are constantly searching for ways to modify these variables to optimize and, ultimately, predict harvest dates to ensure optimum fruit quality.

The sequencing of the grapevine genome has propelled grapevine research into a new era. There is currently a concerted drive to understand how a grapevine adapts on a molecular level to its ever-changing environment, and how these molecular processes ultimately affect the plant. Grapevine research is challenged with finding scientific data to support the wealth of qualitative and anecdotal evidence available for manipulating quality aspects in grapes. This chapter will discuss the complex concept of improving grape quality for winemaking by examining a number of genetic and genomic approaches.

10.2 Viticulture in the context of the broader agricultural sector: a brief overview

Our ancestors unknowingly began the process of plant selection that would lead to the eventual domestication of crop species. Our human progression to the eventual

conscious selection of crop plants on the basis of, for example, seed, size, vigour or yield has culminated in the current situation with our dependence on a few, carefully selected groups of edible plants. This domestication relied on the selection of certain desirable traits, constituting the first phase of plant improvement. Most methods of plant improvement still rely on the existence of variation and the subsequent selection of 'favourable' variants. Historically, or rather until quite recently, this variation could only be exploited if it could be selected for on some pre-determined basis, which generally relied on the physical expression of the variation in an observable phenotype (Murphy, 2007). The advances since the late 1990s, in especially the genomics field, have made our discriminating power for selecting favourable variants faster and easier.

Interestingly, research has shown that certain genetic changes are considered prerequisites for the domestication process. If one considers grains as an example, certain characteristics ensured its selection and subsequent domestication. The few dominant species found today were capable of responding genetically to a prolonged association with humans, and have consequently evolved traits that ensured their continued, and extensive, use. These selected crops evolved to yield plants that were ideally disease-resistant, erect, formed larger seeds (that stayed attached to the plant) and germinated quickly and uniformly when planted ensuring a predictable crop each year, in clear contrast to their wild(er) relatives. These are all traits that have a direct benefit for domestication (discussed in more detail in Murphy, 2007).

Similarly, in grapevine certain morphological and biochemical traits are thought to be associated with the domestication of *Vitis vinifera* from its progenitor species (*V. vinifera* subsp. *sylvestris*) and includes the emergence of hermaphoditism (*sylvestris* is dioecious with the male and female flowers carried on separate plants), uniformity of ripening (berry maturity) within a cluster, higher sugar content and variation in berry colour (Mullins *et al.*, 2004). If one considers only one of these aspects – berry colour – the amount of diversity is visually apparent with a spectrum of varying shades of black, red, pink, grey and white (This *et al.*, 2007).

By the mid-twentieth century, agricultural research and breeding centres had been established in most industrialized countries. At this point in time, the significant yield benefits that had previously been attained through mechanization (by implementing tractors, harvesters, etc.), were being surpassed by the successes achieved via plant breeding. Since it was logistically easier to distribute improved seed stocks, genetic knowledge and improved plant breeding techniques than tractors, these progresses and successes spread rapidly to the developing countries, leading to a global 'green revolution' (Murphy, 2007).

This 'green revolution' in agriculture led to major improvements in a number of areas that included breeding techniques, fertilizers and irrigation systems. The primary driving force behind this revolution was an effort to keep food production ahead of the ever-increasing population growth, and the threat that our population growth would eventually outstrip its available resources. Although grapes are not regarded as a staple food, they are affected by the consequences of population growth on the availability of arable land.

Apart from a growing population, we are also faced with global warming (and the associated natural phenomena), changing legislation regarding the use of pesticides/herbicides and an international movement towards environmentally safe practices (environmental awareness). The limitations of traditional breeding approaches in this regard are obvious: increased input costs and practices, some of which are not sustainable, and include the use of chemicals with potentially harmful secondary effects on human health and the environment. This limitation is evident in the amount of pesticides used in Europe (for example), where grapevine covers an area of 3.7 million ha of the total 105 million ha area used for agricultural production in the European Union (EU). Despite accounting for only 3.5% of the total agricultural area in the EU, grapes receive approximately 15% of the pesticides applied to major crops. This is in stark contrast to the international trend away from the use of agrochemicals (Pesticide Action Network Europe, 2008).

Grapevine production is dependent on a number of natural resources: solar energy, suitable climate, water and soil. Viticulture (and agriculture as a whole) is experiencing increasing scrutiny from government, regulators and consumers regarding responsible environmental practices. This scrutiny translates into both economic and social pressure to align current production methods to sustainable production practices. This has led to the development of environmentally sustainable viticultural practices aimed at protecting and preserving these natural resources. Guidelines are provided in resolutions from a number of officiating bodies that includes the Organisation Internationale de la Vigne et du Vin (OIV; Resolution CST 1/2008: Castellucci, 2008) and the International Federation of Wine and Spirits (FIVS) Global Wine Sector Environmental Sustainability Principles (GWSESP) (Caplan, 2006). A number of sustainability programmes are already in existence and include the Integrated Production of Wine (South Africa), the Sustainable Winegrowing Practices (USA), the Sustainable Winegrowing (New Zealand) and the Australian Wine Industry Stewardship (Australia). These environmental sustainability programmes need to satisfy three aspects of sustainable development: economic, environmental and social sustainability. The current challenge for viticulturists lies in aligning environmentally sustainable objectives with cost-effective practices for the provision of a quality product (be it grape or wine). Before this challenge can be met, the questions that need to be addressed are: what constitutes a 'quality product' for viticulturists and oenologists and, once defined, how can it be improved?

10.3 Grape and wine quality

10.3.1 Defining and assessing grape and wine quality

Quality refers to the 'degree to which a set of inherent characteristics fulfils requirements', where the requirements may differ from one person to the next. The term 'wine quality' is particularly difficult to define. In contrast to many modern beverages that rely on a single consistent flavour and/or aroma, wine quality is more dependent on a subtle array of sensations (Bisson *et al.*, 2002). The quality

(or even the perceived quality) can be affected by a number of contributing factors that include: the cultivar, the region of production and even something as trivial as the reputation of the producer.

To compound the matter further, wine quality can be evaluated in a number of different, and usually diverse, ways but also from a number of different perspectives: the consumers, producers, marketers and scientists all have their own set of norms and criteria. From the consumer's perspective, quality may refer to the value in terms of sensory and image perception or personal preference. A marketing professional could define quality as consumer satisfaction. To the winemaker, quality may refer to the diversity of flavour and the wine's ability to age. Typically subjective terms feature in any discussion on wine quality, and can include terms such as: subtlety, purity, potential, mouthfeel, complexity, balance, harmony, uniqueness. Most of these descriptors are more artistic than scientific, and this makes research into grape and wine quality particularly difficult.

The term 'grape composition' has been suggested as an alternative to 'grape quality' for scientific studies since the metabolite composition of both grapes and wine can be objectively measured and quantified (Carmona *et al.*, 2008). Broad measures of quality are routinely used in a vineyard, and much has been published on the topic. The uniformity of ripening, the exposure to sun, the disease state of the vines, certain berry characteristics (e.g. size, seeds, colour and taste) can all be visually assessed in the vineyard. Chemical analyses are also relied on to assess quality and include measurements of sugars (as an indicator of ripeness), pH (acidity) and anthocyanins (colour). These quality measurements should therefore be regarded as a series of objective measures rather than one single measure of quality.

Human appreciation could be regarded as the most important indicator of wine quality, but its subjectivity, and therefore associated imprecision, hampers the identification of chemical factors responsible for quality. The challenge to grape and wine producers is to understand (and possibly predict) consumer preference and produce 'quality' wines (whilst implementing sustainable production practices).

The final grape quality (i.e. composition) can be influenced by a number of interacting factors that include the environment (both macro- and microclimate), site selection/location, the physiology and genetics of the vine (clonal selection of scion and/or rootstock), as well as various viticultural practices and harvest parameters (too numerous and diverse to discuss, reviewed to some extent in Jackson and Lombard, 1993). In turn, the final wine quality is influenced by primarily the grape quality, as well as the physiology and genetics of the micro-organisms involved in the fermentation process (yeast and bacteria), various oenological practices and ultimately sensory perception.

Wine is a complex mixture of thousands of compounds: most of these are produced by the grapevine, either in the leaves (e.g. sugars and tartaric acid) or in the berry (e.g. malic acid and phenols), or are products of the winemaking process itself (via bioconversions or wine-associated microorganism-derived). The grape berry has been described as a sophisticated biochemical factory, capable of

importing and accumulating water, micronutrients, sugars, amino acids (the primary metabolites) and synthesis of flavour and aroma compounds (secondary metabolites) (Lund and Bohlmann, 2006). Wine flavour and aroma is thus the result of the complex assortment of hundreds of these compounds, with both the absolute concentrations, as well as the ratio of the respective compounds to each other, playing a role in the perceived flavour and aroma spectrum that is considered characteristic of a grape variety and/or wine style. The relative assortment of these compounds in the berries of each grape cultivar defines the 'varietal character'. The most notable examples are the methoxypyrazines in Sauvignon blanc and the monoterpenes in Gewürztraminer grapes and wines.

For winemaking purposes, the grape berry can be divided into three major types of tissue: flesh, skin, and seeds. Each of these tissues varies considerably in composition. Berry development can be divided into two discrete successive sigmoidal growth periods separated by a lag phase. These stages are well described in a number of reviews (Mullins *et al.*, 2004; Adams, 2006; Conde *et al.*, 2007).

Briefly, the first period of sigmoidal growth starts at bloom and proceeds for approximately 60 days. Several solutes that accumulate in the berry during the first growth stage are important for wine, the most notable being acids, tannins and certain aroma compounds (the pyrazines). Tartaric and malic acid provide wine with acidity and are therefore critical to wine quality. Hydroxycinnamic acids are also formed and are the precursors to volatile phenols, as well as being involved in certain browning reactions in wine. Proanthocyanins (tannins), primarily present in the skin and seed tissues, are responsible for the bitter and astringent properties of red wine. They are considered important in red wine colour stability. The aroma compounds known as methoxypyrazines are present at this stage and are responsible for the 'herbaceous" character of unripe wines (Bogart and Bisson, 2006).

After a lag phase, the berry enters a ripening phase that begins with a phenomenon known as *véraison*. Many of the solutes formed during the first phase are reduced during the second phase of berry development. For example, malic acid is metabolized, and the amounts of methoxypyrazines and tannins are similarly reduced. There is a drastic shift during this stage to fruit ripening and a drastic increase in sugars (both glucose and fructose) takes place. Certain secondary metabolites that are regarded as major determinants of wine quality are formed during this phase. They include the anthocyanins and volatile flavour/aroma compounds such as terpenoids, especially monoterpenes, sesquiterpenes and C_{13}-norisoprenoids.

Since ripening has a direct bearing on the quality of the final product, there have been numerous studies attempting to elucidate the underlying control mechanisms (Mullins *et al.*, 2004; Agasse *et al.*, 2009). Understanding when various components accumulate in the berry during ripening is crucial for understanding how, for example, viticultural practices impact the plant or how a plant responds to stresses. It should be noted, however, that berry development and, specifically, the formation of compounds having sensory importance are still far from being understood. Studies regarding these compounds are limited as a result of the complex nature of chemical analytical research. Advances in the extraction and sensitivity of analytical techniques will accelerate the field of grapevine metabolomics and lead to the

identification of candidate compounds. Improvements in both chemical analysis and sensorial analysis are required to determine the impact of an identified compound(s) to wine quality. Linking these compounds (and combinations of compounds) to related genes, proteins, and metabolic pathways is the challenge currently facing grapevine research (Lund and Bohlmann, 2006).

10.3.2 From vineyard management to wine (style): the genetic link(s)

Understanding how a grapevine adapts on a molecular level to its physical environment (due to viticultural management practices or abiotic/biotic stresses) is a prerequisite if predictive measures of a plant's status and/or berry quality are to become part of the vineyard decision management system. The ability to analyze the molecular 'phenotype' of the grapevines will assist researchers in quantifying the impact of viticultural practices.

Genomics is improving our knowledge of the genetic makeup of living organisms and has accelerated research in plant sciences and related fields. The field of genomics can be regarded as the study of all the genes of an organism at various levels: DNA (genotype/genome), mRNA (transcriptome) or protein (proteome) levels. Genomics facilitates the characterization of gene functions and interactions and gives us the potential, for the first time, to study the complex frameworks between genes controlling metabolic pathways and, ultimately, their relationship to phenotype.

Genomics approaches will accelerate grapevine improvement by identifying the contributing pathways. This can be done on a number of different levels and includes identifying transcriptional, translational or metabolic pathways that correlate with berry quality (e.g. the biosynthesis of compounds known to contribute to quality, such as sugars, organic acids, terpenoids and flavonoids).

Most traits of plant breeding interest are complex and quantitative in nature. The identification of genes involved in the control of quantitative traits, and the understanding of their functional and regulatory organization, is required before a trait of interest can be improved (Bernardo, 2008). Comprehensive genomic information facilitates molecular breeding with resolution that has previously not been possible. Genomic research will provide the information required to modify a plant's phenotype by identifying the regulatory genes. Future approaches such as marker-assisted selection (MAS) and transgenesis will facilitate the transfer of the genes for desirable traits into elite or classic cultivars of *V. vinifera*, with the ultimate goal of improving agronomic performance while preserving traditional quality traits.

10.4 Improving grape quality for winemaking

10.4.1 Grapevine genetic resources

Germplasm collections

The number of grapevine cultivars is estimated at between 5000 and 8000. These

cultivars are, however, known by 14 000 to 24 000 different names (i.e. homonyms and synonyms) (Moreno-Sanz *et al.*, 2008). Of this total, international wine production relies predominantly on only 300–400 cultivars. There has been a concerted effort to characterize and conserve marginal and/or endangered grapevine cultivars in germplasm collections. These germplasm collections are genetic resources and should be regarded as the raw materials required for crop improvement. For conventional breeding, researchers need access to this natural genetic diversity to generate new improved varieties of agronomic and oenological importance.

Most countries with a viticulture history/industry have invested time and effort in grape germplasm collections. A number of on-line germplasm collections are available and generally have the mandate to preserve and ultimately improve the utilization of relevant germplasm in breeding programmes. Databases are required to make germplasm collections more accessible for breeders by structuring the required information in searchable, non-redundant formats. In order for the accessions (i.e. genotypes/germplasm) in these databases to be useful, a standard set of protocols, nomenclature and consensus descriptors needs to be established.

The United States Department of Agriculture (USDA) has two large grape collections, one in Geneva, New York, and the other in Davis, California. In conjunction with Cornell University, the Geneva location specializes in the colder climate grapevines. The *Vitis* germplasm collections of the USDA can be accessed via the Genetic Resources Information Network (GRIN) (http://www.ars-grin.gov/npgs/). The collection consists of 4035 accessions representing 50 species. Approximately 30% of this total (i.e. 1283 accessions) represents *V. vinifera* subsp. *vinifera* (database updated September 2008).

The European central *Vitis* database is maintained by the Federal Research Centre for Cultivated Plants at the Institute for Grapevine Breeding in Germany and represents the germplasm collections of 14 EU countries (Austria, Bulgaria, Croatia, Czech Republic, France, Germany, Greece, Hungary, Italy, Moldavia, Portugal, Slovenia, Spain, and Switzerland), consisting of 18 contributing partners. The establishment of the database was funded by the European Union Project GENRES #81 titled 'European Network for Grapevine Genetic Resources Conservation and Characterization'. This germplasm collection has 28 200 accessions, of which 12 431 represent *V. vinifera* L. subsp *vinifera* accessions and can be accessed via GENRES (http://www.en-vitiside/index.php). It is also included in the European Internet Search Catalogue (EURISCO).

The *Vitis* International Variety Catalogue (VIVC) of the Institute for Grapevine Breeding Geilweilerhof has compiled an inventory of *Vitis* species, varieties and other *Vitis* genotypes existing in international grapevine collections from 130 institutions in 45 countries and can be accessed via http://www.vivc.bafz.de.

Research co-ordination

The grape genetics research community formed the International Grape Genome Program (IGGP) for the purpose of co-operation and co-ordination in increasing knowledge of the grape genome. From their website (http://www.vitaceae.org),

the goal of the IGGP is to 'understand the genetic and molecular basis of biological processes in *Vitis*' (International Grape Genome Program, 2002). This understanding is fundamental for the development of new cultivars with improved quality and reduced economic and environmental costs. Traits that are considered of primary interest for the associated industries are pathogen and abiotic stress resistance, quality traits for fruit and wine grapes and reproductive traits that affect yield. The IGGP is a collaborative effort, and it is encouraged that data generated within the programme be publicly accessible via sequence repositories such as the National Center for Biotechnology Information (NCBI). The IGGP has been involved in coordinating the generation of markers (through the *Vitis* Microsatellite Consortium, VMC), genetic mapping, BAC libraries, physical mapping, genome sequencing, expressed sequence tags (ESTs) as well as transcriptional profiling, functional analysis and bioinformatics.

10.4.2 Grapevine genomic resources

The years since 2000 have seen major advances in plant genome studies. This is largely due to the rapid advances made in genome sequencing projects. To date four plant genomes have been sequenced: *Arabidopsis* (The Arabidopsis Genome Initiative, 2000), rice (Goff *et al.*, 2002; Yu *et al.*, 2002), poplar (Tuskan *et al.*, 2006) and grape (Jaillon *et al.*, 2007; Velasco *et al.*, 2007). Several other projects have targeted ESTs, and there has been an enormous increase in molecular tools providing information on the genetic basis of traits. Collectively, these sequences are a valuable genomic resource that will drive plant improvement forward.

The rapid development of genomic technologies and the associated applications has provided the tools and the comparative information to facilitate characterization of plant genomes. The functional information accumulated (and still accumulating) in *Arabidopsis* offers a model system for the functional analyses of other plant genes. These comparative genomic possibilities provide a framework in which to efficiently identify and functionally analyze important grapevine genes controlling key traits.

Comparison of the four completed plant genomes based on gene homology revealed that only 7914 genes are conserved amongst these plant genomes, and that almost 50% of the approximately 30 000 genes were unique to grapevine (Velasco *et al.*, 2007). In order to study these interesting genes of unknown function, grapevine ideally needs to become a model plant.

Grapevine: a model plant?

Can grapevine be considered as a model plant? This question is probably best addressed by examining what the requirements are for a plant to be considered a model organism. Firstly, model organisms have to have biological data available that makes them attractive to study as examples for other species that are more difficult to study directly. These can be grouped into genetic models, experimental models and genomic models. Model organisms are often selected based on the fact that they are easy to work with in a research environment. This includes character-

istics such as a short life-cycle, available techniques for genetic manipulation/transformation, the availability of genetic resources (inbred strains/mutants) and relatively simple growth requirements. Conservation of physiological mechanisms and potential economical benefit could also be considered.

Although the biology of the grapevine might exclude its use as a model organism, being a highly heterozygous perennial plant with a relatively long reproductive cycle, it has become clear that it is necessary to develop the genomic tools to accelerate the acquisition of knowledge about characteristics of agronomic importance such as disease resistance, tolerance to abiotic stresses and berry ripening and quality (International Grape Genome Program, 2002). The grapevine can actually be considered suitable for genomic studies for a number of reasons. The genotypes of grape varieties are highly heterozygous and nearly all modern cultivated varieties (cultivars) are hermaphroditic, self-fertile and out-cross easily. Grapevine is a diploid plant with $2n = 38$ chromosomes and a relatively small genome of approximately 475–487 Mbp (approximately three to four times the genome size of *A. thaliana*). *Vitis* is considered a representative of a group of deciduous woody perennial plants that produce non-climacteric fruits that are characterized by secondary metabolism compounds responsible for colour, flavour and aroma (International Grape Genome Program, 2002). Furthermore, the fact that grapevine is a basal eudicot family, with unique shoot, flower and fruit architecture, makes it an attractive subject for basic comparative genomic studies.

A number of interesting mutants have been described that may have potential for grapevine genetic studies. Pinot Meunier is an old cultivar and is considered a mutant of Pinot noir (Boss and Thomas, 2002). Pinot Meunier is a periclinal chimera having two distinct cell layers (the L1 and L2 layers). Pinot Meunier is similar to Pinot noir except that the leaves are more hairy and the vine is slightly stunted. Although Pinot Meunier exhibits normal internode length, it was discovered that plants generated from cells from only the outer L1 layer exhibit short internodes due to a mutation resulting in a decreased sensitivity to the phytohormone gibberellic acid. The gibberellic acid insensitive (VvGAI) mutation has been shown to be responsible for this phenotype. Plants generated from cells from the L2 layer, however, were phenotypically normal (in fact indistinguishable from Pinot noir).

This research led to the subsequent development of the 'Dwarf' (in Australia; Boss and Thomas, 2002) and 'Pixie' (in the USA; Cousins and Tricoli, 2006) grape varieties from the L1 cell layer of Pinot Meunier. This mutant variety's traits make it potentially useful for genetics, genomics and breeding. As its name suggests, mature clusters of the Dwarf/Pixie mutants typically measure slightly less than 10 cm. Since the dwarf varieties can be grown in the greenhouse to maturity, this characteristic reduces the amount of space needed for grapevine experimentation. This mutant also has the ability to initiate fruit throughout the year, and typically forms flower buds, blooms, immature fruit and ripe fruit on the same vine. This trait would accelerate research by allowing glasshouse studies on grapevine flowers and berries at all stages of development throughout the year. Although the Dwarf/Pixie mutant has many characteristics that make it an attractive candidate

Table 10.1 A comparison of the NCBI Entrez records (July, 2009) for *Arabidopsis thaliana* (NCBI taxonomy ID: 3702) and *Vitis vinifera* (NCBI taxonomy ID: 29760)

NCBI database	Description	Number of records	
		Arabidopsis thaliana	*Vitis vinifera*
Nucleotide	A database containing sequences from several sources, including GenBank, RefSeq and PDB	252 422	119 402
Nucleotide EST	Expressed sequence tags database (dbEST) contains sequence data on 'single-pass' cDNA sequences	1 527 298	353 941
Nucleotide GSS	A database of Genome Survey Sequences (GSS) that are similar to the ESTs, with the exception that most of the sequences are genomic in origin	493 859	229 272
Protein	The protein entries are compiled from a variety of sources, including SwissProt, PIR, PRF, PDB and translations from annotated coding regions in GenBank and RefSeq	158 456	79 831
Structure	The Molecular Modeling Database (MMDB) contains 3D macromolecular structures	385	11
Genome Sequences	The Genome database provides views for a variety of genomes, complete chromosomes, sequence maps with contigs and integrated genetic and physical maps.	7	22
Genome Projects	A database of large-scale sequencing, assembly, annotation and mapping projects	1	1
Popset	A set of DNA sequences that have been collected to analyze the evolutionary relatedness of a population	584	7
3D Domains	3D Domains are compact structural domains identified automatically in the Entrez macromolecular 3D structure database (MMDB)	1414	33

Domains	The Conserved Domain Database (CDD) contains protein domain models imported from outside sources, such as Pfam and SMART, and curated at NCBI	62	–
GEO Datasets	A database with curated gene expression and molecular abundance datasets assembled from the Gene Expression Omnibus (GEO) repository	1048	8
GEO Expressions	A database of individual gene expression and molecular abundance profiles assembled from the Gene Expression Omnibus (GEO) repository	1 597 738	–
UniGene	A UniGene entry is a set of transcript sequences from the same transcription locus, as well as information on protein similarities, gene expression, cDNA clone reagents and genomic location	30 576	23 166
UniSTS	A comprehensive database of sequence tagged sites (STSs) derived from STS-based maps	809	912
PubMed Central	The US NLM digital archive of biomedical and life sciences journal literature	14 198	600
Gene	A database of genes from RefSeq genomes that are defined by sequence and/or located in the NCBI Map Viewer	33 500	28 214
HomoloGene	A system for detection of homologs among the annotated genes of sequenced eukaryotic genomes	11 208	–
SRA Experiments	The Short Read Archive (SRA) stores sequencing data from next generation sequencing platforms	41	1

for a 'laboratory' grapevine, due to the importance of phytohormones to a number of plant processes, the altered gibberellic acid sensitivity in these mutants may preclude its use in a number of studies.

A second potentially useful grapevine genotype is the *fleshless berry* (*flb*) mutation that may advance our understanding of the molecular and developmental processes involved in fruit growth and development (i.e. the differentiation of an ovary into fruit). The *flb* mutation impairs the differentiation and division of cells of the inner mesocarp responsible for flesh in grapevine berries and is described in detail in Fernandez *et al.* (2006a,b). The *flb* mutant has normal synchronous flowering and normal flower set, but severely altered ovary development (referred to as 'wrinkle') and subsequent berry morphogenesis, characterized by an up to 20 times reduction in the weight of the berry at ripening. Interestingly, this mutation does not affect the fertility, size or number of seeds produced. The genetic basis of the mutation is being investigated, but is still unknown. The mutation is not due to a deficiency in growth regulators, but could be due to a lack in hormone signal reception and/or transduction. The cloning and characterization of the *FLB* gene will provide valuable insights into fruit traits of importance in fleshy fruits.

Most of the tools required for genetic studies are currently available for grapevine, and include microsatellite markers (simple sequence repeats, SSR), ESTs, single nucleotide polymorphisms (SNPs), genomic sequences (BAC-end sequences, BES), two complete genome sequences as well as genetic and physical maps (Troggio *et al.*, 2008). Table 10.1 compares the status of *V. vinifera* genetic resources available from the NCBI to those of the most well-studied plant genetic model, *A. thaliana*. These resources will be discussed individually in the following sections.

Whole genome sequences

The draft sequence of grapevine represents the fourth draft produced for flowering plants (after *Arabidopsis*, rice and poplar), the second for a woody species (poplar) and the first for a fruit crop. Two research groups independently sequenced the grapevine genome. Vigna/Vigne published a draft of the grapevine sequence in 2007 (Jaillon *et al.*, 2007). This French–Italian collaboration sequenced the genome of a highly inbred nearly homozygotic Pinot noir line, PN40024 (estimated at 93% homozygous). The PN40024 line was developed by French viticulturist by inbreeding a Pinot noir clone. Several generations of selfings produced lines with simplified genomes, but with stunted growth phenotypes. Although these lines were of no commercial value for winemaking, and were referred to as 'pathetic, hardly a grape', the simplified genome was particularly appealing for sequencing since it would simplify the arduous task of sequence assembly (Travis, 2008). The consortium subsequently sequenced and assembled 6.2 million end reads, to provide a draft whole genome sequence of 487 Mbp (at approximately 8.4× genome coverage). The sequences generated in this project are deposited in the NCBI Trace Archive (NCBI project ID: 18785) and Genoscope.

The Pinot noir Grapevine Genome Initiative (PNGGI) published a second draft grapevine sequence in 2007 (Pryer *et al.*, 2002; Velasco *et al.*, 2007). This consortium, coordinated by the Istituto Agrario di San Michele all' Adige (IASMA;

now the Edmund Mach Foundation), sequenced the genome of ENTAV115, a heterozygous commercial variety of Pinot noir with an estimated 11.2% of the sequence differing between homologous regions. The whole genome shotun (WGS) sequence (representing 10.7× genome coverage) and assembly was performed by Myriad Genetics using Sanger sequencing (at 6.5× genome coverage) and 454 pyrosequencing technologies (4.2× genome coverage) (NCBI project ID: 18357). Although technically harder to assemble, the heterozygous ENTAV115 variety generated sequences that provide useful information for identifying polymorphisms (Zharkikh *et al.,* 2008). These polymorphisms proved useful for anchoring sequences to chromosomes (in genetic maps) as well as for future breeding purposes.

The availability of the draft grapevine genome sequences opens up the possibility of molecular breeding (see subsection 'Molecular breeding' in section 10.5.2). The large germplasm collections and the fertility of hybrids between wild and domesticated species make it possible to introduce selected genes via conventional breeding. The availability of appropriate molecular markers allows selection of the desired trait(s) without the loss of alleles important for grape and wine quality.

It is currently thought possible that the characterization of function and regulation of conserved plant genes will be achieved in the next decade (the so-called 'post-genomic' era). The current challenge is to integrate data of transcription (temporal and spatial regulation), translation, protein interactions, metabolism, transport, etc., to ultimately understand how these pathways control plants. Advanced analytical techniques are required to process data from a wide variety of sources further using integrative multidisciplinary approaches (including computational biology, chemometrics and systems biology).

Comparative genomic studies

Functional genomics analyzes the function of individual genes within a genome. The functions of genes can often be predicted by studying their parallels in other species. Comparative genomics is the analysis and comparison of genomes from different species, with the aim of gaining an understanding of how species have evolved and determining the function of genes and non-coding regions of the genome. Many features can be analyzed with comparative genomics, and these include: sequence homology, gene position, the length and number of exons in genes, the percentage of non-coding DNA and highly conserved regions (Hardison, 2003; Caicedo and Purugganan, 2005).

Comparative genomics exploits both similarities and differences in the proteins, RNA and regulatory regions of different organisms to infer how selection has acted upon these elements. Elements that are responsible for similarities between different species are conserved (stabilizing selection), while elements responsible for differences should be divergent (positive selection pressure). Due to neutral selection pressure, elements that are not crucial for the evolutionary success of an organism are not conserved (Hardison, 2003). Comparative genome analyses between model plants (e.g. *Arabidopsis*) and sequenced plant genomes have already facilitated cross-utilization of genetic resources, providing clues to the

evolutionary events that are associated with the divergence of distantly related genomes (Pryer *et al.*, 2002).

A joint project of the USA's Department of Energy's Joint Genome Institute and the Center for Integrative Genomics has made available a tool to facilitate comparative genomic studies amongst green plants (Phytozome: http://www.phytozome.net). Phytozome provides a centralized web-based access point to plant genomes, and includes tools for visualization of genomes and associated annotations, sequence analysis and data retrieval. Clusters of orthologous and paralogous genes are constructed at phylogenetic 'nodes'. These nodes correspond to significant evolutionary events, and clustering is used to group genes into sets representing the ancestral genes that existed prior to the node event. Phytozome currently provides access to nine sequenced and annotated green plant genomes, and includes: *A. thaliana*, *Populus trichocarpa* (poplar), *Medicago trunculata*, *Glycine max*, *V. vinifera*, *Sorghum bicolour* and *Oryza sativa* (rice). Eight of the available plant genomes have been clustered into gene families at six evolutionarily significant nodes. The Phytozome database contains 30 434 protein-coding transcripts from the 487 Mbp draft sequence of grapevine (from Genoscope, September 2007 release; Jaillon *et al.*, 2007). The genes are annotated using PFAM, KOG, KEGG and PANTHER assignments. The publicly available annotations from RefSeq, UniProt, TAIR, JGI are hyper-linked and searchable within Phytozome.

There are, however, still a number of limitations to comparative genome analysis. Plant genes that are members of large multigenic families currently complicate functional analysis. Genetic redundancy and genes with unique functions (genus- and/or specie-specific) further hinder the process of assigning functions to genes. Assigning functions to genes based on sequence homology, however, still provides putative functions that could provide the clues to understanding biochemical functions and the underlying biological roles.

Physical and genetic maps

There are published physical maps for two highly heterozygous grapevine cultivars: one for Pinot noir (Troggio *et al.*, 2007) and the other for Cabernet Sauvignon (Moroldo *et al.*, 2008). The physical maps for the two sequenced genomes (PN40042 and ENTAV115) are also currently under construction. Both published physical maps are based on restriction enzyme BAC fingerprinting. The technologies required for producing BAC fingerprints have improved significantly in the last few years, from using one to five restriction endonucleases, and a shift from gel-based to sequence-based electrophoresis to generate the profiles for the fingerprints (Lamoureux *et al.*, 2006).

The Cabernet Sauvignon physical map was constructed using 29 727 BAC clones assembled into 1770 contigs. This 715 684 kbp represents almost 1.5× coverage of the genome size. DNA molecular markers were used to anchor 395 of the 1770 contigs (corresponding to 255 476 kbp) to the 19 linkage groups. The utility of the constructed physical map was subsequently illustrated by mapping candidate genes for non-host and host resistance, and for defence signalling pathways. These complex gene families were mapped and the findings supported

Table 10.2 Overview of publicly accessible microarray experiments for *Vitis vinifera*

Database: accession	Description of the experiment	Platform	Grapevine cultivar	Reference
PLEXdb: VV1	Effects of vines exposed to salinity, PEG or cold: Vines grown in a hydroponic drip system were treated with 120 mM salt (10:1 NaCl:CaCl), polyethylene glycol (PEG), cold (5 °C) or unstressed	Affymetrix® *Vitis vinifera* (Grape) Genome Array V. 1.0	*Vitis vinifera*: Cabernet Sauvignon	Tattersall *et al.*, 2007
PLEXdb: VV2	Long-term effects of vines exposed to salinity and water-deficit: Potted vines in the greenhouse were exposed to irrigated controls, non-irrigated water-deficits and saline treatments for 16 days	Affymetrix® *Vitis vinifera* (Grape) Genome Array V. 1.0	*V. vinifera*: Cabernet Sauvignon	Cramer *et al.*, 2007
PLEXdb: VV3	Tissue differences between well-watered or water-deficit berries: Transcriptional analysis of pulp, skin and seeds of field grown grape berries – this experiment was conducted on well-watered and water-deficit plants	Affymetrix® *Vitis vinifera* (Grape) Genome Array V. 1.0	*V. vinifera*: Cabernet Sauvignon	Grimplet *et al.*, 2007
PLEXdb: VV5	The effect of water-deficit treatment on berry development: Transcriptomic analysis of berry development in two cultivars under well-watered and water-deficit stress	Affymetrix® *Vitis vinifera* (Grape) Genome Array V. 1.0	*V. vinifera:* Chardonnay Cabernet Sauvignon	Deluc *et al.*, 2007
PLEXdb: VV7	Transcript profiles of the red wine cultivars Carménère naturally infected with GLRaV-3 were compared to virus-free grapevine plants	Affymetrix® *Vitis vinifera* (Grape) Genome Array V. 1.0	*V. vinifera*: Carménère Cabernet Sauvignon	Espinoza *et al.*, 2007 Deluc *et al.*, 2007
PLEXdb: VV9	The effect of high temperature on berry development: Berry development from one week before *véraison*, until maturity at two temperatures: High day temperatures (max. 35 °C) vs control (max. 25 °C)	Affymetrix® *Vitis vinifera* (Grape) Genome Array V. 1.0	*V. vinifera*: Cabernet Sauvignon	Not published

Table 10.2 *cont.*

Database: accession	Description of the experiment	Platform	Grapevine cultivar	Reference
PLEXdb: VV11	Global gene expression analysis of grapevine berries during development and ripening	Affymetrix® *Vitis vinifera* (Grape) Genome Array V. 1.0	*V. vinifera*: Pinot noir	Pilati *et al.*, 2007
PLEXdb: VV12 GEO: GSE6404	The effect of fungal infection on a susceptible grapevine: Response of grapevine to powdery mildew infection at 0, 4, 8, 12, 24 and 48 hours post-inoculation	Affymetrix® *Vitis vinifera* (Grape) Genome Array V. 1.0	*V. vinifera*: Cabernet Sauvignon	Fung *et al.*, 2008
PLEXdb: VV13 GEO: GSE6404	The effect of fungal-infection on a resistant grapevine: Response of grapevine to powdery mildew infection at 0, 4, 8, 12, 24 and 48 hours post-inoculation	Affymetrix® *Vitis vinifera* (Grape) Genome Array V. 1.0	*V. aestivalis*: 'Norton'	Fung *et al.*, 2008
GEO: GSE11406	Expression data in individual grape berries during ripening initiation	Affymetrix® *Vitis vinifera* (Grape) Genome Array V. 1.0	*V. vinifera*: Cabernet Sauvignon	Lund *et al.*, 2008
GEO: GSE10906	Transcriptional profiling of healthy grapevine plants vs Phytoplasma-infected plants The study was conducted on grapevine plants grown in the same vineyard (leaf midribs were sampled)	INRA *Vitis vinifera* (Grape): 14k oligo array V. 2.0	*V. vinifera*: Chardonnay	Not published
GEO: GSE3052	Photoperiod induction of endodormancy in axillary buds: A comparison of age-matched axillary buds at one time point during long photoperiod (paradormancy maintenance) and short photoperiod (endodormancy induced)	Yale 12K *Arabidopsis* array Yale 9.2K *Arabidopsis* array	*V. riparia*	Not published

previous hypotheses that NBS-LRR clusters and disease resistance loci were co-localized in grapevine.

There are 17 genetic maps and 11 sequence maps on NCBI (Genome project 12992; Table 10.2). A reference linkage map is required to coordinate linkage groups resulting from individual mapping projects and as a resource for physical mapping. Genetic linkage maps are a prerequisite for study of both qualitative and quantitative trait inheritance and for integration of the molecular information necessary for MAS, map-based cloning and anchoring to physical maps and genome sequences (Troggio *et al.*, 2008). Thus, a key resource forming the basis of classical genetics and genomics of *V. vinifera* is the construction of a dense genetic map based on well-characterized, gene-specific molecular markers.

The genetic maps currently available have all been constructed from a variety of different genotypes for a host of different research purposes. Furthermore, these genetic maps have been constructed using different DNA molecular markers types and/or different DNA molecular marker collections. For research coordination it is therefore necessary to generate a common reference map using a common set of molecular markers and a consensus chromosome nomenclature to integrate all the genetic maps generated. A reference map constructed using 181 DNA molecular markers, was adopted by the IGGP as the integrated reference map for grapevine (Riaz *et al.*, 2004). A subsequent reference integrated map generated by Vezzulli *et al.* (2008b) provided a saturated 'species consensus' map. This map was based on three crosses of the *V. vinifera* parent cultivars Syrah, Pinot noir, Grenache, Cabernet Sauvignon and Riesling and was constructed using 1134 DNA molecular markers (Vezzulli *et al.,* 2008b). These reference maps are particularly useful for targeting genomic regions when more intensive mapping efforts are required, such as for localizing quantitative trait loci (QTLs) or for MAS.

Quantitative trait loci (QTL)

Most quality-related characteristics relevant for crops show phenotypic variation because they are controlled by multiple genes and are referred to as QTL. A QTL can be defined as a region of a chromosome (locus) present in a population of segregating progenies from a biparental cross. The QTL is associated with a phenotype, and can be positioned on a genetic linkage map (Paran and Zamir, 2003).

A number of mapping projects have identified DNA molecular markers associated with QTLs and their localization in genetic linkage maps. Identification and localization of QTLs relies on genetic maps with a high density of evenly distributed DNA molecular markers. As discussed, several grapevine genetic maps are available (Table 10.3) and a number of QTLs have to date been identified. Most QTLs are, however, related to disease-resistance or quality parameters in table grapes (e.g. seedlessness, berry weight, etc.). This is in part due to the fact that conventional breeding programmes were used to improve table grape cultivars, and phenotypical markers for traits regarded as desirable for the industry received the most focus. Very few QTLs potentially affecting wine quality have thus far been identified, and include flavour/aroma (monoterpenes) and berry colour.

Table 10.3 Selected maps reported for Grapevine

Markers and mapping population	Summary of the map/integrated map	Reference
Genetic map: 274 markers (274 SSR) 96 F1 *Vitis vinifera* L. cv. Syrah × Grenache IGGP reference map (recommended)	220 loci mapped onto 19 LG spanning 1406.1 cM with an average marker disance of 6.4 cM	Adam-Blondon *et al.*, 2004
Genetic map: 153 (152 SSR, 1 polymorphic EST) 153 F1 *V. vinifera* L. cv. Riesling × Cabernet Sauvignon	153 loci mapped onto 19 LG spanning 1728 cM with an average marker distance of 11 cM	Riaz *et al.*, 2004
Genetic map: 515 markers (502 SSR, 13 other markers) 46 F1 *V. vinifera* L. cv. Chardonnay × Bianca* 95 F1 *V. vinifera* L. cv. Grenache × Syrah 114 F1 *V. vinifera* L. cv. Riesling (selfed) 139 F1 *V. vinifera* L. cv. MTP2223-27 × MTP2121-30 153 F1 *V. vinifera* L. cv. Riesling × Cabernet Sauvignon	515 loci mapped onto 19 LG spanning 1647 cM with an average marker distance of 3.3 cM	Doligez *et al.*, 2006
Genetic map: 994 (483 SNP [derived from EST and BES sequences], 132 SSR, 379 AFLP) 94 F1 *V. vinifera* L. cv. Syrah × Pinot noir	994 loci mapped onto 19 LG spanning 1245 cM with an average distance of 1.3 cM	Troggio *et al.*, 2007
Genetic map: 1767 markers (same as 1, with 799 additional SNP markers) 94 F1 *V. vinifera* L. cv. Syrah × Pinot noir	1767 loci mapped onto 19 LG spanning 1276 cM with an average marker distance of 4 cM	Velasco *et al.*, 2007

Genetic map: 1134 markers (350 AFLP, 332 BES, 169 EST, 283 SSR) 94 F1 *V. vinifera* L. cv. Syrah × Pinot noir 96 F1 *V. vinifera* L. cv. Syrah × Grenache 87 F1 *V. vinifera* L. cv. Cabernet Sauvignon × Riesling	1134 loci mapped onto 19 LG spanning 1443 cM with an average marker distance of 1.27 cM	Vezzulli *et al.*, 2008b
Physical map: 49 536 BAC clones *V. vinifera* L. cv. Pinot noir	49536 BAC clones fingerprinted, 367 anchored contigs covering 351.73 Mbp	Troggio *et al.*, 2007
Physical map: 30828 BAC clones *V. vinifera* L. cv. Cabernet Sauvignon	29727 BAC clones fingerprinted, 1770 contigs covering 715.684 Mbp, (395 anchored contigs covering 255.476 Mbp)	Moroldo *et al.*, 2008

*Doligez *et al.* (2006) noted that the cultivar Bianca is a Villard Blanc × Bouvier cross containing approximately 21% of non-*vinifera* genetic background originating from other *Vitis* species

Battilana *et al.* (2009) identified genes affecting monoterpene content by combining metabolite profiling analysis with a QTL and candidate gene approach. Positional cloning is commonly used to identify and isolate genes genetically controlling important traits. The candidate gene approach is an alternative method that is particularly useful for plants species with large genomes and/or long generation times. It is also less expensive and labour-intensive than the positional cloning approach. The candidate gene approach therefore links annotated genes to QTLs (reviewed in Paran and Zamir, 2003). These genes are manually selected from sequences (annotated WGS sequences, ESTs, etc.) that are known to play a potential role in the measured phenotype, or have a structural similarity to known genes. The selected genes (referred to as candidate genes) are subsequently linked to the QTLs using DNA molecular markers. In this study, the candidate genes were selected based on gene ontology terms in sequence databases and the candidate genes were linked to QTLs for monoterpene formation. They showed that the candidate gene, *DXS* (1-deoxy-D-xylulose 5-phosphate synthase) co-localized with a major monoterpene QTL on linkage group 5. DXS is the first enzyme in the plastidial MEP/DOXP pathway of terpene biosynthesis (discussed in Section 10.6.1).

Allelic variation in the transcription factor, *VvmybA1*, has been examined in over 200 accessions of cultivated grapevine including several well-characterized fruit colour mutants (This *et al.*, 2007). The authors showed the insertion of a Ty3–*gypsy*-type retro-transposon, *Gret1*, in the promoter region of the *VvmybA1* gene led to a white-fruited phenotype in more than 200 *V. vinifera* accessions analyzed. They further showed that polymorphisms in the *VvmybA1* gene were strongly associated with red or pink fruited varieties.

Due to the time and cost requirements of high-resolution linkage mapping required for QTLs, alternative approaches are constantly investigated to identify gene–trait associations in organisms. 'Genome-wide association studies' (GWAS) are a promising technique used to analyze if selected DNA molecular markers correlate with the trait(s) of interest. This technique requires DNA molecular markers that are evenly distributed across the genome, and a measureable trait found in a wide variety of cultivars. GWAS therefore looks for associations between phenotypes of interest and the DNA sequence variation present in the genotype (typically assessed by SNP-based genotyping, discussed on p. 350). Grapevine is considered promising for GWAS since it has a relatively small genome (475–487 Mb), and significant trait variation within the genus.

As a first step to GWAS in grapevine, researchers investigating grape diversity at the USDA research station (Geneva) have identified 9000 SNPs by next-generation Illumina/Solexa pyrosequencing of 17 grape cultivars (10 *V. vinifera* cultivars and seven wild *Vitis* species) (USDA-ARS, 2008). The 9000 SNPs were selected from a total of 110 000 'high-quality' SNPs identified. The 9000 selected SNPs were used to construct a custom genotyping array. This '9K *Vitis* SNP array' is currently being used to genotype 2000 grape cultivars in the USDA germplasm collection (representing 1000 *V. vinifera* and 1000 wild *Vitis* species). These data will provide information on the degree of genetic variation within the *Vitis* genus, and potentially facilitate future GWAS.

10.4.3 Applying molecular tools to grapevine

Gene expression and transcriptomic data

Innovation and improvements in reagents (enzymes, antibodies, etc.) and the associated technologies (detection, automation, etc.) has resulted in rapid improvements in gene expression profiling techniques, from the gel-based hybridizations (northern hybridizations), to the relative semi-quantitative gel-based endpoint polymerase chain reaction (PCR) methods to high-throughput real-time PCR monitoring systems. These methods are, however, logistically limiting in the number of transcripts that can be processed manually.

The development of 2D-arrays where genes, gene fragments or synthesized probes are placed in a systematic order allowing them to be made available as probes for high-throughput, parallel assays has made transcriptomic analyses possible. The transcriptome can be regarded as the 'complete' set of transcripts in a cell, and their quantity, for a specific developmental stage or physiological condition. An understanding of the transcriptome is required for understanding the functional elements of the genome. Transcriptomic studies have been used to: catalogue transcripts (mRNAs, non-coding RNAs and small RNAs); determine the structure of genes (start of transcription, 5' and 3' ends, splicing patterns and other post-transcriptional modifications); and quantify the expression levels of transcripts (McEntyre, 2002).

Various transcriptomic technologies have been developed, and the approaches are either hybridization-based or sequence-based. A number of systems are currently commercially available, the Affymetrix Genechip® (hybridization-based) and Illumina (formerly Solexa) (sequence-based) systems being most commonly cited.

The Affymetrix GeneChip® *Vitis vinifera* Genome Array (v 1.0) contains approximately 16 000 probes, representing 14 000 *V. vinifera* and 1700 other *Vitis* sequences. Sixteen pairs of 25-mer oligonucleotide probes were used to quantify the level of transcription of each sequence represented on the microarray. The transcript sequences used for the design were sourced from GenBank, dbEST and RefSeq, and sequence clusters were created from the NCBI UniGene database (Build 7, Oct 2003). The relevant sequence and annotation information is available on the Affymetrix website (http://www.affymetrix.com/) or from NCBI Gene Expression Omnibus (GEO) (GEO Platform GPL1320). The Affymetrix GeneChip® *V. vinifera* Genome Array has been cited in a number of publications (Table 10.3).

Grimplet *et al.* (2007) used the Affymetrix microarray to compare the tissue-specific mRNA expression profiles of the pulp, skin and seed of mature grape berries (*V. vinifera* L. cv. Cabernet Sauvignon) in well-watered and water-deficit treated grapevines. These results revealed novel insights into the tissue-specific expression mRNA expression patterns of genes differentially expressed in berry tissues. The largest difference in tissue-specific expression was observed between the seed and pulp/skin. Overexpression of transcripts associated with flavonoid biosynthesis, pathogen resistance and cell wall modification as observed in

exocarp tissue (relative to the other berry tissues investigated). This was expected since this tissue is involved in both pathogen defence and pigment production. Overexpression of transcripts involved in cell wall function and transport processes as observed in the primarily nutritive mesocarp tissue. The seeds showed higher transcript abundance of genes encoding phenylpropanoid biosynthetic enzymes, seed storage proteins and late embryogenesis abundant proteins. The transcript abundance of most functional categories decreased in the pulp and seed tissues from berries from the water-deficit stressed vines (relative to well-watered vines). Interestingly, in the skin of berries from the water-deficit stressed vines, transcripts of several functional categories including general phenylpropanoid and ethylene metabolism, pathogenesis-related responses, energy and interaction with the environment were significantly overexpressed. From a grape quality perspective, the authors reported that water-deficit stress had a profound effect on transcript profiles of genes associated with specifically the biosynthesis of aroma and colour metabolites in both skin and pulp tissues that would ultimately affect wine quality. A number of viticultural practices are known to affect the flavour, aroma and colour of grapes. A notable example is the partial rootzone drying (PRD) treatment. It would be interesting to see how this viticultural management practice affects the plants on the molecular level (e.g. transcriptomic, proteomic and metabolomic level) and how these molecules correlate to quality.

Deluc *et al.* (2007) investigated the transcriptional network(s) controlling berry development and potentially influencing the organoleptic properties of wine. The expression profiles of berries of *V. vinifera* cv. Cabernet Sauvignon at seven distinct stages of berry development were analyzed. The stages ranged from small pea-size berries, through *véraison* to mature berries. The authors reported on significant changes in expression patterns of transcription factors, abscisic acid (ABA) biosynthesis and calcium signaling genes and identified putative factors involved in developmental events (such as *véraison*), phytohormone partitioning within berry cells, aroma compound production, pathway regulation and sequestration of flavonoid compounds. Interestingly, an alternative pathway for glucose and triose phosphate production, that is active from *véraison* to ripe berries, is proposed based on the analysis of sugar metabolism gene expression patterns.

Pilati *et al.* (2007) used the Affymetrix microarray to identify genes involved in fruit ripening and to investigate the influence of seasons on the process. Gene expression was monitored from berries sampled before and after *véraison* in three consecutive growing seasons. The authors reported the over-representation of five functional categories. Cell wall organization and biogenesis, carbohydrate and secondary metabolisms and stress response were significantly induced during the ripening phase, while photosynthesis was strongly repressed. About 19% of the core gene set was characterized by genes involved in regulatory processes, such as transcription factors and transcripts related to hormonal metabolism and signal transduction. Auxin, ethylene and light were identified as the main stimuli affecting berry development. An oxidative burst was observed from *véraison* that was characterized by the accumulation of H_2O_2 and the regulation of a number of reactive oxygen species (ROS) scavenging enzymes.

Lund *et al.* (2008) used the Affymetrix microarray to analyze 32 individual *V. vinifera* cv. Cabernet Sauvignon berries from two vines grown under controlled conditions in a greenhouse. The progression of ripening initiation was analyzed by examining global patterns of transcription in individual berries to identify transcripts/genes associated with signalling, by focusing on genes annotated with signal transduction functions. The authors identified two genes correlated with ripening initiation and suggested a role for these two genes in abscisic acid signalling during ripening initiation.

It must be noted that with only 14 000–16 000 transcripts represented on the Affymetrix GeneChip® *Vitis vinifera* Genome Array, only approximately 50% of the putative transcripts identified in the grapevine genome sequence are represented. Current initiatives to develop more comprehensive (representative) platforms are underway, and include the Roche Nimblegen® microarrays as well as the next-generation 'sequence-by-synthesis' (pyrosequencing) technologies, for example Illumina (formerly Solexa).

Next-generation pyrosequencing technologies generate many short reads of DNA fragments (30–400 bp, depending on the technology) in a reduced timescale and have lowered the cost per nucleotide. This technology has a number of genomics applications and has been used to investigate genetic variation, genomic rearrangements, DNA methylation and transcription factor binding sites. RNA Sequencing (or RNA-Seq) is a relatively recent development and employs these next-generation sequencing methods to sequence cDNA (Wang *et al.*, 2009). The obtained cDNA sequences can be used to analyze the 'transcriptional landscape' of a number of eukaryotic genomes. Research using this technique for grapevine is, however, limited. A recent publication by Denoeud *et al.* (2008) showed the power of this technology for gene modelling by using RNA-Seq and an annotation pipeline 'Gene Modelling using RNA-Seq' (G-Mo.R-Se). By using the available PN40042 grapevine genome sequence and approximately 175 million Solexa/Illumina® RNA-Seq reads generated from four tissues (leaf, root, stem and callus), the authors were able to identify new exons (in known loci) and alternative splice forms, as well as entirely new loci.

A number of public sequence repositories house grapevine expression data. The NCBI Gene Expression Omnibus (GEO) is a gene expression repository supporting MIAME compliant data submissions. MIAME describes the 'Minimum Information About a Microarray Experiment' that is required to interpret the results unambiguously or to reproduce the experiment. The MIAME guidelines are endorsed by the Microarray Gene Expression Data (MGED) group and are accepted by major scientific journals and adapted by many microarray databases (Brazma *et al.*, 2001; Mukherjee *et al.*, 2005; Zimmermann *et al.*, 2006). The online GEO database is curated and is a resource for gene expression data browsing, query and retrieval. There are currently four GEO datasets for *Vitis* (http://www.ncbi.nlm.nih.gov/geo/) (Table 10.3).

The *Vitis* Expression Database (VitisExpDB) is an on-line database containing annotated ESTs and gene expression data for both *V. vinifera* and non-*vinifera* grapevine species. VitisExpDB contains approximately 320 000 ESTs, their BLAST

annotation details and gene ontology based structured vocabulary. Putative homologs for each of the ESTs in other varieties along with information on their percent identity, phylogenetic relationship and common primers to amplify these putative homologous sequences are available. The VitisExpDB has microarray expression profiles generated based on a high density 60-mer gene expression chip consisting of ~20 000 non-redundant sets of ESTs in host plant *Vitis* interaction with *Xyllela fastidiosa*. The microarray data were generated from 36 hybridization experiments from three time points: early (one week), mid (six weeks) and late (10 weeks) stages of disease development from both infected and non-infected tissues of resistant and susceptible genotypes. The expression profiles are searchable and can easily be mapped onto metabolic pathways. VitisExpDB is accessible via http://cropdisease.ars.usda.gov/vitis_at/main-page.htm.

PLEXdb (Plant Expression database) is a public resource for gene expression for plants and plant pathogens. PLEXdb integrates gene expression data sets with structural genomics and phenotypic data. PLEXdb has a grapevine-specific MIAME/Plant-compliant database, GrapePLEX, containing microarray experiments from a range of different conditions that range from biotic- and abiotic stress to different developmental stages (Table 10.2). Both the raw and processed/normalized expression data are available and the database provides gene annotation for plant microarrays (Affymetrix and other platforms) and can be accessed via http://www.plexdb.org/.

Proteomics data

The proteome represents all proteins involved in a particular pathway, organelle, cell, tissue, organ or organism that can be studied to provide biological information for that particular system. The identification, characterization and quantification of all proteins is currently, however, practically impossible with a single extraction. A number of reviews provide useful insights on the topic (Roberts,2002; Hegde *et al.*, 2003; Carpentier *et al.*, 2008). A complete proteomic analysis requires individually optimized extraction protocols to isolate the different protein fractions: polar, non-polar, soluble, membrane-bound, etc. protein fractions. To compound the extraction-associated problems, the individual proteins within a single extraction can add an additional layer of complexity. If one considers only the soluble protein fraction, the solubility of the different proteins from a specific extraction protocol can vary, requiring extraction with both polar and non-polar protocols. Furthermore, proteins that vary at the extremes of abundance (low and high), size (small and large) or pI (basic and acidic) might not resolve in two-dimensional (2D) gel electrophoresis (extensively reviewed in Jorrin-Novo *et al.,* 2009).

Nonetheless, the *V. vinifera* proteome has been studied in a number of systems. The grape berry proteome has received much attention since it (the berry) contains the key compounds associated with grape and wine quality. Sarry and co-workers (2004) compared the berry mesocarp (i.e. the berry without the skin and the seeds) proteome at ripeness of six grapevine cultivars (representing four *V. vinifera* and two other *Vitis* species). In the *V. vinifera* cv. Gamay proteome, 67 of the 300

proteins detected could be identified and assigned to three functional classes based on the biological process, and included: metabolism and energy state (34%), defense and stress responses (19%) and primary metabolism (13%). The study confirmed the presence of a number of proteins involved in stress-related responses including defense to pathogens (e.g. chitinases and thaumatin-like proteins), reactive oxygen scavenging systems and survival in anaerobic conditions. The study also supported the apoplastic pathway of sugar loading during ripening in grapevine, as previously reported.

Similarly, Deytieux and co-workers (2007) used 2D gel electrophoresis to analyze the proteins isolated from *V. vinifera* cv. Cabernet Sauvignon grape skins during three distinct berry ripening stages: 46 days after anthesis (DAA, corresponding to *véraison*), 55 DAA (when all the grapes in the bunch were coloured) and 94 DAA (corresponding to full maturity). They observed a general decrease in proteins involved in energy and general metabolism and a decrease in the levels of specific heat-shock proteins during development. They also showed an overexpression of proteins involved in anthocyanin biosynthesis at the end of the colour change (55 DAA) in comparison to the period of full ripeness (94 DAA).

Marsoni and co-workers (2008) used 2D gel electrophoresis coupled to mass spectrometry to study somatic embryogenesis in grapevine by analyzing proteins that were differentially expressed in embryogenic versus non-embryogenic callus of *V. vinifera* cv. Thompson seedless. The expression patterns of 35 proteins differed significantly between the two samples. Of the 35 identified proteins, 31 could be assigned to five functional classes based on the biological process, and included: metabolism and energy state (26%), protein processing (19%), cell proliferation (16%), stress response (16%) and signalling (3%). From their findings, they concluded that the embryogenic status in grapevine is related to the ability to control oxidative stress.

The availability of genomic sequences has accelerated the identification of proteins in the last few years. Proteomic studies are currently restricted by the amount of proteins (and genes) that can actually be analyzed at any time point, and are dependent on the research question being addressed as well as certain technical limitations (Jorrin-Novo *et al.*, 2009). Although published in 2002, the quote from J. Roberts (Department of Biochemistry, University of California, USA) still holds true today: 'we know just enough about plant proteomes to imagine the breathtaking scope of our ignorance…comparing our understanding of the nature and changes in proteinaceous particles in plants with that of planets and stars in astronomy, our grasp on and of the plant proteome may be likened to the time when man first noticed changes in the heavens. And there is no less cause for excitement' (Roberts, 2002).

Metabolomics data

Although highly informative, transcriptomic and proteomic analyses have limitations. For example, changes in the transcriptome and/or proteome do not necessarily result in changes in the biochemical phenotypes (or metabolome). The metabolome represents the final 'omic' level in a biological system and can be considered as the

complete set of metabolites in a biological sample. The metabolome is dynamic and changes constantly in response to changing conditions (e.g. development, stress, treatment).

Although the definition of metabolomics describes a non-selective analytical approach to identify and quantify all metabolites in a biological system, it is currently not possible using a single analytical method. Due to this shortcoming, metabolomics currently relies on 'composite metabolite profiling' in which separate technology platforms are used to assess the metabolites belonging to different metabolic pathways (Stitt and Fernie, 2003).

There are a number of metabolomic analyses published on grapevine. Figueiredo *et al.* (2008) used transcript and metabolic profiles of two cultivars, Regent (a resistant interspecific hybrid: (Silvaner × Müller-Thurgau) × Chambourcin) and Trincadeira (a susceptible *V. vinifera* cultivar) using one-dimensional and 2D nuclear magnetic resonance (NMR) techniques to identify several compounds such as amino acids, carbohydrates, organic acids and phenolic compounds. The metabolomic data were used, in conjunction with transcriptomic data, to identify metabolic pathways involved in plant defence against pathogenesis. The metabolic profile identified an accumulation of the compounds inositol, glutamine, glutamate, alanine, and caffeic acid in the resistant variety. The accumulation of glutamine and the up-regulation of the phenyl-ammonium lyase (*PAL*) gene were considered as evidence of an activated PAL pathway, resulting in an accumulation of caffeic acid. Caffeic acid has previously been shown to be involved in fungal resistance. The authors discuss the potential implementation of this approach to discriminate between resistant and susceptible grapevine cultivars.

Similarly, Grimplet *et al.* (2009b) correlated transcriptomic, proteomic and target metabolite gas chromatography–mass spectroscopy (GC–MS) analysis of 32 polar compounds (amino acids, sugars, organic acids) to study the grape berry tissues (the pericarp consisting of the skin and pulp and the seeds) under well-watered and water-deficit stress conditions. The relatively limited metabolomic analysis showed that 18 of the 32 metabolites analyzed exhibited tissue-specific differences with the skin accumulating high concentrations of caffeic acid, proline, shikimate and gluconate. The water-deficit stress treatment caused increases in alanine, catechine, myoinositol, shikimate and sucrose, and a decrease in the levels of glutamate and tartrate in pulp tissue. The authors discuss the influence of water-deficit stress on the tissue-specific accumulation of enzymes involved in the biosynthesis of flavour and aroma compounds in wine (organic acids, specific sugars, phenylpropanoids, proanthocyanins and volatile compounds).

Metabolites are the end products of cellular regulatory processes, and their levels can be regarded as the response of biological systems to change (e.g. genetic or environmental). The current problem facing metabolomics is the fact that metabolites have a greater variability in the order of atoms and subgroups than, for example, the 4-letter codes for genes or the 20-letter amino acid codes for proteins. In contrast to DNA and proteins, metabolites cannot be sequenced. The composition, arrangement of the atoms and the stereochemical orientation has to be elucidated for each metabolite studied (Fiehn, 2002). Although daunting, ad-

vances in our ability to extract, analyze and interpret metabolomic data will provide the most valuable information in grape quality-related aspects.

Systems biology

For organisms with sequenced genomes, integrated studies that analyze data on a genomic, proteomic and metabolomic level (using advanced bioinformatic/statistical analyses) have become more informative. This integrative approach has led to a new field of study: systems biology. This approach is holistic since it focuses on the biological system and analyzes the complex interactions that exist within the biological systems. These interactions are studied systematically with the ultimate aim of gaining insight into the organizational principles that are at work at both the cellular and 'organismal' level (reviewed in Kitano, 2002). Currently this approach to studying biological systems still has numerous challenges on a number of levels that include the extraction protocols, visualization, data analysis, interpretation and even data storage.

A proper understanding of a biological question relies on integrative and comparative analyses using model systems. The complexity of the questions raised for crop sciences requires researchers to translate diverse genetic, genomic and phenotypic information to address research questions/problems. To compound this issue further, the data from these diverse data types from genomic, transcriptomic, proteomic, biochemical and physiological experiments are typically dispersed throughout constantly evolving web-based information repositories. These data resources are often in various data formats in incompatible databases.

There are a number of platforms available for the analysis and visualization of transcriptomic, proteomic and metabolomic data. It is currently possible to carry out analysis of the co-expression of molecules previously linked in a metabolic network (Saito *et al.*, 2008). Grimplet *et al.* (2009a) (subsequently published in Grimplet *et al.* (2009c)) analyzed data from sequencing projects, functional annotations (based on homology to gene annotations in other organisms) and existing metabolic pathways to create a model for molecular interactions in grapevine. By analyzing the available sequences, the authors found 30 433 putative proteins in the genomic sequence (from http://www.vitisgenome.it), 34 134 RNA sequences (tentative contigs and singletons from TIGR – the Institute for Genomics Research) and 34 522 RNA sequences (from other non-*vinifera* sources). A total of 39 423 unique sequences were identified by aligning the transcripts to the genomic sequences. Of the 39 423 sequences, 27 678 had homology to transcripts from other organisms: 21 901 could be functionally annotated (i.e. having a precise function of which 13 149 could be mapped); 3049 had an 'unclear function' and 2728 were totally unique ('unknown'). Based on KEGG and available literature, 5503 genes could be mapped into 89 metabolic pathways (e.g. carbohydrate, energy, lipid, secondary metabolites, etc.). A further 2481 genes could be grouped into 67 transcription factor families; 974 genes were involved in genetic information (e.g. ribosomes, transcription, DNA replication, RNA polymerase, etc.); 1947 signal genes (e.g. auxin, cytokinin, flowering, cell cycle, circadian rhythm, etc.); 448 structural genes (e.g. cell wall metabolism and cell wall proteins, etc.); and 3537 genes in 15 transport

pathways (based on the KEGG, literature or transcription factor databases). This equates to a total of 13 149 grapevine genes mapped to 198 discrete pathway maps.

These results are the basis of a proposed 'grapevine metabolic network'. The proposed network will be based on the MetNet software (Wurtele *et al.*, 2003; http://metnet.vrac.iastate.edu/), and will be able to handle extensive information pertaining to molecules. The MetNet database will allow the grapevine research community to annotate molecules, and the database is not restricted to only enzymatic reactions. This approach should accelerate the efficiency and quality of grapevine genomic annotation and multivariate data analysis.

It is important to keep in mind that systems biology is an emerging field relying on data from multiple sources, each with their own inherent limitations. Even at the most basic level of systems biology, the genome, the most characterized plant genomic model (*A. thaliana*) still has more than 15% of the genes functionally unknown (based on sequence similarities to other organisms), and only approximately the same percentage has been experimentally characterized (The *Arabidopsis* Information Resource (TAIR): http://www.arabidopsis.org). This, combined with the current limitations of transcriptomics, proteomics and metabolomics discussed previously, should be kept in mind when attempting to describe biochemical functions and biological roles in plants based on, for example, putative gene function assignments (Fiehn, 2002).

10.5 Grapevine improvement

Over the last few centuries, wine grape production was improved mainly by advances in agronomic and management techniques and an ever-increasing reliance on agrochemicals. As a consequence, production costs of modern/intensive viticulture are higher and include a large energy cost component that can vary from one viticultural region to the next. Grapevine improvement faces unique challenges that hamper its progress. Most crop species have been improved primarily by the generation of hybrids. A hybrid is the progeny of an intraspecific or interspecific hybridization (cross) between genotypes. In grapevine this process is hampered by a number of factors. First, the traits that are important for wine quality are generally polygenic. The possibility of recombining all positive traits of both parentals in one hybrid is very rare. Second, grape and wine producers are market-driven, and the market has a proclivity for a limited amount of traditional cultivars (Mullins *et al.*, 2004). A hybrid is a combination of both parents and therefore represents a 'new' cultivar and cannot be referred to as either of the parents (in marketing terms). Clonal selection has had numerous successes, but is in turn limited by the genetic diversity found within a cultivar and cannot tap into the genetic diversity found in other grapevine cultivars (genus and species).

Transgenic plants have the most potential for grapevine cultivar improvement; since only the trait of interest is conferred and there is no 'reshuffling' of two diverse genotypes, the resultant transgenic plant will ideally maintain the character of the transformed plant/cultivar (apart from the transformed trait). Transgenic

technology is also not limited to the genetic diversity available within a species or genus, since candidate genes can be sourced from theoretically any genetic source. This transgenic technology, however, has limited support in the public sector and the potential benefits are currently far outweighed by consumer resistance to its application in crop species. These methods, and the advances in technologies available to breeding, will be discussed.

10.5.1 Clonal selection

Introduction of hybrids (as new cultivars) into the international market remains a difficult process and is hampered by a discerning consumer and the lack of acceptance by both producers and consumers towards hybrids. This discrimination is due to the fact that modern viticulture for wine production is historically dominated by a number of traditional or classic varieties (cultivars). These cultivars have a long-standing reputation for producing quality wines. The ten classic international varieties include: Cabernet Sauvignon, Chardonnay, Chenin blanc, Merlot, Pinot noir, Riesling, Sauvignon blanc, Semillon and Syrah (Shiraz). The term 'noble grape' is a further distinction that is still used to describe cultivars traditionally associated with high-quality or superior wines. Historically, the noble grapes comprised only six varieties: the red cultivars Pinot noir, Cabernet Sauvignon, and Merlot; and the white cultivars Sauvignon blanc, Riesling and Chardonnay. The styles of wines produced by these cultivars are still desirable and relatively firmly set in consumer preferences. For these mostly non-scientific reasons, clonal selection has been relied on for improvement of these cultivars.

Clonal selection relies on the cumulative genetic variation that exists within clones of a specific cultivar. This genetic variation exists because grapevine cultivars dedicated to wine production are the result of selection of superior genotypes of ancient origin that have most likely been generated by spontaneous crosses during the cultivation/domestication of grapevine. These cultivars have been propagated vegetatively and therefore each cultivar is a unique, heterozygous genotype. This vegetative propagation has resulted in grapevine cultivars that have accumulated a substantial amount of somatic mutations (Mullins *et al.*, 2004).

Although clonal selection has been successfully implemented in most cultivars, this method of grapevine improvement has its limits since it relies exclusively on relatively minor mutations that exist within a cultivar. Visual examples of this are the somatic colour mutants of Pinot noir: Pinot blanc and Pinot gris (Pinot grigio). Pinot noir berries are black while Pinot blanc berries are white. The colour of Pinot gris can vary, but is typically grey. The colour of Pinot blanc berries is thought to be due to a deletion of the *VvmybA1* gene causing inhibition of anthocyanin biosynthesis (Yakushiji *et al.*, 2006). The mutation responsible for the Pinot gris phenotype is not known, but is thought to be due to a deletion or a somatic recombination in the colour locus (Walker *et al.*, 2006).

Since the clonal selection method relies on an observable phenotype, results can be affected by variation from a number of different sources (e.g. the vineyard, the

winery, the taste panel, etc.) that can obscure results, especially when searching for objective quality-related characteristics (Bouquet *et al.*, 2008).

10.5.2 Conventional breeding

Increased production efficiency and improved fruit quality for grapevine has been primarily based on the modification of management and/or growing conditions of specific elite genotypes, by pruning, utilizing training systems and grafting onto rootstocks. Conventional breeding has made significant progress in the development of rootstocks, but has had very little impact on the scion (specifically for wine grapes). Although the choice of rootstock has been implicated in affecting the quality of the resulting wine (Gawel *et al.*, 2000), most rootstock breeding has, however, focused on resistance to biotic or abiotic factors and stresses (Mullins *et al.*, 2004).

Conventional breeding approaches are typically limited by the availability of natural genetic variation in crops or related wild species. Fortunately the genus *Vitis* has large reserves of genetic variation, and the potential for grapevine improvement by breeding is theoretically limitless. Two main obstacles hamper progress in grapevine breeding. First, grape is a woody perennial plant with an extensive youth phase. For grapevine breeding this means that a relatively long time is needed to go from seed to seed (i.e. the reproductive cycle) and therefore years are required for recurrent backcrosses. Key characteristics for grapevine, such as productivity and quality, can therefore only be evaluated after many years of selection and analysis. Second, the grapevine genome exhibits a high degree of heterozygosity (Bouquet *et al.*, 2008).

Of a number of potential targets (reviewed in Vivier and Pretorius, 2002), grapevine breeding has focused on two main goals: improved resistance to pathogens/stress and improved quality (berry or wine). These two goals are usually interdependent since the resistant genotypes of the wild species usually have poor fruit quality and, conversely, commercial cultivars with quality berries are typically more susceptible to pathogens. Hybrids with improved fungal disease resistance have been selected from the progeny of crosses between *V. vinifera* and mostly wild *Vitis* species with resistance traits. Years of recurrent backcrossing with elite grapevine cultivars and selection for both wine quality and other desirable traits has led to new cultivars that produce quality wines and additional traits (Mullins *et al.*, 2004). An example of an intraspecific hybrid that is commercially viable is the German cultivar Muller-Thürgau, long considered to be a Riesling × Sylvaner cross, which was developed to combine the complexity of the Riesling grape with the earlier ripening character of Chasselas de Courtillier (Regner *et al.*, 2001, Sefc *et al.*, 1997). Similarly, the South African cultivar Pinotage (Pinot noir × Cinsaut) was crossed in an attempt to combine the robust growth character of Cinsaut (previously Hermitage) with the quality grapes of Pinot noir (Mullins *et al.*, 2004). Although neither of these hybrids exhibited all the desired traits, they did produce grapes of acceptable quality for winemaking; they still cannot compete in the international wine market with the elite cultivars.

The efficiency of grapevine breeding programmes also relies on the availability of suitable screening methods for a range of characteristics that include: fruit and wine quality, yield, disease resistance, winter hardiness, and tolerance to salinity. It is at this step that the genome sequence, and specifically molecular markers, will improve breeding programmes. Conventional breeding has made, and will doubtlessly continue to make, enormous improvements to crops, but conventional breeding has its limitations. It is clear that alternative ways need to be explored to improve crops as agriculture experiences ever-increasing pressure from a number of fronts.

Molecular breeding

The rapid progress in plant genomics research has opened a new era in plant breeding. This can be attributed to: improvement in the supporting technologies (most notably the high-throughput technologies that include sequencing and bioinformatics); national and international investment in grapevine research (e.g. the Franco–Italian sequencing project); publicly available/accessible grapevine sequences (genomic and cDNA sequences); the availability of two draft grapevine sequences; development of DNA molecular markers; and the development and integration of genetic and physical maps. This has resulted in an improvement of our ability to identify genetic loci for key QTLs, the development of technologies for detecting genetic variation, the transfer of knowledge and technologies from model to crop species and the application of tools to assist breeding programmes (e.g. MAS, pyramiding and tilling) (Xu and Crouch, 2008). Conventional breeding efforts can be further improved by implementing collaborative approaches that incorporate functional, comparative and structural genomics.

The genetic gains from conventional breeding in major crop species have reached an apparent plateau. DNA molecular markers associated with qualitative and quantitative traits are used for the indirect selection of genes of interest. MAS is one such indirect selection process where the trait of interest is selected for based on a marker linked to it (instead of the presence of the trait itself). MAS selection is only possible once a DNA sequence (i.e. DNA molecular marker) is associated with a trait that is of interest to the breeder (Reece and Haribabu, 2007). The assumption is that the linked allele associates with the gene and/or QTL of interest (Xu and Crouch, 2008). Improvements in DNA molecular marker techniques are adding to the selection power of MAS, by providing both more reliable types of markers and an increasing list of trait-associated loci. MAS can be useful for traits that are typically difficult to measure, exhibit low heritability and/or are expressed late in development (Xu and Crouch, 2008). Mapped genes and QTLs can be used for MAS (Morgante and Salamini, 2003; Costantini *et al.*, 2008). Gene pyramiding is also possible using MAS and has been applied successfully in other species to enhance resistance to disease and insects by selecting for two (or more) genes. Since the genotype is analyzed for discrimination/selection, the advantage of using DNA molecular markers is that it allows the selection for QTL-linked markers that have the same phenotypic effect.

DNA molecular markers

Genetic markers were originally used to determine the order of genes on a chromosome (genetic mapping). These markers have gradually evolved from morphological markers, through isozyme markers to the current DNA molecular markers. Since genome-wide analysis is not feasible using morphological or isozyme markers, DNA markers have been continuously improved from the hybridization-based detection, to PCR amplification, to the current high-throughput sequencing-based systems.

DNA molecular markers exploit naturally occurring polymorphisms in DNA sequences. These polymorphisms are found in DNA sequences due to base pair deletions, substitutions, additions or variable repeated patterns. The ideal DNA molecular marker will have a number of desirable properties/characteristics that include: polymorphic, co-dominant inheritance, a random distribution throughout the genome, abundant in the genome, easily detectable, cost-effective and reproducible (both intra- and inter-laboratory reproducibility). DNA molecular markers can further be grouped into discrete categories, based on the method used to detect the DNA polymorphisms: Southern hybridization-based techniques (e.g. restriction fragment length polymorphism – RFLP); PCR-based techniques (e.g. RAPD, microsatellites); techniques based on both RFLP and PCR (e.g. amplified fragment length polymorphism – AFLP); and techniques generating sequence information (e.g. SNPs) (Schlotterer, 2004; Bouck and Vision, 2007).

From genomic studies, it is evident that thousands of phenotypically neutral, random DNA markers can be generated for any species where sufficient sequence information is available (both whole genome sequences and/or expressed sequences) and have been used successfully in a number of studies (e.g. biodiversity, trait mapping, etc.). Gene targeted markers can be regarded as a subset of the random DNA markers and, as the name suggests, they are derived from polymorphisms that occur within genes/coding sequences. As more and more data are generated from sequencing projects and the associated structural and functional biological studies, it is becoming possible to identify and develop so-called 'functional markers' that are derived from polymorphic sites within genes affecting phenotypic trait variation (e.g. the *DXS* gene for monoterpene content or the *VvMYBA1* gene for colour discussed in Section 10.4.2) (Andersen and Lubberstedt, 2003). These markers require functional analysis of individual genes and are therefore still limited pending the experimental characterization of the respective candidate genes.

DNA molecular markers have been used for a number of different applications that include: germplasm characterization, genetic diagnostics, characterization of transformants, genomic studies, organization and phylogenetic studies. DNA molecular markers are used to link plant breeding and plant biology. They have been applied to a number of applications: assessing genetic diversity (e.g. germplasm identification, classification and management); assembling plant genomes (e.g. by development of integrated maps); analysis of complex traits (quantitative inheritance patterns); MAS and cultivar development.

Table 10.4 A selection of *Vitis vinifera* microsatellite/simple sequence repeat (SSR) collections cited in literature

SSR collection	Number of loci	Origin	NCBI dbSTS accessions	Reference
VVS	9	*V. vinifera* L. cv. Sultana	G64021-25, BV722862-65	Thomas *et al.*, 1993
VVMD	4	*V. vinifera* L. cv. Pinot noir	No	Bowers *et al.*, 1996
VVMD	22	*V. vinifera* L. cv. Pinot noir	No	Bowers *et al.*, 1999
VrZAG	18	*V. riparia*	BV722835-44; 52–56; 58–60	Sefc *et al.*, 1999
VMC	371	*V. vinifera* L. cv. Sultana	Non-consecutive accessions	Vitis Microsatellite Consortium
scuVV	124	*V. vinifera* L. cv. Chardonnay	Not available	Scott *et al.*, 2000
VVI	169	*V. vinifera* L. cv. Syrah	BV140581-771	Merdinoglu *et al.*, 2005
UDV	118	*V. vinifera* L. cv. Sultana	BV096965-7072	Di Gaspero *et al.*, 2005

Microsatellites/simple sequence repeats

Microsatellite (or simple sequence repeat (SSR)) markers are based on repeated sequences of one- to six-base core sequences (typically two to four), found interspersed in the genome. Since the sequences flanking the repeats are conserved, but the length of the repeat itself varies, these markers can be detected by PCR using a pair of primers flanking the microsatellite. Each microsatellite tags a single locus (but having multiple allele sizes) in the diploid genome. Microsatellites are particularly useful because of their: (i) abundance in genomes, (ii) high degree of variability in the repeat sequence, and (iii) reproducibility. The use of microsatellites for grapevine genetics includes: the identification of cultivars, the relatedness of cultivars and the analysis of the parentage of crosses (Sefc *et al.*, 1997; Arnold *et al.*, 2002; This *et al.*, 2004; Goto-Yamamoto *et al.*, 2006).

Although microsatellite markers are a robust and useful tool in studying grapevine genetics, their development is time-consuming and expensive (prior to the draft grapevine genome sequence being released). The grapevine research community made a concerted effort since 2000, through the IGGP VMC, to develop microsatellite markers for the grapevine research community. This multinational consortium consisted of 20 research groups from ten countries that were co-ordinated by AgroGene S.A. in Moissy Cramayel, France.

There are large series of microsatellite markers available, and the most commonly cited are listed in Table 10.4. The availability of the two whole genome sequences of grapevine has made microsatellite discovery quite simple. Screening of the nearly homozygous grapevine line (PN40024), for example, identified 26 962 microsatellites (Jaillon *et al.*, 2007).

Single nucleotide polymorphisms (SNPs)

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation. A SNP is a single base pair mutation at a specific locus in a diploid consisting of two alleles. The vast majority of differences between individuals are point mutations due to SNPs. SNPs are a highly abundant class of DNA sequence polymorphisms found in both plant and animal genomes; they are more prevalent and have a higher frequency of occurrence than microsatellites. Furthermore, SNPs are genetically more stable than microsatellites. The major advantage of SNPs, however, is the potential to screen for the presence of the polymorphism without gel-based electrophoresis utilizing high-throughput methods, such as microarray technology (Lijavetzky *et al.*, 2007; Pindo *et al.*, 2008). The commonly used first generation DNA molecular markers (e.g. RFLP and RAPD) are all gel-based (Gupta *et al.*, 2001).

SNPs can be used for the same purposes as currently available markers (i.e. RFLPs, AFLPs and RAPDs), and include: construction of linkage maps, DNA fingerprinting, diversity analysis and trait mapping (Salmaso *et al.* 2008; Vezzulli *et al.*, 2008a,b). Unlike the other DNA-based markers, SNPs are directly based on known sequence polymorphisms, and considerable amounts of sequence data are required to develop a SNP marker. It is this characteristic of SNPs that makes them ideal for markers based on candidate genes. SNPs are evolutionarily conserved, and are therefore used in QTL analysis and in association studies in place of microsatellites.

The sequence information that is currently available for grapevine, BAC-end sequences (BES), shotgun sequencing, ESTs and the two completed genome sequences, makes it possible to identify millions of electronic SNPs (eSNPs). Velasco *et al.* (2007) identified more than two million SNPs in the heterozygous ENTAV115 grapevine genome sequence of which more than 87.6% (i.e. 1 751 176 SNPs) could be mapped to chromosomes and at least one SNP could be detected in 86.7% of anchored genes. On average, the SNP frequency in the genome was 4 SNPs/kb in all 19 linkage groups. These eSNPs are a valuable resource for high-throughput genotyping. Grapevine SNP marker information is available from the NCBI SNP database (dbSNP accession numbers from 79088086–79088470 [Build 130]).

Expressed sequence tags (ESTs)

Sequencing projects are primarily preoccupied with obtaining the sequence of an organism (sequencing and assembly) and subsequently identifying the complete set of genes of an organism (annotation). The ultimate scientific goal is, however, to gain a fundamental understanding of how genes are regulated and the ways in which genes interact in the complex biological system (the organism). To accomplish the latter goal, researchers need to identify and study the protein(s) encoded by genes.

An EST is a short sequence of a transcribed mRNA-derived cDNA sequence. The number of ESTs has increased rapidly. There are currently 353 941 *V. vinifera* and an additional 27 482 non-*vinifera* ESTs in Genbank (Table 10.1). An EST is

produced by unidirectional sequencing of a cloned mRNA transcript. The resulting sequence is usually a low-quality fragment of approximately 500–800 nucleotides. Since the cDNA clones are derived from mRNA, the EST represents an expressed gene (or transcript). ESTs are useful for identifying the coding sequences in the genomic DNA sequence of an organism. ESTs are also useful for refining the predicted transcripts of genes, which in turn helps in the prediction of protein products and possible function. Although the quality of the sequence information derived from ESTs is quite low, it is sufficient for designing probes for the DNA microarrays that are used for expression profiling.

In silico methods have been used to assess the expression level of a transcript by analyzing the EST frequency. The EST frequency is the number of transcripts of a gene of interest in a library as a fraction of the total number of transcripts in the library. EST frequency can also be used to identify co-regulated genes whose expression is correlated with stages of development or which have similar regulation in response to abiotic/biotic stresses. Co-regulated genes provide the basis for gene networks and are particularly useful for inferring the function of unknown genes using the 'guilt-by-association' principle (Pereira, 2000).

There are a number of databases that curate *V. vinifera* ESTs. Most notable are those of the NCBI (Table 10.1) and TIGR. The TIGR DFCI Grape Gene Index (VvGI) integrates data from international grapevine EST sequencing projects and represents a non-redundant collection of genes and data on their expression patterns, cellular roles, functions and evolutionary relationships.

Both NCBI (via Unigene) and TIGR (via Gene Indices) assemble ESTs into contigs. Many ESTs from a cDNA library are often partial sequences that correspond to the same mRNA transcript. Contigs are therefore assembled to reduce the total number of ESTs. It should be kept in mind that this assembly process may yield artifacts (e.g. a contig may in fact contain two distinct gene products). The completed genome sequence can be used to identify and rectify these errors (by analysis of the two loci).

These genomic tools provide methods to infer biological information in the form of expression data. Ultimately, this information must be correlated to the formation of quality-related compounds (e.g. metabolites and enzymes) before improved cultivars or improved viticultural practices can be designed.

10.5.3 Grapevine transformation and biotechnology

Genetic transformation requires an effective transformation and regeneration system, gene constructs for transformation and, preferably, a fundamental understanding of the pathway/trait that is being manipulated. Relative to other plant species, grapevine is considered recalcitrant to genetic transformation. Various factors in the transformation system have hampered progress in the past. These factors are diverse and range from cultivar-specific differences to selection regimes. Although not a routine process for all cultivars, numerous successes have been reported for grapevine transformation (Mullins *et al.*, 1990, 2004; Perl *et al.*, 1996; Bouquet *et al.*, 2008). *Agrobacterium*-mediated transformation is the most

widely used system for introducing gene constructs into grapevine, and the use of somatic embryogenic calli (or cell suspensions) has proven the most successful (reviewed in Mullins *et al.*, 2004 and Bouquet *et al.*, 2008). Embryogenic calli have been obtained from different tissues, including embryos (zygotic), leaves, ovaries and anther filaments. Anther filaments are the most widely used tissue for transformation (Mezzetti *et al.,* 2002). The retention of the embryogenic potential of cell cultures during maintenance has, however, proven problematic with the embryogenic potential typically decreasing during the repeated subcultures, embryo development and the subsequent regeneration into plants.

If one was tasked with improving wine quality, the major scientific challenge for generating transgenic grapevine would be the complexity of the targeted genetic traits. For a polygenic trait, this translates into a large number of genes that need to be manipulated to effect change. Furthermore, the lack of information on the biochemical and physiological processes that link available transcriptional data of candidate genes with the phenotypic expression of the trait under investigation adds even more complexity to an already complex problem.

Transgenic grapevines

Advances in molecular biology and the associated technologies have led to the isolation of a number of genes that are associated with agronomically important traits. Transgenic plants are generated by transforming cassettes expressing a gene(s) of interest into the plant genome (generally via Agrobacteria or biolistic bombardment). Transgenic plants are recombinant, since they contain heterogenous DNA, and are therefore regarded as a class of genetically modified organism (GMO). There have been a number of targets identified for grapevine biotechnology that can be broadly grouped into the following categories: pests and diseases (fungal, bacterial and viral diseases); abiotic stress tolerance (frost tolerance, drought, salinity tolerance, enhanced photoprotection); quality traits (enhanced colour and sugar concentration, resistance to browning); and health benefits (increased concentrations of provitamins and antioxidants). The majority of reports to date have focused on pests and diseases (Mullins *et al.*, 2004; Bouquet *et al.*, 2008).

There have been a number of field trials (and applications for field trials) for transgenic grapevine, with the majority targeting agronomic traits, such as: resistance to pathogens (bacterial-, fungal- and viral-resistance), weed control (herbicide tolerance) and adaptation to climate (cold-hardiness). The traits targeted for quality include sugar concentration and colour. The remaining traits targeted plant development (modified flower and fruit development to increase yield).

CSIRO (Australia) has a number of transgenic grapevines under evaluation (CSIRO Plant Industry, 2002). The CSIRO research group have targeted candidate genes that could potentially affect berry quality for wine-making by targeting genes involved in flower and berry development (*SH4*), sugar accumulation in ripening berries (*INV*) and flavonoid biosynthesis (*DFR* and *UFGT*, discussed in Section 10.6.2). The overexpression of the *V. vinifera* cv. Shiraz *SH4* gene encoding an agamous/shatterproof-like MADS domain transcription factor should

provide insight into the role/function of this gene. MADS-box genes are involved in the regulation of flower and fruit development, and transgenic grapevines can potentially generate novel flower and/or fruit characteristics. Invertases are involved in the regulation of berry sugar composition and break down sucrose into fructose and glucose. Overexpression and silencing/down-regulation of the *V. vinifera* cv. Sultana *INV* gene, encoding an invertase β-fructofuranosidase, should produce transgenic grapevine with berries with altered sucrose concentrations.

Irrespective of the commercial viability of these transgenic grapevines currently under evaluation, the outcomes of the genetic, biochemical and physiological characterization of these plants will provide insights into the *in planta* role of these genes and their respective biosynthetic pathways. The use of transgenic plants (including grapevines) thereby provides a valuable genetic tool for analyzing gene function (functional biology).

10.6 Current research on quality aspects

From a grape and wine quality perspective, the research contributing to our understanding of quality components in grapevine can be grouped into a number of themes. Certain themes, central to quality, have received much attention, and include: flowering and berry-ripening, terpenoids (flavour and aroma) and flavonoids (colour). Two of these themes will be discussed in more detail.

10.6.1 The terpenoids and flavour and aroma

Terpenoids are among the most abundant and structurally diverse group of natural metabolites. They are multifunctional and are involved in functions that range from universal primary metabolites (e.g. membrane structure, redox reactions, light harvesting and photo-protection, regulation of growth and development) to family/genus-specific secondary metabolites. These secondary metabolites are typically involved in plant defence or communication and include plant–environment, plant–insect, plant–microorganism and plant–plant interactions. From a human perspective, terpenoids have been exploited as medicines, flavours, fragrances, cosmetics, dyes, insecticides, etc. (reviewed in Dudareva *et al.*, 2006). Specifically from a grape and wine quality perspective, the monoterpenoids, sesquiterpenoids and the carotenoid/tetraterpenoid-derived C_{13}-norisoprenoids contribute significantly to the varietal character of grapes and the subsequent wine. Although some of these grape-derived terpenoid compounds are found as free volatiles, the majority are stored as non-volatile, water-soluble sugar conjugates (glycosides) (Lund and Bohlmann, 2006).

All terpenoids are formed from the C_5 isopentenyl diphosphate (IPP) and its more reactive isomer dimethylallyl diphosphate (DMAPP). Plants use two separate and biochemically independent pathways for the formation of IPP: the chloroplastic methyl-erythritol-4-phosphate (MEP) pathway (also referred to as the 1-deoxy-D-xylulose 5-phosphate (DOXP) pathway) and the acetate-mevalonate

(MVA) pathways in the cytosol. The acetate-MVA pathway is responsible for the production of sesquiterpenes and triterpenes, while the DOXP/MEP pathway produces the monoterpenes, sesquiterpenes, diterpenes, tetraterpenes, plastoquinone and the prenyl side chains of chlorophyll (Dubey *et al.*, 2003).

Prenyltransferases convert the IPP/DMAPP to the terpenoid prenyl diphosphate precursors: geranyl diphosphate (GPP), farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP). The terpene synthases (TPS) subsequently convert the prenyl diphosphates to the various terpenoids found in plants: DMAPP to hemiterpenes, GPP to the C_{10} monoterpenes, FPP to the C_{15} sesquiterpenes and GGPP to the C_{20} diterpenes.

The genes, enzymes and intermediates of the DOXP/MEP pathway have been studied in a number of plant species (Dubey *et al.*, 2003; Ganjewala *et al.*, 2009). For example, all the genes encoding enzymes of the MEP pathway with homology to the *Escherichia coli* MEP pathway enzymes have been identified from *A. thaliana*. Similarly, the grapevine genome sequencing and annotation allowed the positioning of the genes onto metabolic maps as well as determining the copy number of the respective genes in the grapevine genome. More than a hundred terpenoid-encoding genes could be identified in the grapevine genomic sequence (Pinot noir ENTAV115). Of the 124 genes identified, 110 could be mapped to linkage groups, and could be functionally assigned to carotenoid biosynthesis (24), abscisic acid metabolism (24), prenyltranferases (5), DOXP/MEP biosynthetic pathway genes (9) and MVA pathway genes (8) (Velasco *et al.*, 2007).

Analysis of the Pinot noir (ENTAV115) genome sequence by Velasco *et al.* (2007) identified 89 functional terpene synthase encoding genes and 27 pseudogenes. The contribution of each of these 89 functional genes to the flavour/aroma profile of wine remains to be investigated, but underlines the genetic complexity of only a single aspect of grapevine quality (i.e. monoterpenes).

Martin and Bohlmann (2004) identified, isolated and functionally characterized a monoterpene synthase ((–)-α-terpineol synthase) from grapevine by *in silico* screening of full-length cDNA EST libraries . The gene product was functionally characterized by heterologous expression in *E. coli* (Martin and Bohlmann, 2004). An *in vitro* enzyme assay and gas chromatography–mass spectrometry (GC–MS) was used to identify the enzyme as an (–)-α-terpineol synthase. The authors concluded that *V. vinifera* has the genetic and biochemical capacity to enzymatically form monoterpenes directly from GPP. The identification and characterization of terpene synthases provides researchers with the genetic information required for the development of molecular markers (functional markers) for quality-associated traits in grapes (flavour and aroma).

The monoterpene concentration of grapevine cultivars has also received attention in breeding programmes due to the contribution of these aromatic compounds to the ultimate varietal character, and hence quality, of the grapes and wine. As discussed previously, Battilana and co-authors (2009) combined metabolite profiling with QTL analysis and a candidate gene approach to identify key genetic factors underlying Muscat aroma in grapevine. The authors reported co-segrega-

tion between candidate genes and QTLs for the content of the main aromatic monoterpene compounds (i.e. linalool, nerol and geraniol). Three genes were identified of which two mapped within a QTL for linalool content on linkage group 10. The third gene co-localized with a major QTL for the level of monoterpenes on linkage group 5. This gene encoded a 1-deoxy-D-xylulose 5-phosphate synthase (DXS), the first enzyme in the plastidial DOXP/MEP pathway. The authors suggested a role for DXS in regulating the metabolic flux through the DOXP/MEP pathway.

10.6.2 The flavonoids and colour

The phenolic compounds in wine include a large group of chemical compounds referred to as polyphenolics. Polyphenolics are involved in a number of quality-related traits in wine and include the taste, colour and mouthfeel of wine. Polyphenolics can further be grouped into two categories: the flavonoids and the non-flavonoids. In white wines, the most abundant polyphenolics are hydroxycinnamic acids and flavan-3-ol monomers. In red wines, where up to 90% of phenolic content is made up of flavonoids, the most abundant polyphenols are tannins and anthocyanins (reviewed in Adams, 2006; Downey *et al.*, 2006; Conde *et al.*, 2007).

Three classes of flavonoids are present in grapes and wine: anthocyanins, flavonols and proanthocyanidins (tannins). Generally one can assign the colour in red grapes and wine to the anthocyanins; flavonols function as UV-protectants and free-radical scavengers; and tannins provide astringency, stability and structure to wine.

Grape flavonoids are predominantly found in the skin and seeds (specifically the seed coat). Only hydroxycinnamic acid can be found in significant amounts in the flesh of white cultivars. The biosynthesis of phenolics that contribute to the quality of wine are all formed via the phenylpropanoid pathway. This pathway is relatively well studied in plants (including grapevine) and most of the genes in the pathway have been identified (Boss *et al.*,1996; Adams, 2006; Castellarin and Di Gaspero, 2007; Jeong *et al.*, 2006; Lijavetzky *et al.*, 2006). Studies have shown that, although all the genes are expressed in red cultivars, two genes encoding a phenylalanine ammonia lyase (*PAL*) and UDP-glucose:flavonoid 3-O-glucosyl transferase (*UFGT*) are not expressed in white cultivars. The expression of two genes encoding for Myb-family transcription factors regulates anthocyanin biosynthesis and is responsible for the skin colour variation in grape berries. VvMYBA1 regulates specifically *UFGT* transcription and it is only expressed in cultivars with coloured skin. A Gret1 retroelement insertion in the promoter of *VvMYBA1* gene has been shown to inhibit anthocyanin biosynthesis in the berry (Kobayashi *et al.*, 2005). The Gret1 insertion inhibits the expression of *VvMYBA1*, consequently preventing it from inducing the expression of the final gene of the phenylpropanoid pathway, *UFGT*. This Ty3-*gypsy*-type retrotransposon insertion event in the *VvMYBA1* promoter was found in over 200 *V. vinifera* accessions and is strongly associated with the white-fruited phenotype (Cadle-Davidson and Owens, 2008).

In a cross population segregating for berry colour, the *VvMYB1A* gene co-localized with colour and could be mapped to linkage group 2.

Velasco *et al.* (2007) identified genes known to encode for enzymes of the phenylpropanoid pathway based on gene predictions from the draft grape genome. The authors found that the majority of the genes involved in the phenylpropanoid pathway are typically organized in gene families. Using comparative genome analysis (between grape, poplar and *Arabidopsis*), the authors further noted that, within the phenylpropanoid pathway, grape and poplar have relatively large gene families when compared to *Arabidopsis* (where the genes are typically single copy). This was as expected since poplar and grapevine are both woody perennials and use a host of flavonoids for primary and secondary metabolic functions.

As mentioned previously, a research group from CSIRO (Australia) has a number of transgenic grapevines under evaluation in field trials (CSIRO Plant Industry, 2002). They have targeted candidate genes that could potentially affect anthocyanin biosynthesis and consequently the berry quality for wine making. The research group has overexpressed and silenced/down-regulated the *V. vinifera* cv. Lambrusco *dfr* gene encoding a dihydroflavonol reductase. The DFR enzyme is involved in the biosynthesis of the flavonoids: anthocyanins and tannins. DFR catalyzes the conversion of dihydroflavonols to leucoanthocyanins and leucodelphinidins, that serve as substrates for the subsequent steps in the anthocyanin pathway and proanthocyanin (tannin) pathway. Anthocyanins, localized in the skin of red and black grape berries, are liberated during the winemaking process and determine the colour of the wine. The berries from these transgenic grapevines are expected to have an altered anthocyanin and/or tannin profile. The same group has also overexpressed the *V. vinifera* cv. Shiraz *UFGT* gene encoding a UDP glucose flavonoid 3-O-glucosyl transferase. The UFGT enzyme is responsible for the glucosylation of cyanidins and delphinidins to anthocyanins, and this enzyme has been identified as the determinant factor for grapevine berry colour. Both the quality and quantity of the colour in grape berries determine the colour of the resultant wine, and the berries of the transgenic grapevine are expected to have altered berry colour.

10.7 Conclusions and future trends

The current advances in grapevine genetics and genomics will lead to an improved understanding of the molecular basis of quality in grapes (and wine). Although significant advances have been made in elucidating the biochemistry of the flavour and aroma composition of grapes (e.g. the carotenoids, monoterpenes and flavonoids), there are still many questions unanswered. Isolating and characterizing the genes involved in a biosynthetic pathway is still a long way from understanding a biochemical network. Systems biology approaches are particularly promising to analyze the networks involved in grapes quality (e.g. flavour, aroma and colour). Although the sequencing of the grapevine genome has accelerated the identification of genes, proteins and enzymes involved in

quality-associated compounds, the regulation, coordination and interaction of these molecules are still not understood. Analysis of a plant's transcriptome, proteome and metabolome to stress (biotic or abiotic), the environment or viticultural practices will provide researchers with a snapshot of the plant's molecular response to its surroundings. Correlations between these three diverse molecular levels could provide the basis for beginning to understand quality in a grape berry.

The availability of the human genome sequence should also not be overlooked, and could assist researchers in probing the genetic factors that influence the perception of flavour and aroma compounds in individuals. Human taste and olfactory receptors have already been identified, and differences in the genes encoding these receptors could be correlated to food preferences, eating pattern, and even alcohol preferences (Polaskova *et al.*, 2008 and references within). An increased understanding of genetic variation in the human genome would therefore provide us with the genetic basis for taste and olfactory preferences of individuals and population groups. This is no small task since it is known that significant variation exists between individuals in the number and distribution of taste receptors in the mouth, which will directly affect the perception of taste(s). Furthermore, even if individuals had the same amount of receptors, further variation can be found in the amount and rate of saliva produced in the mouth. This will affect the ultimate perception of bitterness, sweetness, saltiness, astringency and sourness. Since the perception of the quality of wine is determined by the complex interaction of numerous tastes produced by the various wine components, the task of defining quality in scientific terms remains particularly daunting and will require advances in a number of disciplines.

Grapevine researchers currently have the tools to begin to understand the genetic basis of quality: DNA molecular markers, whole genome sequences, the ability to analyze the transcriptome and, to a lesser extent, the proteome and metabolome, large germplasm collections, a transformation system for generating transgenic grapevine and an internationally organized grapevine research community. The challenge facing grapevine research is correlating the large data sets currently being generated from such divergent fields as genomics, plant physiology, chemical analysis, bioinformatics and sensorial studies to the central theme of grape quality.

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11

Viticultural and vineyard management practices and their effects on grape and wine quality

A. G. Reynolds, Brock University, Ontario, Canada

Abstract: This chapter discusses the impacts of various cultural practices on fruit composition and wine quality, with an emphasis on aroma compounds. It is framed within four basic pillars of the Cool Climate Paradigm of winegrowing: (i) keep the fruit warm; (ii) keep the leaves exposed to sunlight; (iii) achieve vine balance; (iv) minimize water stress. Practices that are particularly relevant to these basic pillars include hedging and basal leaf removal; training systems; vine spacing; crop control and shoot density; vineyard floor management; irrigation. Fruit exposure, canopy manipulation, pre-fermentation practices, and vineyard site may influence in particular the concentration of aroma compounds in grapes and wines. These differences can sometimes be confirmed organoleptically in wines. In the case of monoterpenes: (i) potentially volatile terpenes (PVT) are more responsive to viticultural and oenological practices than free volatile terpenes (FVT); (ii) FVT and PVT are rarely correlated with soluble solids, titratable acidity, or pH, and thus cannot be predicted by standard harvest indices; (iii) losses in FVT and PVT can occur between the berry and juice stages, hence the desirability of skin contact; (iv) FVT and PVT concentrations can, in some cases, be related to wine tasting results.

Key words: canopy management, crop control, basal leaf removal, training systems, monoterpenes, norisoprenoids, methoxypyrazines, anthocyanins, phenols.

11.1 Introduction

Wine quality is the result of a complex set of interactions, which include geological

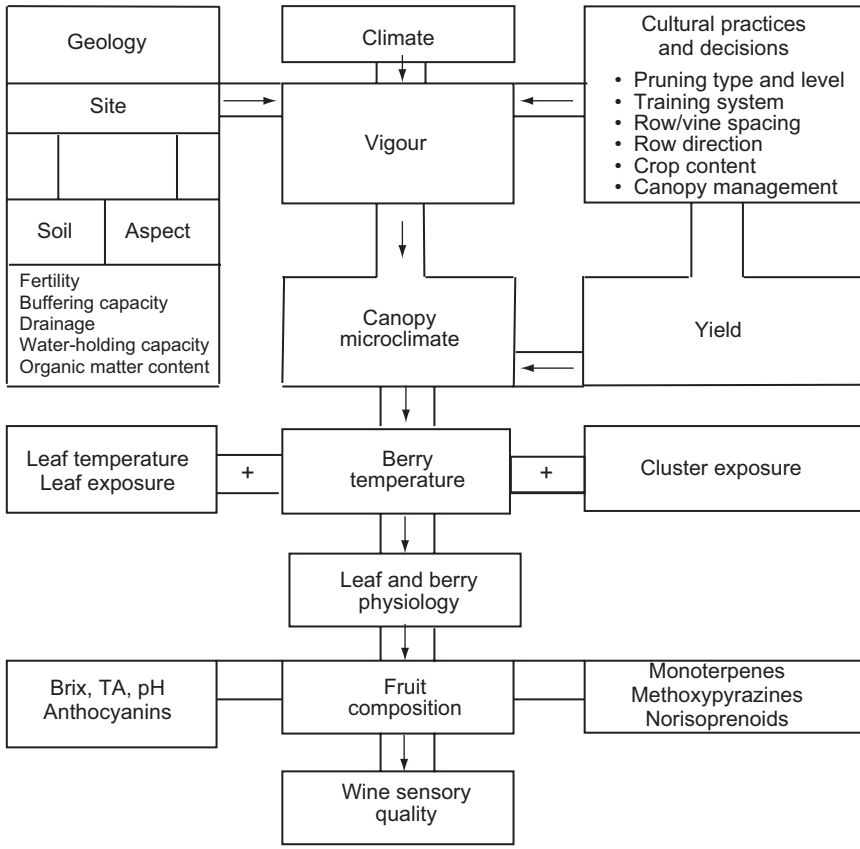

Fig. 11.1 A conceptual view of factors contributing to vine balance and wine quality (modified from Smart *et al.*, 1985a).

and soil variables, climate, and many viticultural decisions (Fig. 11.1). Taken as a whole, they may be described as the terroir effect (see Chapter 9). This chapter will specifically focus upon those viticultural practices beyond soil and climate that influence grape composition and wine quality.

It is very likely that winegrowers have recognized for centuries that specific viticultural practices result in better fruit composition and hence better wines. Specific practices such as trimming vine canopies to minimize shading of fruit; removal of leaves in the fruit zone, again for purposes of fruit exposure; adaptation of particular training systems to optimize fruit composition; and reduction of crop levels to increase rate of fruit maturation are but four specific practices that have been used by winegrowers before the advent of scientific research in this field.

A specific common goal among many of these viticultural practices is that of fruit exposure. It is one of four 'pillars' of what the author refers to as the 'Cool Climate Paradigm', which is a set of general principles common to most premium winegrowing regions of the world. These are: (i) keep the fruit warm,

presumably by either natural cluster exposure provided by an appropriate training system or by canopy management practices such as basal leaf removal; (ii) keep the leaves exposed to sunlight, again, by viticultural practices such as appropriate training, shoot density, and row and vine spacing; (iii) achieve vine balance between vegetative growth and crop size; (iv) Avoid water stress. These pillars will be addressed throughout this chapter as they pertain to various cultural practices.

Research into viticultural practices that affect wine quality did not appear in the literature until the early to mid-1980s (Smart, 1982). Part of the reason for this was the development during that time of sensory descriptive analysis (Williams *et al.*, 1982), which allowed viticulturists and sensory scientists to collaborate on field-based projects with an ultimate goal of microvinification of fruit from individual field treatments and subsequent sensory evaluation of those wines. Among the first of these studies were those of Smart *et al.* (1985a,b) in Australia, who clearly showed the positive impact of alleviating canopy shade upon fruit composition and wine quality in Shiraz through accommodation of vine vigour by canopy division. In France, Carbonneau *et al.* (1978) and Carbonneau and Huglin (1982) put forward a similar idea by the introduction of the Lyre system for *Vitis vinifera*. Later work that followed this theme included evaluation of divided canopies in California (Kliewer *et al.*, 1988), British Columbia (Reynolds and Wardle, 1994; Reynolds *et al.*, 1995c, 1996b), and New Zealand (Smart and Smith, 1988; Smart *et al.*, 1988).

The original basis for these then-radical ideas was the work of Shaulis *et al.* (1966) in Concord, who clearly showed that optimization of cluster and leaf light environment by the Geneva Double Curtain (GDC) divided-canopy system led to substantial improvements in fruitfulness, yield, and fruit composition. This work was verified in Australia by Shaulis and May (1971) using Thompson Seedless. It led to a revolution in the viticultural world that might be described by the Small Vine (Old World) philosophy of narrow rows, close vine spacing, and narrow, vertical trellises, versus the Large Vine (New World) concept that might include divided canopies, wide rows, and wide vine spacing as a combined utility for accommodation of vine vigour.

11.2 Fruit exposure and fruit composition

11.2.1 General effects of fruit exposure on fruit composition

A primary pillar of the Cool Climate Paradigm is that fruit must be kept warm. In general, exposing fruit to the sun will increase fruit temperature along with the enzymatic activities therein. Consequently, when compared to shaded fruit, exposed fruit will normally contain lower malic acid concentration (Lakso and Kliewer, 1976), lower methoxypyrazines (Allen *et al.*, 1991), higher anthocyanins and phenols (Crippen and Morrison, 1986b), higher monoterpenes (Reynolds and Wardle, 1989b), and occasionally higher soluble solids (Kliewer and Lider, 1968; Crippen and Morrison, 1986a; Reynolds *et al.*, 1986b). However, there are two

main components to fruit exposure, light and temperature, and it has been exceedingly difficult to separate the effects of these factors.

Our understanding of the significance of light and temperature on grape composition began during the 1950s. Most of the early studies on effects of light focused on soluble solids and organic acids. For example, Ribéreau-Gayon (1959a,b) was perhaps the first to show experimentally that excluding light to vines would lead to increases in titratable acidity (TA) and decreases in soluble solids in the fruit. Kliewer and Schultz (1964) examined the influence of three light environments (21%, 30% and 100% of full sun) and found that higher TA and concentrations of most organic acids were higher in shaded fruit. Tartaric and citric acid concentrations were highest under full sun conditions. More C^{14} label incorporation was observed in the organic acid fraction of berries from shaded vines as well. This pattern was similar, but not as pronounced, in the nearly-mature fruits; somewhat more label incorporation into the acid fraction was observed in immature berries. The percentage of C^{14} in malate under the different light treatments paralleled that found in the organic acid fraction as a whole. Tartaric and citric acids, on the other hand, displayed more label when grown in full sun. The overall proportion of label in malic acid far exceeded that found in tartaric acid, as was previously reported by Stafford and Loewus (1958). Although air temperatures of the Kliewer and Schultz (1964) experiment and similar ones by Schultz and Lider (1964) were similar among all light treatments, the fruit temperature was greatly affected. In both cases, the apparent differences in malic acid metabolism due to the various light treatments were typical of those expected under appropriately-corresponding temperature regimes. Only the higher C^{14} incorporation into tartaric acid in high light is suggestive of a true light response.

Shading of vines during fruit maturation increased TA, malate, and tartrate, as well as lowering soluble solids in a study conducted by Kliewer *et al.* (1967). Wine quality was also increased by shading, owing, presumably, to better acid balance. Again, the effects of the light factor were integrated completely with those of temperature. Total accumulated solar and sky radiation varied directly with the degree of shading, and leaf and berry temperatures under full sunlight were as much as 10 °C greater than those in the shade. Similar studies in Germany by Klenert (1974, 1975), whereby both light intensity and within-canopy air temperature were reduced, indicated that shading vines pre-*véraison* lowered soluble solids due to a delayed stage III of berry growth, and retarded acid synthesis also. Post-*véraison* shading led to slower degradation of malate. Similarly, Hofäcker and Alleweldt (1976) and Hofäcker *et al.* (1976) both showed positive correlations between light intensity and soluble solids and negative correlations between light intensity and TA.

Controlled environment studies and better experimental design permitted Kliewer (1971) and Kliewer and Lider (1970) to investigate actual light intensity effects on fruit composition. The latter group failed to show any light effects that were independent of those due to temperature, other than decreased tartrate concentrations under high light conditions. Vines grown in high temperatures had lower malate and TA, as well as lower pH, regardless of light intensity. Kliewer

(1971) attempted to ascertain independent light effects, and showed that low light increased the concentrations of TA, malic acid, and tartaric acid. Monobasic salts of malic and tartaric acid were also in higher concentration under low light conditions. Little if any explanation was offered for these findings.

Several seminal experiments were conducted during the 1960s and 1970s in which natural cluster exposure in the field has been studied. Typical fruit compositional differences were reported by Kliewer and Lider (1968), who examined soluble solids and TA of Thompson Seedless clusters grown in either the sun or the shade. Almost as much variation was observed between the front and rear of clusters within a single exposure treatment as between treatments. Tartaric acid concentrations were affected little by cluster exposure. Considerable differences in temperature were also documented between sun and shade clusters, as well as between the front and rear portions of berries and clusters. It appears likely that the extremely large heat load that exposed grape berries are capable of accumulating was for the most part responsible for the large composition differences observed between exposed and shaded fruit. Subsequent studies in Switzerland by Koblet *et al.* (1977) and Vautier *et al.* (1978) demonstrated similar effects of cluster exposure to the sun on fruit composition and quality. Fruit exposed to the sun was 6 °C higher. 'Sun' fruit also had higher pH and soluble solids while displaying lower TA, malate, and tartrate. Higher tartrate was observed in illuminated clusters of Chasselas. Higher soluble solids and lower TA were also observed for exposed clusters of 14 cultivars by Gaprindashvili (1981) in Moldavia.

Among French–American hybrids, Reynolds *et al.* (1986b) examined five cluster exposure categories (totally exposed; exposed east side of canopy; exposed west side of canopy; partial shade; full shade) on the hybrid cultivar Seyval blanc in New York. As expected, exposed clusters on the west side of the canopy were warmest during the day, and consequently had lowest TA and malate, highest tartrate, and highest soluble solids. In Ohio, Hummell and Ferree (1997) examined the response of berry set, yield, and fruit composition to low light environments in Seyval blanc and De Chaunac grapevines in the greenhouse. Potted vines of with either one or two clusters were subjected to high or low light conditions, created by 80% shade cloth and supplemental lighting. Seyval blanc and De Chaunac clusters grown under low light developed their golden and blue–red colour more slowly than clusters grown under high light conditions. Overall, vines subjected to low light conditions produced clusters with lower soluble solids, pH, and potassium ion concentrations compared to vines grown under high light conditions. The soluble solids and pH of Seyval blanc clusters were more sensitive to light than De Chaunac. Titratable acidity and tartaric acid concentrations of Seyval blanc were less sensitive to low light compared to De Chaunac clusters.

Among the many studies with *Vitis vinifera* cultivars, Crippen and Morrison (1986a) likewise reported that exposed Cabernet Sauvignon clusters had higher soluble solids, but on a total sugar per berry basis, however, there was no difference. The shaded berries were larger and had higher water content than did the exposed berries, effectively lowering soluble solids. Pre-harvest, anthocyanins

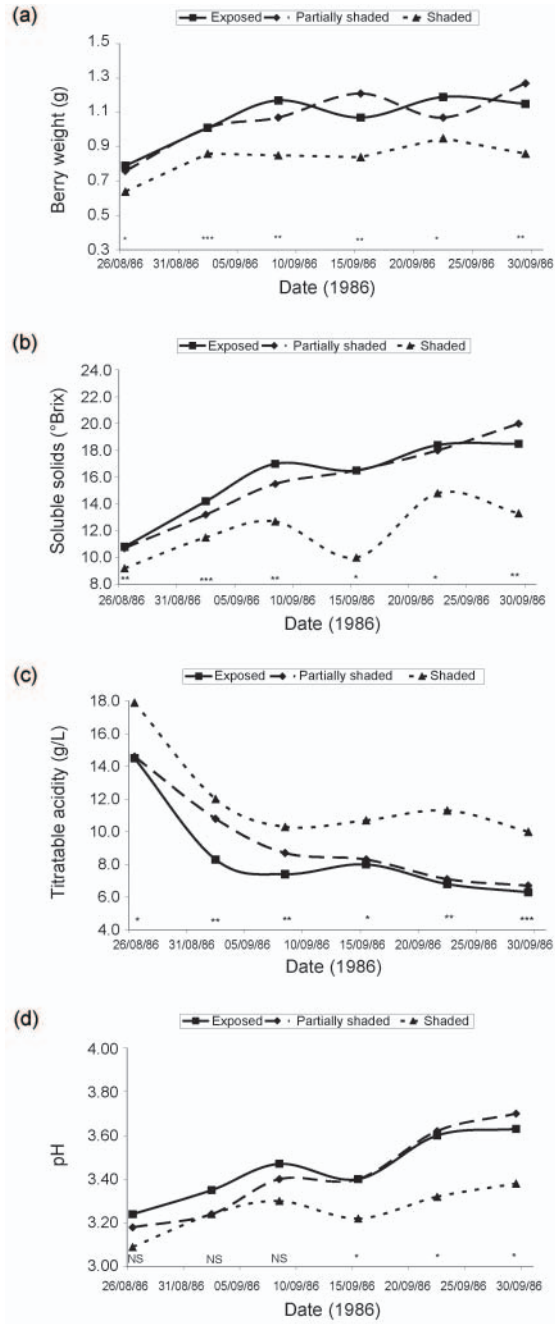

Fig. 11.2 Effects of cluster exposure on berry weight and composition of Gewürztraminer, Kaleden, British Columbia, 1986: (a), berry weight (g); (b), soluble solids (°Brix); (c), titratable acidity (g/L); (d), pH. *,**,***, ns: Significant at $p < 0.05$, 0.01, 0.001, or not significant, respectively (redrawn from Reynolds and Wardle, 1989b).

were higher on both a concentration and per berry basis; but by harvest, there were no differences. Morrison and Noble (1990) used natural shading (from parallel donor vines) in an attempt to separate the effects of cluster shading and leaf shading. Selective shading was achieved by removing leaves from the donor vines, thereby maintaining a constant leaf area to fruit weight ratio. Shading the leaves resulted in lower soluble solids, as well as increased TA, potassium and pH. Leaf shading also decreased both the rate of pre-*véraison* malate accumulation and the rate of post-*véraison* malate decline. Malate, potassium, and pH were higher in fruit from the leaf shading treatments at harvest. Shading clusters did not affect sugar, acid, or potassium accumulation, but shading of the berries resulted in reduced anthocyanin and phenol concentrations. Sensory evaluation of the aroma of crushed fresh grapes detected differences between the aroma of the control and each of the shade treatments. Similarly, wines made from fruit of the control treatment were different in aroma and flavour from wines made from the three shading treatments. In Gewürztraminer in British Columbia, exposed clusters contained lower TA and pH, and substantially higher soluble solids (Fig. 11.2), and free and bound monoterpenes (Reynolds and Wardle, 1989b).

More recently, Bergqvist *et al.* (2001) assessed the effects of sunlight exposure on berry growth and composition of two red wine grape cultivars (Cabernet Sauvignon and Grenache) grown in the central San Joaquin Valley of California. Sunlight exposures ranged from mid-day photosynthetically active radiation (PAR) < 10 μmol/(m^2 s) (shaded) to > 600 μmol/(m^2 s) (fully exposed) from berry set to harvest. Experimental clusters were evenly distributed between the north (afternoon shaded) and south (afternoon exposed) sides of the canopy. Fruit response to sunlight varied based on cluster location within the canopy, and these results were at least partially due to large differences in berry temperature. At the same exposure level or PAR, mid-day berry temperature was generally 3–4 °C greater for clusters on the south side of the canopy compared to clusters on the north. Soluble solids initially increased with greater sunlight exposure, then declined when mid-day PAR exceeded 31–50 and 51–100 μmol/(m^2 s), respectively, for clusters on the north and south sides of the canopy. Juice TA generally declined as sunlight exposure increased, with Cabernet Sauvignon clusters on the north side of the canopy maintaining greater acidity at the same exposure level than clusters on the south. Juice pH declined as exposure increased on the north side of the canopy, while sunlight had little effect on pH for clusters on the south. These results suggested that the effects of light on fruit composition are heavily dependent upon the extent to which berry temperature is elevated because of increased sunlight exposure.

11.2.2 Fruit exposure effects on phenolic analytes

There is considerable evidence that anthocyanin production is positively correlated with light levels, both on the canopy in general (Kliewer, 1970, 1977; Keller and Hrazdina, 1998; Spayd *et al.*, 2002), and on the fruit specifically (Spayd *et al.*,

2002). Others results have been either more complicated or entirely contrary to these results, suggesting that there may be varietal differences in the synthesis of anthocyanins. Among those who have conducted studies using artificial shading, Kataoka *et al.* (1984) found that complete shading (in an aluminium foil bag) of Kyoho clusters during ripening resulted in inhibited phenylammonium lyase (PAL) activity and no anthocyanin accumulation in the berries. The same experiment found that complete shading of Super Hamburg berries had little or no effect upon PAL activity nor on anthocyanin synthesis. Likewise, Downey *et al.* (2004) found that shading clusters inside opaque boxes had no effect on anthocyanin accumulation in Shiraz berries in two of three years. Differences occurred between shade and exposed fruit in the relative accumulation of different anthocyanins; shaded fruit contained relatively more peonidin and cyanidin glucosides. Condensed tannins were not affected by shading, but flavonols were reduced by shading. Similarly, Yamakawa *et al.* (1983) found considerable anthocyanin accumulation in *Vitis* cell suspension in the absence of light, but light irradiation enhanced anthocyanin accumulation.

Cortell and Kennedy (2006) examined changes in flavonols, proanthocyanidins, and anthocyanins in Pinot noir in Oregon in shaded and exposed treatments. Light exclusion boxes were installed on pairs of clusters on the same shoot (shaded treatment), while a second set of clusters on an adjacent shoot were considered as the exposed treatment. Cluster shading resulted in a substantial decrease in flavonols and skin proanthocyanidins and minimal differences in anthocyanins. Shaded and exposed treatments were similar at *véraison* in terms of seed proanthocyanidins; however, by harvest, the shaded treatment had higher extension and terminal subunits compared to the exposed treatment. Shaded fruit was lower for all skin proanthocyanidins at both *véraison* and harvest. Shading caused an increase in the proportion of (–)-epicatechin and a decrease in (–)-epigallocatechin at harvest in berry skins. Seed proanthocyanidins in shaded fruit contained a lower proportion of (+)-catechin and a higher proportion of (–)-epicatechin-3-*O*-gallate and a lower proportion of (+)- catechin and (–)-epicatechin-3-*O*-gallate and a higher proportion of (–)- epicatechin. For anthocyanins, the shaded treatment had proportional reductions in delphinidin, cyanidin, petunidin, and malvidin and a large increase in peonidin glucosides. Model wine extractions from the two treatments paralleled differences in the fruit with a lower concentration of flavonols, anthocyanins, and proanthocyanidins in the shaded treatment, while the skin proanthocyanidin percent extraction was 17% higher in the exposed model extraction than the shaded treatment.

Among those assessing natural canopy sun and shade conditions, Crippen and Morrison (1986b) found differences between sun-exposed and shaded Cabernet Sauvignon fruit in terms of soluble phenol content per berry and in both anthocyanin concentration and total content per berry pre-harvest. There were no differences at harvest, however. Total soluble phenols increased until *véraison*, and then decreased from *véraison* to harvest. The percentage of polymerized phenols decreased during early berry growth, and then increased from *véraison* to harvest.

Temperature has also been found to affect anthocyanin production in grape

berries. In general, high temperatures (above 35 ºC) have been inhibitory to anthocyanin synthesis (Kliewer 1970, 1977; Kliewer and Torres 1972; Kataoka *et al.*, 1984; Spayd *et al.*, 2002). Kliewer and Torres (1972) also determined that diurnal flux in temperature also affect fruit coloration. Day–night temperature differences of greater than 10 ºC were generally found to be inhibitory to fruit coloration, beyond the detrimental effects of high temperature on coloration.

Bergqvist *et al.* (2001) assessed the effects of sunlight exposure and related temperature effects on phenolic analytes in addition to berry growth and composition of Cabernet Sauvignon and Grenache. Anthocyanins increased linearly as sunlight exposure on the north side of the canopy increased, but declined when cluster exposure on the south exceeded 100 μmol/(m^2 s). Total phenols generally followed a similar pattern. The results suggest that the effects of light on fruit composition, including phenolic analytes, are heavily dependent upon the extent to which berry temperature is elevated because of increased sunlight exposure. Authors concluded that prolonged exposure of clusters to direct sunlight should be avoided for maximum berry colour in the central San Joaquin Valley and other warm regions.

In Piemonte, Italy, Chorti *et al.* (2007) assessed five fruit exposure levels to Nebbiolo: vines exposed to normal light conditions throughout the season; fruit-zone shaded vines from fruit-set to *véraison*; fruit-zone shaded vines from fruit-set to harvest; fruit-zone shaded vines from *véraison* to harvest; and fruit-zone leaf-removed vines at fruit-set. Fruit-zone shading during the initial phase of berry development decreased berry size and consequently impacted concentration of many analytes. Fruit-zone shading delayed accumulation of soluble solids and, when applied from *véraison* to harvest, reduced total anthocyanin concentration, but it did not affect total flavonoid accumulation. Cluster-zone leaf-removal obviously increased cluster exposure and decreased total anthocyanin and total flavonoid accumulation.

The separation of light and temperature effects on phenolic analytes was achieved by Spayd *et al.* (2002). Anthocyanin and phenolic profiles of berry skins from Merlot in the Yakima Valley of Washington were examined in terms of the individual influences of sun exposure and temperature. To accomplish this, and therefore to separate light and temperature effects, west-exposed clusters were cooled to the temperature of shaded clusters and shaded clusters were heated to the temperature of west-exposed clusters. Berry temperature was increased as much as 13 °C above ambient and shaded cluster temperatures when clusters were exposed to sunlight, regardless of aspect. However, maximum fruit temperatures were higher for clusters on the west side of the canopy (often > 40 °C) because ambient temperatures were higher after 1200h. East-exposed clusters had higher total skin monomeric anthocyanins (TSMA) concentrations than west-exposed or shaded clusters. Exposure to sunlight increased TSMA concentrations regardless of temperature. Cooling sun-exposed clusters increased TSMA concentrations, while heating shaded clusters decreased TSMA in a warm season, but had no effect during a cooler ripening season. Ultraviolet (UV) light barriers did not influence either cluster temperature or TSMA concentrations, and decreased TSMA concen-

trations in berry skins from west-exposed clusters were due to temperature and not to UV radiation. Exposure to solar radiation increased concentrations of the 3-glycosides of quercetin, kaempferol, and myricetin. Sun-exposed clusters, regardless of aspect, had almost 10 times greater concentrations of total flavonols than shaded clusters. UV-light barriers reduced individual and total flavonol concentrations, suggesting that their synthesis might be partly light driven; temperature had little to no effect on their concentrations.

As Joscelyne *et al.* (2007) suggest, few of the aforementioned studies have investigated the impact of vine shading on the sensory attributes of the resultant wine. This study examined the effects of canopy exposure levels on phenolic composition plus aroma, flavour, and mouthfeel aspects in Cabernet Sauvignon and Shiraz wines, whose source vines were subjected to different levels of canopy exposure in the Sunraysia region, Victoria, Australia. Canopy exposure treatments included a control (standard vineyard practice), exposed (achieved with a foliage wire 600 mm above the top cordon), highly exposed (using a foliage wire with leaf plucking in the fruit zone), and shaded treatment (using 70% shade cloth). Spectral and descriptive analyses showed that anthocyanins, other phenolics, and perceived astringency were lower in wines made from shaded fruit; however, the reverse was generally not observed in wines of exposed and highly exposed fruit. Descriptive analysis also showed wines from the shaded fruit were different from other treatments for a number of flavour and aroma characters.

Another aspect given little attention in the literature is that of spatial variability in fruit exposure resulting from different vine vigour levels, and the relationship between vigour and phenolic analytes. Cortell *et al.* (2007) examined fruit exposure effects on Pinot noir anthocyanins from a standpoint of vigour-induced fruit exposure effects. High vigour zones in two vineyards had lower soluble solids and higher TA, and there was a trend for lower anthocyanin concentration in the high vigour zones. In one year, there was a higher proportion of malvidin-3-*O*-glucoside and lower proportions of the other four anthocyanins (delphinidin-, cyanidin-, petunidin-, and peonidin-3-*O*-glucosides) commonly found in Pinot noir. In both years studied, one site had proportionally higher peonidin-3-*O*-glucoside and lower malvidin-3-*O*-glucoside than the other site. Authors opined that some of these differences might have been related to the higher exposure and temperatures found in site B compared to site A, which were found also in the low vigour zones.

It is reasonable to assume that significant enzymes responsible for anthocyanin and phenol synthesis are up-regulated by enhanced light and temperature environments. Downey *et al.* (2004) focused exclusively on light effects on the synthesis of phenolic analytes and the genes encoding those enzymes responsible. Opaque boxes were applied to clusters of Shiraz grapes prior to flowering to determine the effect of sunlight on berry development and accumulation of flavonoids. The boxes were designed in particular to exclude light and to minimize changes in temperature and humidity. There was no effect of shading on sugar accumulation and berry weight. Chlorophyll concentration was lower in the shaded fruit, which appeared pale yellow until *véraison*. The fruit coloured normally in the shaded

clusters, and in two of three seasons there was no change in anthocyanin concentration. Anthocyanin composition was altered in the shaded fruit, which had a greater proportion of the anthocyanins cyanidin and peonidin glucosides. Shading had no effect on the concentrations of condensed tannins in the skin or seeds of ripe fruit, but it reduced flavonols in the skin. In the exposed fruit, flavonol concentration was highest around flowering then declined as the berries grew, but there was an increase in flavonols per berry during ripening. When the boxes were applied before flowering, shaded fruit had much lower concentrations of flavonols throughout berry development, and at harvest the flavonols were < 10% that in exposed fruit. The authors mentioned two key genes that appeared to be up-regulated during berry development. First, expression of the gene encoding UDP-glucose flavonoid-3-*O*-glucosyl transferase, a key gene in anthocyanin synthesis, increased after *véraison* and was similar in both shaded and exposed fruit. Second, a gene encoding flavonol synthase was expressed at flowering and during ripening in exposed grapes, but its expression was reduced in shaded fruit. The authors suggested that shading had little effect on berry development and ripening, including accumulation of anthocyanins and tannins but, as Spayd *et al.* (2002) indicated, shade substantially decreased flavonol synthesis.

11.3 Effects of viticultural practices on fruit composition and wine quality

11.3.1 General effects on fruit composition and wine quality

A second important pillar of the Cool Climate Paradigm is to keep leaves well-exposed to sunlight. This may be accomplished in many ways, but ultimately the vigour of the vine must be accommodated such that canopy shade is avoided. Carbonneau *et al.* (1978) and Smart *et al.* (1985a,b) were among the first to take a viticultural experiment 'from the field to the glass' to conclusively demonstrate the positive impact of canopy management for minimization of canopy shading, in this case, use of divided canopies. Smart *et al.* (1985a,b) varied the degree of shade in Shiraz grapevine canopies by four treatments [control; shade; severe hedging (slash); Geneva Double Curtain (GDC)] to create a naturally occurring vigour gradient, hence providing different canopy microclimates. Constraining foliage into a smaller volume ('shade') increased shading over control vines, and GDC training and slashing reduced it. A shaded canopy microclimate was associated with increased potassium concentration in the leaves, petioles, and stems at *véraison*, as well as reduced soluble solids and higher malic acid and potassium concentrations in fruit. Wines from these musts had higher pH and potassium and reduced proportions of ionized anthocyanins. Correlation studies between fruit exposure and various fruit composition variables confirmed these results. An eight-character visual scorecard of grapevine canopies was evaluated and used to describe the canopies. A conceptual model was proposed to explain how soil and climatic factors and cultural practices could affect canopy microclimate. High vine vigour had similar effects on must and wine composition as shading treatments.

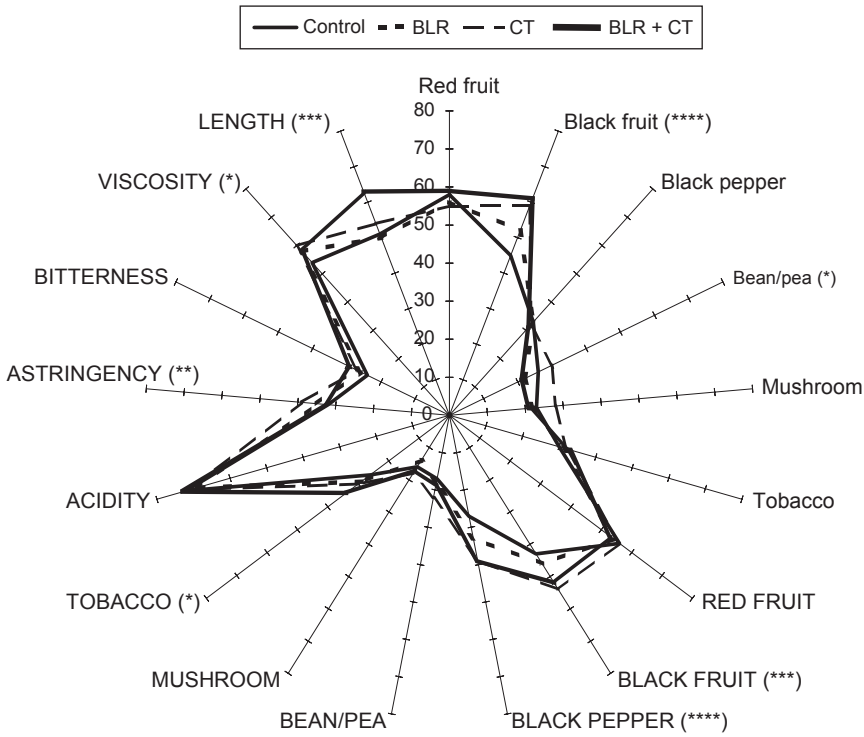

Fig. 11.3 Sensory attributes of Cabernet Franc wines, 2004, in response to four canopy management treatments, Pillitteri Estate Winery, Niagara-on-the-Lake, ON. *,**,***,****, ns: significant at $p < 0.05$, 0.01, 0.001, 0.0001, or not significant, respectively. BLR = basal leaf removal; CT = cluster thinned.

Canopy management

Practices such as hedging the tops and sides of vigorous canopies and removing leaves in the fruit zone have likely been traditional techniques in Europe for centuries. They have only been addressed through research since the mid-1980s. Those who focused on growing season canopy management include studies by Wolf *et al.* (1986), who showed that basal leaf removal on Chardonnay vines in New York increased soluble solids and lowered TA. Depending on the severity of hedging, there may be little or no impact on fruit composition (Reynolds, 1988a,c, 1989a; Reynolds *et al.*, 1996a), but either severe and/or late season hedging may have negative consequences in terms of delayed fruit maturity (Reynolds and Wardle, 1989a,c). Smith *et al.* (1988) reduced TA using fruit zone leaf removal, and found substantial increases in several free and glycosylated aroma compounds in Sauvignon blanc berries from leaf-removed treatments. Reynolds and Wardle (1989a) and Reynolds *et al.* (1996a) found that leaf removal reduced soluble solids in some situations; however, they also found reduced TA and increased levels of free and bound monoterpenes in Gewürztraminer (1996a) and Riesling (1996b), in several early-maturing muscat cultivars (1995a) and, most recently, Chardonnay

musqué (2007a). Since many of these studies focused on aroma compounds, they will be addressed in detail later in this chapter. Wine sensory quality has been favourably affected in many situations using canopy management treatments such as these (Reynolds *et al.*, unpubl.; Fig. 11.3).

Crop control

A third essential pillar of the Cool Climate Paradigm is that the vine must be balanced with respect to vine vigour and crop size. The terms *yield*, *crop level*, and *crop load* are often used interchangeably in both the popular and scientific literature. Their precise definition is required prior to discussing effects of crop reduction and balance. For purposes of this chapter, the following definitions will be adhered to: crop size [yield per vine or per unit of land area (acres or hectares)]; crop level [the number of clusters retained per shoot, per unit of cane pruning weight or per unit of canopy length (feet or meters)]; crop load or Ravaz Index [the ratio of crop size:vine size, assuming both are in the same units (pounds or kilograms)]. A ratio in the range of of 10 to 12 seems to be the presently accepted maximum crop load to maintain optimum wine quality. Crop load is one of many factors that determine the ability of a vine to mature its fruit. Growers routinely manipulate crop load in order to optimize both yield and fruit maturity. It has long been perceived that high crop loads can lead to inferior quality of grapes and wine.

It should also be said that although *vine size* (weight of cane prunings) is a common estimate of vine vigour, it is, more accurately, an estimate of carbohydrate reserves, referred to by Winkler *et al.* (1974) as *capacity*. Vigour, on the other hand, is frequently used as a synonym for vine size, but it is in actuality a measure of rate of growth rather than total growth, *per se.*

An attempt to define vine balance was first made by Ravaz (1930) using the F/V formula, whereby F = weight of fruit per shoot and V = the weight of the vegetative portion of the shoot. Using the cultivar Aramon, he demonstrated that an $F/V \leq 4$ would lead to a balanced vine without compromise to fruit quality. Branas *et al.* (1946) provided refinement to this formula by including probable alcohol with weights of fruit and pruned canes:

$$Ev = Ra + Sb + Dc$$

where *l'expression végétative* (*Ev*) is equal to the sum of the weight of fruit (*récolte*, *Ra*), the weight of pruned canes (*sarments*, *Sb*), and the probable alcohol (*degré alcoolique*, *Dc*). According to Galet (1983), this formula appeared to be applicable only to Aramon and was eventually abandoned. However, Bravdo *et al.* (1984, 1985a) redefined the basic concept of Ravaz as crop load, i.e., the ratio of yield per vine to weight of cane prunings per vine. Over-cropping effects in Carignane, based upon diminished wine quality, began to appear when crop loads exceeded 10 to 12 (1984). Their related work with Cabernet Sauvignon (Bravdo *et al.*, 1985a) suggested that reducing crop loads < 10 had no impact with respect to improving wine quality.

Traditionally, pruning has been the main vehicle for maintaining vine balance. In the early 1930s, Winkler in California (1934) helped defined how pruning and

cropping influenced yield and growth of the grapevine. The major conclusions were: (i) increasing crop size per vine decreases capacity (vine vigour + crop potential); (ii) dormant pruning increases shoot vigour by concentrating growth into fewer shoots; (iii) increasing shoot vigour reduces fruitfulness, hence shoot number, crop size, and fruitfulness are highly correlated. These points underscored the need to regulate both vigour and crop size by pruning to mature fruit consistently. Moreover, it was shown once again that a balance between vegetative growth and reproductive growth was essential to avoid over-cropping. Over-cropping will not only reduce shoot vigour (hence vine capacity) but will also ultimately reduce yield and wine quality.

The balanced pruning concept that was primarily used for *V. labruscana* cultivars embraces one of Winkler's important points, that being that a large vine can carry more buds than a small one. Although *V. labruscana* are not traditionally considered as wine grapes, this seminal work nonetheless has great relevance to our discussion of wine quality. Partridge in Michigan first examined this balanced pruning concept with Concord vines (1925). He noticed that many vineyards contained a large range of weight of cane prunings ('vine size'; Winkler's 'vine capacity') from 0.1–6.9 pounds (0.05–3.1 kg) per vine. By pruning the big vines lightly, and the small vines severely, the growth among vines became more consistent. Formulae were derived which prescribed pruning to 30 nodes if vine size was 0.5 kg; 40 nodes for 1 kg, 50 nodes for 1.5 kg, etc. It was also noticed that, as vine size increased, although node number was concomitantly increased, yield began to increase at a decreasing rate once vine size exceeded 1.5 kg (Partridge, 1925). Although not explicitly stated, the apparent reason for this effect was due to reduced fruitfulness of individual shoots on the most vigorous vines as a result of shade. Upshall and van Haarlem (1934) showed in a similar fashion that over-pruning very vigorous vines led to reductions in soluble solids and yield, and increases in juice TA. They concluded that excessive pruning encouraged vegetativeness and, consequently, recommended pruning according to vine vigour.

Shaulis and Oberle in New York (1948) revisited this concept, where they refined the original balanced pruning formula for Concord and Fredonia. Pruning severities were varied from 20 + 10 (20 nodes for the first 0.5 kg of cane prunings and 10 nodes for every 0.5 kg thereafter) to 40 + 10 for Concord and from 20 + 10 to 50 + 10 for Fredonia. They concluded that decreased pruning severity consistently increased yield for both cultivars. However, subsequent work (Shaulis and Robinson, 1953) suggested that Concord fruit composition was compromised if pruning severity decreased to 40 + 10; hence, the 30 + 10 level was recommended thereafter. These pruning formulae were modified to account for cluster size differences in Catawba (Tomkins and Shaulis, 1955) and Delaware (Shaulis and Jordan, 1960). It became clear that decreasing pruning severity did not provide linear increases in yield, due to substantial concomitant decreases in the percentage of node and canopy leaf exposure (i.e. shade). Moreover, shade rather than yield was implicated as the major limitation to fruit composition in high-yielding, highly vigorous vines (Kimball and Shaulis, 1958). These conclusions led to the develop-

ment of the Geneva Double Curtain training system (Shaulis *et al.*, 1966), and, consequently, showed that vine balance was attainable through means other than manipulation of pruning level.

French–American hybrid cultivars developed popularity during the 1950s in the Northeast USA and Canada. They presented a new problem to grapegrowers because their base buds were so fruitful. Moreover, cluster weight of many French–American hybrids (e.g. de Chaunac, Seyval blanc) exceeds 300 g, and this merely compounds the fruitful base bud situation. Balanced pruning is not an effective means of controlling crop and maintaining vine balance, since these fruitful base buds represent a major propensity to over-crop (Bradt, 1962, 1964; Fisher *et al.*, 1977). The initial consequence was that severe pruning of low-vigour vines often exacerbated the low-vigour problem due to the excessive crop load imposed by the fruitful base shoots. Another crop regulation strategy was therefore necessary to regulate crop and maintain wine quality. Flower cluster thinning was therefore introduced to compensate for the shortcomings associated with balanced pruning. Research in Ontario showed that yields of Chelois (Bradt, 1962), de Chaunac (Bradt, 1964; Fisher *et al.*, 1977), and Verdelet (Bradt, 1962) were increased by moderate balanced pruning and thinning compared to severe or balance pruning alone. Fisher *et al.* (1977) reported that thinning to one cluster per shoot consistently increased vine size, yield, and fruit-soluble solids over a very long period. These responses were subsequently confirmed by research with de Chaunac in British Columbia (Looney, 1981; Reynolds, 1989b).

A strategy was tested in New York whereby base shoots were removed from de Chaunac vines to reduce crop level. Unfortunately, this procedure removed more leaf area than it did excess crop, and consequently led to lower soluble solids than untreated and flower cluster thinned vines (Pool RM, pers. comm.). Subsequent work with Seyval blanc in New York (Reynolds *et al.*, 1986a) showed that a better approach for large-clustered cultivars with fruitful base buds was to maintain a reasonable shoot density (15–20 shoots/m row), and 'balance thin' at bloom. Hence, vines carried the same shoot number regardless of vigour, but low-vigour vines were more severely thinned. In this manner, all vines theoretically carried the same leaf area, but weaker vines were encouraged to grow by limiting the crop: leaf area ratio. By retaining a fixed shoot density and balanced thinning, bunch rot was minimized, and yield, vine size, winter hardiness, and wine grape quality were optimized. Among nine shoot density × thinning combinations, the best appeared to be 13–20 shoots/m row and 37 clusters/kg cane prunings. This assumed 'average' vines (*ca.* 1 kg cane prunings/vine) would be thinned to 1.5 clusters per shoot, less vigorous ones to one cluster per shoot, and very vigorous ones to two clusters per shoot.

This strategy was appropriate for de Chaunac as well, although better fruit composition (including increased anthocyanins) was achieved when thinning was combined with 15 + 10 balanced pruning, more so than when combined with 20 nodes/m row (Reynolds, 1989b). Balanced pruning alone was ineffective in the absence of cluster thinning.

Timing of cluster thinning has been examined in some jurisdictions. Flower

cluster thinning, which is popular among French–American hybrid cultivars, has the advantages of being fast, because the canopy is still only partly formed, and hence the clusters are easily seen. However, yield compensation is optimized by flower cluster thinning; by removing flower clusters, berry set is enhanced in the remaining clusters as a result of reducing competitiveness between sinks. Moreover, again due to reduced sink competition, berry weights are normally greater at harvest in flower cluster thinned vineyards than in non-thinned vineyards. This may be a disadvantage if an objective is to maximize skin:juice ratios for colour, tannin, and flavour extraction. Another disadvantage is increased cluster compaction, with concomitant increases in bunch rot. A third potential disadvantage is stimulation of vine vigour, which leads to more canopy shade and higher fruit TA (Reynolds *et al.*, 1986a, 1994a,c). Nonetheless, flower cluster thinning has been associated with increased yield, vine size, vine hardiness, fruit-soluble solids, flavour compounds, anthocyanins, and sometimes wine quality. In cultivars with cluster weights < 150 g, usually yield compensation is insufficient to overcome the decrease in cluster number, hence yields are often not increased and, in cultivars such as Chardonnay (Reynolds *et al.*, 2007b,c), Gewürztraminer (Reynolds and Wardle, 1989a), Pinot noir (Reynolds *et al.*, 1994c), and Riesling (Reynolds *et al.*, 1994a,b), yield can sometimes go down.

Post-set thinning is more laborious (hence more expensive), because the canopy is nearly formed. Yield compensation is reduced; berries have set, therefore no increases are expected for berries per cluster. Retained clusters are less compact than those on flower cluster thinned vines, and bunch rot infection is consequently lower (Reynolds *et al.*, 1986a). Increases in cluster weight and berry weight are nonetheless expected, since removal of competing clusters is done during the cell division stage. Yield, soluble solids, vine size, and vine hardiness also increase. Wine quality may not be improved by post-set thinning compared to flower cluster thinning, but anecdotal evidence seems to indicate that it is at least as effective. Note also, however, that the longer thinning is delayed, the more berry size is reduced (Weaver and Pool, 1973; Reynolds *et al.*, 1986a, 2007c).

Véraison thinning has minimal impact in terms of yield compensation; in fact, yields per vine are usually reduced. Although effective in reducing crop size, it is very expensive, and the expense may not justify the added returns, since costs are incurred on labour as well as lost yield. The benefits are increased wine quality, decreased yield, and no impact on vine size and winter hardiness. A study by Ough and Nagaoka (1984) showed that cluster thinning of Cabernet Sauvignon in the Napa Valley to one-third the original crop had minimal impact on fruit composition and wine quality. Roberts (1994) reported a substantial improvement in Pinot noir wine quality in response to two thinning treatments in the Carneros region of California. The most severe thinning treatment was equivalent to 6.3 tons/ha, which was considered uneconomical in California at that time, but about ideal in Burgundy. Reynolds *et al.* in Ontario (2007b) showed that *véraison* cluster thinning Chardonnay musqué (clone 77 ENTAV) advanced fruit maturity (25.3 °Brix; 7.1 g/L TA; 3.67 pH) compared to hedged controls (23.9 °Brix; 7.9 g/L TA; 3.59 pH) and leaf-pulled treatments (24.1 °Brix; 6.8 g/L TA; 3.62 pH).

Moreover, *véraison* thinning increased dried fruit aroma and flavour in the wines. However, Reynolds *et al.* (2007c) also examined several times of thinning of Chardonnay musqué, and found no substantial changes in either fruit composition or wine sensory properties.

If cluster thinning has an impact upon vine size, it may be argued that it is therefore affecting vine balance. However, thinning is frequently carried out at *véraison*, at which time vegetative growth should have ceased. Fruit composition and wine quality may therefore be impacted, but vine size remains unchanged.

Many studies have been conducted to assess the efficacy of crop reduction. Many attempted to separate effects of pruning and cluster thinning, much as Winkler had done in the 1920s. One example in California involved crop levels (i.e. clusters per vine) that were set by both removing grape clusters (normally-cropped), and by leaving more shoots (high-crop) (Weaver *et al.*, 1961). The adjustment of crop by leaving more shoots introduced shading effects that made it difficult to separate the effects due to crop from the effects due to shading. The over-cropped red wine grapevines in particular tended to produce grapes of lower soluble solids and higher TA when compared to normally cropped vines. Over-cropping, however, was not found to be always detrimental to colour.

Later work by Kliewer and Weaver (1971) was more effective in the separation of the effects due to pruning and the effects due to cluster thinning. This work investigated the effect of leaf area per unit weight of fruit on growth, composition, and coloration of Tokay grapes on vines in which the crop level was adjusted by pruning or by pruning and cluster thinning (Kliewer and Weaver, 1971). Crop weights per vine and leaf area per fruit weight were, respectively, negatively and positively correlated to soluble solids and degree of coloration. Significant curvilinear equations ($r^2 = 0.76–0.92$) were obtained for relationships between leaf area per unit weight of fruit and fruit maturity (soluble solids), berry weight, fruit coloration, and proline in berries. Regression analysis indicated that fruit maturity, berry weight, fruit coloration, and proline concentration in berries were maximum at 11–14 cm^2 leaf area/g fruit. Severe over-cropping occurred on non-pruned and non-thinned vines, and on vines pruned but not cluster thinned (3.8–5.0 cm^2 leaf area per g fruit, respectively). This led to substantial reductions in pruning weights, total leaf area per vine, berry and cluster weights, soluble solids, fruit coloration, and proline and arginine concentrations in berries, compared with those for vines that were pruned and thinned to 18 clusters per vine (12.6 cm^2 leaf area/g fruits). Overall, this study showed that cluster thinning could have a profound effect on both berry sugar and coloration. This study also demonstrated that crop levels need to be matched by photosynthetic capacity. A subsequent study in California (Kliewer *et al.*, 1983) addressed the influence of two crop levels (not thinned and cluster thinned to one per shoot), combined with two irrigation regimes (none and frequent), and two levels of potassium fertilization (0 and 2.2 kg potassium sulphate per vine per year) on growth and yield of Carignane vines. Irrigation increased yields by 25.6%, whereas cluster thinning reduced yields by 21.5%. Irrigation and crop thinning increased both berry weight and berry number per cluster compared to no irrigation and no crop thinning, respectively.

The leaf area/crop weight ratio idea was re-investigated by Kliewer and Dokoozlian (2005). A wide range of leaf area/crop weight ratios were tested by either pruning to different levels of nodes per vine, imposing different degrees of defoliation, and/or use of cluster thinning on four grape cultivars (Thompson Seedless, Tokay, Chenin blanc, and Cabernet Sauvignon). For single-canopy training systems, the leaf area/crop weight ratio required for maximum level of total soluble solids, berry weight, and berry coloration at harvest ranged from 0.8–1.2 m^2/kg, whereas for horizontally divided-canopy systems, this ratio was reduced to 0.5–0.8 m^2. Optimal crop yield/pruning weight, pruning weight per m canopy length, leaf area (m^2) per m canopy length, and leaf area density (m^2/m^3) for single-canopy systems ranged from 4.0–10, 0.5–1.0 kg/m, 2–5 m^2/m, and 3–7 m^2/m^3, respectively. Similarly, for divided-canopy systems, these ratios ranged from 5.0–10, 0.4–0.8 kg/m, 2–4 m^2/m, and 3–6 m^2/ m^3, respectively. Grapevines with ratios that fell within the ranges given above for each of these five variables were considered well balanced and capable of producing high-quality fruit and wines.

Interactions between crop level and rootstock were investigated by Nuzzo and Matthews (2006). This work was conducted to determine whether the timing of fruit maturity in a high-yielding, non-irrigated Cabernet Sauvignon vineyard could be manipulated through rootstocks (5C Teleki, 1103 Paulsen, 140 Ruggeri, and 110 Richter) and crop level. Four levels of crop were imposed by pruning all vines to four-bud spurs and cluster thinning at *véraison* to achieve 100, 75, 50, and 25% of full crop. The time required to reach 23.5, 24.0, and 25.0 °Brix was linearly dependent on crop level with a rate of about one day per each ton of grapes. Rootstocks and crop levels had no or little impact on fruitfulness, cluster and berry size, and final soluble solids. The reduction in sugar accumulation was a sensitive measure for crop level and was not influenced by rootstock or environmental conditions.

Efficacy of crop reduction has been assessed elsewhere. In Israel, three crop levels were induced by cluster thinning in a high-yielding Carignane vineyard (Bravdo *et al.*, 1984). Reduction of cluster number from about 60 to 40 per vine did not reduce yield, since berry size and berry number per cluster were increased. Pruning weight of the thinned treatment was increased and so was the capacity of the vines. It was noteworthy that further thinning to 20 clusters per vine reduced the yields since the increase in berry size and number was not sufficient to compensate for the reduced number of clusters. Subsequent work with Cabernet Sauvignon employed crop load values that varied between 4 in the severely thinned treatment and 10 in the non-thinned treatment (Bravdo *et al.*, 1985a). Wine quality was higher in the non-thinned as compared to the severely thinned treatment in 2 of 5 years. The pruning weight, as expected, was negatively correlated with crop level and crop load, and positively with the duration of harvest delay. Potassium concentration in leaves, must, and wine was negatively correlated with crop load, crop level, must malic acid and wine colour, and tartaric acid concentration. In a related trial in which crop level and irrigation treatments were combined, Bravdo *et al.* (1985b) found that vines with low crop loads extracted more water in a

continuous irrigation schedule, whereas the vines with high crop load extracted more water in schedules with irrigation cutback before harvest. The interaction between increased irrigation quantity and frequency with either extremely high or low crop load resulted in delayed sugar accumulation. Delayed ripening was associated with low wine quality only in the most intensive irrigation schedule, with the low crop load treatment tending to give the lowest wine quality. The reduced wine quality in the intensive irrigation treatment was expressed by low tasting scores and wine colour and by high pH. Wine potassium was negatively correlated with crop load in all irrigation schedules, although no clear relationship was found between potassium and wine quality.

In British Columbia, Riesling wine quality was improved by flower cluster thinning, along with vine size, soluble solids, and yield consistency (Reynolds, 1989a). It was not a function of yield per vine but instead one of balance; crop loads between 7 and 10 gave highest aroma intensity. Amongst multiple shoot density and cluster thinning levels, 26 shoots/m row was optimal in terms of combining profitable yields with highest wine quality, and cluster thinning was beneficial to manipulate fruit composition and wine quality (Reynolds *et al.*, 1994a,b). At 16 shoots/m, the shoots were more vigorous, leaves were larger, and there was more shaded fruit; yields were lower, but vines were over-vegetative, and cluster thinning did not affect wine quality. At 36 shoots/m, cluster thinning also made no difference; removing crop could not overcome the problems created by the dense canopies. These wine quality differences were supported by differences in the concentrations of monoterpene and C_6 aroma compounds in the wines. Flower cluster thinning of Gewürztraminer also increased concentration of free and bound monoterpene flavourants, but these increases were not linked to wine quality (Reynolds and Wardle, 1989a). Parallel co-ordinated studies in British Columbia and Oregon looked at flower cluster thinning of Pinot noir combined with different shoot densities and Scott Henry training (Reynolds *et al.*, 1994c). Cluster thinning increased cluster weight, berry weight, and berries per cluster, and fruit composition was improved, but yield decreased slightly. Wine quality also was increased by thinning, but only if shoot density and fruit environment were optimal; thinning had no impact on wine quality when shoot density was 20 shoots/m row (Reynolds *et al.*, 1996c).

Mathematical relationships have also been attempted to explain the effects of crop level on fruit composition. Sinton *et al.* (1978) correlated intensity of wine from Zinfandel grapes with crop level and several of the juice components. Soluble solids × pH was selected as the most practical indicator of aroma intensity (r = 0.80). Ratios of the acetate esters to corresponding alcohol also correlated with the aroma ratings. The soluble solids × pH also correlated well with the flavour intensity rating (r = 0.84), which in turn correlated with crop level. Many correlations were calculated between crop level and other variables. However, overall sensory scores could not be correlated with crop level, soluble solids × pH or any number of variables.

Effects of crop load adjustment on sensory attributes of wines have been difficult to assess. The role of yield in the sensory properties of Cabernet Sauvignon

in the Napa Valley in California was tested using pruning and cluster thinning to manipulate yield. Cabernet Sauvignon vines in the Napa Valley were subjected to six winter pruning treatments over two vintages and eight cluster thinning treatments over one vintage, with thinning imposed at *véraison* (Chapman *et al.*, 2004a). These treatments created yields that varied from 4.3 to 22.2 t/ha. Wines made from vines pruned to low bud numbers (hence 'low-yield') were higher in 'veggie' aroma and flavour, bell pepper aroma, bitterness, and astringency than 'high-yield' wines. Conversely, the wines made from vines pruned to high bud numbers were higher in red/black berry aroma, jam aroma, fresh fruit aroma, and fruity flavour than low-yield wines. Regression analysis showed that, in general, 'veggie' attributes decreased in intensity and fruity attributes increased in intensity as node number and yield increased. In contrast, there were few sensory differences detected in wines made from the various cluster thinning treatments, although the yield range was greater in that experiment than in the pruning experiment. The authors concluded that Cabernet Sauvignon aromas and flavours respond to yield manipulation, but do so significantly only when yield is altered early in fruit development. This is an interesting conclusion, since it suggests that cluster thinning at *véraison*, as is practised in most regions of the world, is of no value to wine quality.

The decision to reduce crop size is therefore dependent upon many criteria. The reasons may include:

1. To reduce a potential over-crop situation in a specific year, due to climatological conditions the previous year. For example, high fertility in buds that form in a given year due to high light conditions during and following bloom might lead to excessive crop size the following year; this necessitates thinning some cultivars to prevent over-cropping.
2. To maintain consistent yields and wine grape quality in cultivars with very fruitful base shoots, secondary buds, and tertiary buds.
3. To avoid over-cropping in large-clustered French–American hybrid cultivars such as Chancellor, de Chaunac, Seyval blanc, and Verdelet. Large-clustered *V. vinifera* cultivars such as Cabernet Sauvignon and Merlot also benefit from annual cluster thinning due to their propensity to overcrop.
4. To accelerate fruit maturity in a poor growing season.

Training systems and vine spacing

As previously discussed, research on training systems with respect to their impact on wine quality did not begin until the 1980s. Much of this was inspired by the seminal work of Shaulis *et al.* (1966) on Concord in New York. The most noteworthy of those first studies dedicated to training and wine grape quality include the work of Carbonneau *et al.* (1978), who described the beneficial impact of canopy division (the Lyre trellis) on fruit composition and wine quality of Cabernet Sauvignon in Bordeaux. Smart (1982) and Smart *et al.* (1985a,b) showed the benefits of divided-canopy training on fruit composition and wine quality of Shiraz in Australia.

Studies assessing training systems in North America alone include those on the French–American hybrid Seyval blanc (Reynolds *et al.*, 1985; Reynolds and Wardle, 1994; Reynolds *et al.*, 2004a), Chancellor (Reynolds *et al.*, 1995c, 2004a), Vignoles (Howell *et al.*, 1991), Vidal (Howell *et al.*, 1987), Chardonnay and Cabernet Franc (Vanden Heuvel *et al.*, 2004), and Riesling (Reynolds *et al.*, 1988d, 1996a, 2004b). Generally speaking, changes to training system within a standard vertical shoot positioned system produce minor differences in fruit composition (Reynolds, 1988d; Vanden Heuvel *et al.*, 2004). Increased trunk height in French–American hybrids can increase soluble solids and decrease TA because of enhanced fruit exposure (Reynolds *et al.*, 1985), but this is not always the case (Howell *et al.*, 1987, 1991).

Typically, use of canopy division has had substantial impacts on fruit composition of both French–American hybrids and *Vitis vinifera* wine grapes. Reynolds and Wardle (1994) and Reynolds *et al.* (2004a) showed that Seyval blanc vines subjected to five training treatments [Geneva Double Curtain (GDC); Hudson River Umbrella (HRU); 6-arm Kniffin; mid-wire cordon; Y-trellis] and three vine spacings (1.4, 1.8, and 2.4 m) produced substantial differences in fruit composition. Although yields of divided canopies (GDC and Y-trellis) averaged 42% higher than single-curtain systems, berry weights were lower. The GDC system produced fruit with lowest soluble solids, but also with lowest TA and pH and the lowest percentage of bunch rot. In Chancellor, the GDC system likewise produced fruit with lowest soluble solids, but also with lowest TA and pH and highest anthocyanins (Reynolds *et al.*, 1995b, 2004a). Wine quality from GDC treatments was generally equal to or higher than those from treatments with considerably less yields (Reynolds *et al.*, 2004a). In Riesling, five training treatments were tested [alternate double crossarm (ADC); Lenz Moser; low cordon; low-V (LV); pendelbogen] and three within-row vine spacings (1.4, 1.8, and 2.4 m), with and without basal leaf removal (Reynolds *et al.*, 1996b, 2004b). ADC and pendelbogen training optimized canopy density, and these two systems also had the least number of shaded leaves. Both ADC and LV systems tended to have the greatest number of exposed clusters. Yields of divided canopies (ADC and LV) averaged 55% higher than single-curtain systems, but ADC berry weights were lower. The ADC system produced fruit with lowest soluble solids, but also with lowest TA and pH, and highest free volatile terpene and potentially volatile terpene concentrations.

Few studies have been conducted on row orientation and its effects on fruit composition. Naylor *et al.* (2003) tested north–south (N–S) and east–west (E–W) row orientations with respect to light interception, cluster exposure, and fruit composition of Sauvignon blanc in Marlborough, New Zealand. Total light interception over the day was relatively similar between orientations but differed according to the time of day the measurements were taken. Berry composition was more variable for fruit from E–W rows. Juice aroma was correlated with soluble solids and thus with fruit exposure. Exposed fruit had more total phenols and flavanols than shade fruit irrespective of row orientation but in general was less variable for N–S rows.

Mechanical pruning

Efforts to reduce production costs have led to innovations in mechanization, including mechanical pruning. It has been widely adapted in *Vitis labruscana* vineyards in North America, as well as *V. vinifera* in Australia and Europe. Because the technology was originally developed for, and tested in, *V. labruscana* vineyards, it is highly relevant to our discussion to briefly describe it here.

Interest in mechanized pruning of grapevines evolved in the eastern USA during the 1960s and 1970s, especially in New York, where it was aimed at reducing costs of pruning Concord vines (Shaulis *et al.*, 1973; Pollock *et al.*, 1977). Initially, the technology was developed for use with non-divided canopies, but it was soon modified to accommodate divided canopies, such as the Geneva Double Curtain (GDC) (Shaulis *et al.*, 1973; Pollock *et al.*, 1977; Morris and Cawthon, 1980, 1981; Morris, 1985). Work with *V. vinifera* in Italy (Intrieri, 1979; Baldini, 1982; Cargnello, 1982; Cargnello and Lisa, 1982; Intrieri and Marangoni, 1982), Germany (Maul, 1986), France (Dumartin and Leppert, 1986), and Australia (Freeman and Cullis, 1981; Freeman, 1982; Hollick, 1982; Clingeleffer, 1984; Cirami *et al.*, 1985) has also indicated considerable promise. The original technology developed in New York permitted substantial yield increases in concert with concomitant reductions in production costs (Shaulis *et al.*, 1973; Pollock *et al.*, 1977). In all cases worldwide, mechanized pruning has resulted in a sustainable increase in yield (Morris and Cawthon, 1980, 1981; Freeman and Cullis, 1981; Cargnello and Lisa, 1982; Freeman, 1982; Hollick, 1982; Clingeleffer, 1984; Cirami *et al.*, 1985), along with significant reductions in labour costs. It is normally accompanied by reductions in shoot vigour (Clingeleffer, 1984), vine size (Morris and Cawthon, 1980, 1981; Freeman and Cullis, 1981; Freeman, 1982), cluster weight (Freeman and Cullis, 1981; Clingeleffer, 1984; Cirami *et al.*, 1985), berry set (Clingeleffer, 1984; Cirami *et al.*, 1985), clusters per shoot (Clingeleffer, 1984), berry weight (Freeman and Cullis, 1981; Clingeleffer, 1984; Cirami *et al.*, 1985), pH (Cirami *et al.*, 1985), and soluble solids (Morris and Cawthon, 1980; Freeman and Cullis, 1981; Intrieri and Marangoni, 1982; Cirami *et al.*, 1985).

Successful implementation of mechanized pruning is contingent upon not only the mechanical components but also the training system. Morris (1985) stated succinctly that cordon training is essential. Shaulis *et al.* (1973) and Pollock *et al.* (1977) recommended use of the HRU and GDC systems for vines of moderate and high vigour, respectively, in association with mechanized pruning. Retention of bearing units in the lower 180° hemisphere of the cordon was also recommended by these workers and by those working with *V. vinifera* (Baldini, 1982; Intrieri and Marangoni, 1982). Investigations with French–American hybrids have found both HRU and GDC systems appropriate (Morris *et al.*, 1984).

Researchers in Arkansas adapted the New York technology and thereafter improved upon many features. Although this work was done mostly with Concord, it too is relevant to this discussion because the responses of wine grapes were later found to be similar. In one of the initial studies, yield, vine size, and juice composition were measured on Concord grapes after 6 consecutive years of hand pruning to a 30 + 10 pruning schedule or mechanical pruning followed by no node

adjustment or node limitation by hand pruning to 60 or 90 per vine (Morris and Cawthon, 1981). Limiting nodes per vine to 60 following mechanical pruning maintained vine size, and produced fruit yield and juice composition comparable to balance pruned vines. Retaining 90 or more nodes per vine following mechanical pruning reduced per vine and per node fruit yields and resulted in unacceptable sensory quality, along with uneven ripening of the grapes, which contributed to a problem of low soluble solids and poor juice colour. Continuous use of mechanical pruning of Concord grapevines has proven to be feasible in relatively uniform vineyards that have been shoot positioned and followed by cane selection and node limitation. In fact, most Concord vineyards that are mechanically pruned are indeed subjected to some form of hand follow-up pruning. Michigan has also been an active participant in mechanical pruning research. In southwest Michigan, several strategies for pruning and adjusting the crop level of Concord vines were evaluated by Zabadal *et al.* (2002). Mechanical pruning resulted in yields that were comparable to commercial manual pruning at approximately 13 t/ha. Moreover, the range of yields and fruit-soluble solids for these treatments became greater as pruning severity decreased. Crop adjustment resulted in lower yields and higher fruit soluble solids than mechanical pruning alone. Moderate or minimal mechanical pruning, $\pm$ chemical crop adjustment, did not provide consistently comparable fruit quality to manual pruning. Severe mechanical pruning produced yields and fruit-soluble solids that were similar to commercial manual pruning. The favourable response of severe mechanical pruning was due to compensating factors of a 45% reduction in node fruitfulness and a 75% increase in the number of live nodes compared to commercial manual pruning. Severe mechanical pruning, combined with cane positioning, manual follow-up pruning, and shoot positioning, was considered an acceptable, sustainable practice for managing Concord vines.

Washington State has also been active in mechanization research in Concord. Effects of mechanical pruning and crop adjustment on Concord vine productivity were studied over a 5-year period in two mature vineyards in eastern Washington (Keller *et al.*, 2004). Large, mechanically pruned single-wire or double-curtain-trained vines produced heavy crops and attained satisfactory fruit composition in both warm and cool seasons. Consistent with previous work, these vines could sustain considerably higher yields than are achieved by balance pruning (> 9 t/ha more). Yields of vines with fixed-node pruning (130 nodes) or mechanical pruning with crop adjustment were similar and produced yields 4.6 t/ha greater than those of balance pruned vines. Crop adjustment following mechanical pruning reduced yields but did not improve yield variation and fruit composition and therefore was unnecessary in all but the heaviest cropping season. Balance pruned vines not only produced relatively low yields, but also produced only marginally improved fruit composition, although fruit could be harvested somewhat earlier.

Wine grape studies into mechanized pruning in North America have generally been conducted on winter-hardy hybrid cultivars. Fisher *et al.* (1996) tested mechanical pruning in Ontario for large-clustered cultivars such as De Chaunac. Pool *et al.* (1993) conducted similar work in New York with French–American and other hybrid wine grapes, and found that minimally pruned Chancellor vines

achieved yields in excess of 30 t/ha, compared to 22 t/ha for balance pruned vines. This response of Chancellor to mechanical pruning was confirmed by Reynolds and Wardle (2001) in British Columbia. However, both Fisher *et al.* (1996) and Pool *et al.* (1993) observed delayed fruit maturity as a consequence of the increased crop sizes, and supplemental crop control practices were shown to only partially overcome over-cropping effects. Small-clustered cultivars such as Baco noir showed no negative response to high node numbers (Fisher *et al.*, 1996). However, larger clustered cultivars such as Aurore (Pool *et al.*, 1993), Cayuga white (Pool *et al.*, 1993), De Chaunac (Pool *et al.*, 1993; Fisher *et al.*, 1996), Seyval blanc (Pool *et al.*, 1993; Fisher *et al.*, 1996), and S.V. 23-512 (Fisher *et al.*, 1996) required mechanical crop adjustment in order to achieve adequate soluble solids. In Michigan, Smithyman *et al.* (1997) evaluated three canopy configurations for Seyval blanc grapevines [severe pruning (ca 18 nodes per kg cane prunings); full trellis (45 nodes per vine); and hedge (hand pruned to a 10 cm radius around the cordon)]. The full trellis and hedged vines filled the trellis area earlier, producing a greater leaf area until *véraison*; by harvest, severely-pruned vines achieved a leaf area equal to the other treatments due to new leaf production from laterals. However, the hedged treatment produced canopies that required more time to cluster thin and contained poorly spaced shoots. However, there were no differences in fruit composition among treatments. The authors noted that lower yields and the cost for controlling bunch rot reduced the potential savings of mechanical hedging.

Among other studies with hybrid wine grapes in North America, Reynolds (1988b) in British Columbia subjected Okanagan Riesling vines to two pruning strategies (18 nodes/m row and simulated mechanical pruning; SMP) within each of three training systems [midwire bilateral cordon; Hudson River Umbrella (HRU); and Lenz Moser]. SMP vines tended to have fewer canopy contacts than their manually pruned counterparts. SMP reduced vine size, cluster weight, berries/cluster, and berry weight but led to substantial increases in shoots/vine and yield. Berry TA, soluble solids, and *Botrytis*-infected fruit were also reduced by SMP. Wine quality and aroma intensity were best in wines from manually pruned HRU-trained fruit. SMP tended to reduce wine quality compared to manual pruning.

Interest in mechanical pruning of Concord and hybrids soon spread to *Vitis rotundifolia*. Generally, muscadine grapes have responded similarly to Concord and hybrid grape cultivars. Dixie muscadine grapes (*V. rotundifolia*) in Florida were mechanically pruned and then either adjusted to 800 or 400 nodes per vine (using cuts made 15 to 20 cm from the cordon) or not hand pruned following trimming (Sims *et al.*, 1990). As expected, mechanical pruning with no follow-up hand pruning resulted in much higher yields in the early period of the trial. Increasing pruning severity increased berry weight, soluble solids, and pH. Wine produced from the mechanically pruned vines with no touch-up had a weaker muscadine aroma intensity and a lighter colour in the first year, but wines produced from this treatment in subsequent years were similar to wines from the other treatments, suggesting that mechanical pruning might be a viable practice. Anderson

et al. (1996) investigated the influence of SMP, hand pruning (HP) and SMP every year followed by HP every other year (SMP + HP) on yield and berry composition of muscadine cultivars Noble and Welder over a 6-year period. Pruning did not influence berry composition with the exception of soluble solids of Noble (highest for the hand pruned vines). Yield of Noble was influenced by pruning treatment during most years, and yields for SMP Noble vines decreased linearly with time. Both Noble and Welder SMP + HP vines displayed a strong tendency to bear heavy and light crops in alternate years, with low yields recorded in the years that hand pruning was employed.

Mechanized pruning become extremely widespread throughout much of Australia in the 1980s. Research on mechanized pruning commenced as early as 1971, and reductions in pruning costs during that era were considered to be 75% (Hollick, 1982). Somewhat more recently, the concept of minimal pruning (Clingeleffer 1984, 1988, 1992, 1993; McCarthy and Cirami, 1990) emerged as a rational alternative to both traditional manual pruning and mechanical hedging. Minimal pruning is based on the same premise of mechanical pruning – that pruning simultaneously reduces vine capacity (vigour + crop potential) and yield, while increasing vigour of individual shoots. Therefore, a minimally pruned vine may become self-regulating as a consequence of its yield. Clingeleffer (1984, 1988, 1992, 1993) and others (McCarthy and Cirami, 1990) showed that compared to traditional cane pruning, minimal pruning resulted in shorter shoots, but higher yields, and simultaneous improvements in fruit composition and wine quality as a result of improvements in fruit exposure.

As an example of some of the early Australian work, the effect of hedge pruning Cabernet Sauvignon and Doradillo grapevines was examined by Freeman and Cullis (1981). The vines were hedged to square, offset rectangular, or triangular shapes. The yield and the capacity of hedged vines were generally equal or greater than the manually pruned vines. Hedging increased yields mainly through increases in cluster number per vine. With Cabernet Sauvignon, a triangular hedge initially had lower yields but in later years yielded more than the square and offset hedges. The increase in yield was due to an increase in cluster size, resulting from increases in berry number per cluster, compared to the other hedge shapes. Similar results were obtained with similar trials with Gewürztraminer and Shiraz (Freeman, 1982). Authors indicated that machine pruning could increase the efficiency of pruning 40-fold so that one person could prune up to 400 ha of grapevines. Mechanical pruning of vineyards was rapidly expanding in Australia even 30 years ago; in 1979 about 1000 ha were pruned by machines, and this area is now estimated to be considerably more.

Initial European studies with *V. vinifera* have included numerous investigations by Baldini (1982), Cargnello (1982), Cargnello and Lisa (1982), Intrieri (1982), and Intrieri and Cargnello (1982). Intrieri and Cargnello (1982) described several mechanical pruning treatments on GDC-trained Montuni grapevines in Emilia-Romagna, Italy. All mechanized pruning treatments equalled the manually pruned control in terms of yields and soluble solids. Cargnello and Lisa (1982) described trials carried out since 1975 with espalier-trained Cabernet Franc, Trebbiano, and

Raboso Piave in Veneto, Italy and concluded that mechanical pruning was feasible. They found that mechanical pruning increased yields, as in most other studies, along with decreased cluster weights, but did not reduce soluble solids. They did, however, indicate the need eventually for some form of selective crop thinning. Cargnello (1982) tested mechanical pruning on several training systems (Pergola, Casarsa-Friuli, Sylvoz, and others) in northern Italy and discussed the feasibility of integrating mechanical pruning into a total mechanization of all viticultural operations.

More recently, the influence of SMP and hand pruning was assessed on yield, soluble solids, dry matter production, and total leaf area development of Grenache vines over an 11-year period (Martínez de Toda and Sancha, 1999). SMP was superior to hand pruning for yield, sugar production per vine, and dry matter production. Soluble solids were occasionally reduced by SMP. These effects of SMP could be explained by the larger total leaf area produced and the greater length of time during which the leaves were active. These results were considered especially important in the low-yield model of Mediterranean viticulture with a very limited total leaf area.

The hypothesis that mechanical hedging can be used successfully even on cultivars with low fruitfulness of basal buds was tested on the Croatina cultivar (Poni *et al.*, 2004). Yield per vine increased from 23–49% on hedged vines as compared to short-cane hand-pruned vines. Up to a 30% yield increase, there was no impact on fruit quality, and labour demand was cut by 55–60%. Yield compensation in the hedged vines mostly occurred as reduced budburst; few to no compensation effects were observed for cluster weight and bud fruitfulness. Reduced fruit quality was seen only at the highest node number per vine (75). The authors suggested that mechanical pruning could be used for traditionally long-cane pruned cultivars with related advantages of more balanced growth and ripening and adaptability to full mechanization.

Irrigation, fertilization, floor management

Many experiments have assessed the impacts of irrigation on fruit composition and wine sensory properties. The majority of these have been conducted in arid regions in which irrigation is considered necessary, but a few have also been done in humid regions that normally receive sufficient precipitation but nonetheless frequently experience droughts.

Among those studies conducted in arid climates, trials in Israel by Hepner *et al.* (1985) tested four drip irrigation schedules in a Cabernet Sauvignon vineyard. Seasonal water application in the range of 220–320 mm did not have a considerable effect on wine quality, whereas 400 mm caused a significant quality reduction. Irrigation cut back during the last six weeks before harvest advanced ripening, but this was not necessarily always connected with improved wine quality. Irrigation had a greater effect on growth than on crop level and therefore altered the crop load. Negative relationships were found between wine quality and pruning weight, berry weight, leaf, must and wine potassium concentration, TA, malic acid concentration, and wine pH. Related studies (Hepner and Bravdo, 1985) focused

upon effects of drip irrigation schedules and crop level treatments on potassium status of grape leaves, musts, and wines of both Cabernet Sauvignon and Carignane, and the relationships between potassium, must acids, wine pH, acidity, colour, and wine quality. Leaf, must, and wine potassium were well correlated to each other. Potassium concentration increased with decreasing crop loads, and with increasing amount and frequency of irrigation in Cabernet Sauvignon, the effect being at least partially due to the crop load reduction by irrigation. A positive correlation existed between potassium and must malic acid. The relationships between potassium, acidity, pH, and colour were differently affected by crop load and irrigation, by the absolute potassium level, and perhaps by the cultivars. Other related work (Bravdo *et al.*, 1985b) examined four drip irrigation schedules factorialized with three cluster thinning treatments in Cabernet Sauvignon. Water uptake from soil reserves was increased by restricted irrigation, intensive vegetative growth, and low crop load. Restricted irrigation also increased water uptake from soil reserves located at the periphery of the wetted soil zone. Total water uptake was the highest in the most intensive irrigation schedule, and the total water uptake from the soil decreased as a function of depth and distance from the emitters in all treatments. Vines with low crop load extracted more water in the continuous irrigation schedule, whereas the vines with high crop load extracted more water in schedules with irrigation cutback before harvest. The interaction between increased irrigation quantity and frequency with either extremely high or low crop load resulted in delayed sugar accumulation. Delayed ripening was associated with low wine quality only in the most intensive irrigation schedule, with the low crop load treatment tending to give the lowest wine quality. The reduced wine quality in the intensive irrigation treatment was expressed by low tasting scores and wine colour and by high pH. Wine potassium was negatively correlated with crop load in all irrigation schedules though no clear relation of potassium to quality was apparent.

In Australia, Freeman *et al.* (1981) examined the effect of trickle irrigation (three times weekly replacing 0.6 × evapotranspiration) and no irrigation on leaf water potential, leaf and bunch temperatures, berry composition, and wine quality of Shiraz in the hot, arid climate of Griffith, NSW. Four pruning levels (20, 40, 80, and 160 nodes) were imposed on the two irrigation treatments. Both irrigation and pruning to a high node number delayed sugar accumulation due to an increase in yield. In most years, severe pruning of irrigated vines delayed ripening compared to non-irrigated vines although the yield levels were similar. The delay in ripening in irrigated vines was not completely explained by the increase in yield. The non-irrigated vines reached critical stress levels earlier than the irrigated vines, but accumulated berry sugar more rapidly than the irrigated vines. The differences between the irrigated and non-irrigated vines in grape sugar accumulation, berry TA, and pH occurred early during berry development, possibly at the end of Stage II of berry development when sugar accumulation begins. The TA and pH of juice from berries from irrigated vines were higher than that from non-irrigated vines. Differences were found between wines from the irrigated and non-irrigated vines, but could not be quantified because of the subjective nature of the evaluation procedure used.

Much of California and Washington state vineyards were irrigated by overhead sprinklers and furrows until the 1980s. Consequently, it was not until the 1980s that focus was initiated on effects of drip irrigation on fruit composition and wine quality. In California, one of the first studies involved the response of the Saint Emilion cultivar to drip, flood, and sprinkler irrigation in the southern San Joaquin Valley (Peacock *et al.*, 1977). The principal benefit of drip irrigation was increased efficiency of water use – drip irrigation used less water while maintaining vine vigour, fruit production, and fruit composition similar to results with sprinkler or flood irrigation.

Freeman and Kliewer (1983), also in California, studied the influence of two irrigation regimes (none and frequent), two levels of potassium fertilization (0 and 2.2 kg potassium sulphate per vine per year), and two crop levels (not thinned and thinned to one cluster per shoot) on Carignane grape and wine quality. Irrigation, potassium fertilization, and crop level did not affect the rate of increase in berry weight after the data were transformed to remove the inherent variability of final berry size. Soluble solids in fruits was reduced by irrigation, but the pH and potassium concentrations were increased by irrigation. The rate of increase of berry juice potassium concentration with increasing soluble solids followed a sigmoidal pattern. Potassium accumulated rapidly until fruit reached about 10 °Brix, followed by relatively slow accumulation between 10 ° and 17 °Brix, and then there was a second rapid period of accumulation during the final stages of ripening. The concentration of potassium in non-irrigated fruits at maturities > 10 °Brix was less than in irrigated fruits. The increase in pH at soluble solids concentration > 17 °Brix was associated with increase in potassium concentration. Crop level and potassium fertilization had no effect on grape juice pH, TA, and potassium. Concentrations of arginine and proline in berry juice of non-irrigated vines were less than in irrigated vines on a given date. Irrigation reduced the concentration of anthocyanins in berry skin and wine compared to non-irrigated fruit. Water stress was more important in regulating wine pH than crop level or soil potassium level, suggesting that irrigation management may be a useful method to help reduce high pH problems and improve wine quality.

In the arid Columbia Valley of Washington state, crop loads were altered on Cabernet Sauvignon grapevines exposed to regulated deficit irrigation varying in severity and timing (Keller *et al.*, 2008). Following a dry-down period through fruit-set to stop shoot growth, vines were irrigated at 60–70% of full-vine evapotranspiration until harvest. Other vines either received the same amount of water up to *véraison*, after which the irrigation rate was cut in half, or had their irrigation halved before *véraison* but not thereafter. Clusters were thinned within irrigation treatments during the lag phase of berry growth to achieve a target yield of 6.7 t/ha, compared with a non-thinned control. The severity and timing of irrigation had only minor effects on vegetative growth, yield, fruit composition (soluble solids, TA, pH, potassium, colour), and cold hardiness. The more severe water-deficit treatments slowed berry growth while the treatments were being imposed, but final berry weights were similar. Very few interactive effects of

irrigation and crop load were observed, indicating that the crop load did not influence the response of vines to irrigation.

The southern Mediterranean often has conditions that justify use of irrigation. Salon *et al.* (2006) studied the effects of differential irrigation regimes on vine water status, yield, berry growth and composition, and wine quality on Bobal in Requeŋa (Valencia, Spain). Treatments consisted of a non-irrigated control and four irrigation treatments in which water was applied at different levels from flowering until near harvest. Irrigation increased yield in all seasons mainly because of an increase in berry weight. The concentrations of juice-soluble solids and TA were only slightly affected by the irrigation treatment, and a similar pattern was observed for the ethanol concentration of the wines. The concentration of malic acid in juice and wines increased with irrigation, while tartaric acid concentration in wines decreased. However, the concentration of anthocyanins, total phenols, and colour intensity of the wines decreased with increasing water application in an inverse pattern to that of berry size. Yield, berry weight, anthocyanins, total phenols, and colour intensity of red wines were closely correlated with the water stress integral (which expresses the intensity and duration of stress) calculated from stem water potential determinations.

Irrigation has also been found to have benefits in cool, humid regions that normally have adequate rainfall. In Ontario, five irrigation treatments (non-irrigated control; irrigation cut-offs imposed post-bloom, lag phase, and *véraison*; and full season irrigation) were evaluated in an Ontario Chardonnay vineyard over a 4-year period of which two seasons had lower-than-normal rainfall (Reynolds *et al.*, 2007a). Full season irrigation increased yield by 18–19% over the control due primarily to increased berry weight. However, soluble solids were also increased by irrigation, and full season irrigation showed similar or higher soluble solids than all other treatments in two of four years. Berry TA and pH also fell within acceptable levels for all five treatments. Wines made from irrigated grapes had greater intensities of apple, citrus, and floral aromas and flavours, and less earthy aroma and flavour.

Recently, some innovative new vineyard floor management techniques have been tested. Hostetler *et al.* (2007) investigated the effects of three vineyard floor management strategies–reflective (white) geotextile mulch, black geotextile mulch, and a herbicide strip in vine rows with respect to canopy light and temperature, vine growth, and fruit composition of Cabernet Franc in New York. Black and white geotextiles almost doubled vine size after 2 years. The white geotextile mulch increased sunlight reflected from the vineyard floor into the vine cluster zone, especially early in the growing season, and also increased yields. However, mulch treatments did not affect fruit ripening time or fruit chemical composition at harvest.

Reynolds *et al.* (2008) assessed five *Vitis vinifera* cultivars in Ontario in terms of vine age (> 20 years versus 5 years) and reflective mulch treatments with respect to berry, must, and wine composition as well as wine sensory attributes. Reflective mulch showed few effects on the berry, must, and wine composition of the red wine cultivars; however, mulch increased free and bound terpenes in the Riesling

berries. The red wines made from the mulched vines exhibited the least amount of vegetal aromas and flavours. Reflective mulch led to less perceived acidity in Riesling wines. Berries from young vines tended to have higher soluble solids and lower TA, pH, and total phenols than those from old vines, but these effects appeared to be transient. Wines made from young vines were higher in TA, and lower in pH, colour intensity, and anthocyanins than those from old vines, but again these effects were transient. Wines produced from young Cabernet Sauvignon and Cabernet Franc vines exhibited more intense vegetal aromas and flavours than those from old vines in one season.

11.3.2 Effects of viticultural practices on phenolic analytes

Growing season canopy manipulation

Many studies have also been done on the effects of canopy manipulation on red wine grape composition (Smith *et al.*, 1988; Reynolds *et al.*, 1995b, 1996a,b). Among research into growing season canopy manipulation, a New Zealand study unequivocally found that basal leaf removal (either 50% or 100% of leaves in fruit zone) increased both total phenol and anthocyanin concentrations in Cabernet Sauvignon, with the greatest increase (~50% over control) occurring when the treatment was done five weeks after flowering (Smith *et al.*, 1988). Mazza *et al.* (1999) also found that leaf removal resulted in higher phenolic concentration and colour density over the control. Conversely, Iacono *et al.* (1994) found that when 40% of basal leaves were removed from around the grape clusters there was a reduction in soluble solids, no change in malic or tartaric acid, and no change in anthocyanins, even though available radiation to the clusters was dramatically increased above the control. Shading of the entire canopy (with a 50% shade cloth), on the other hand, lowered the anthocyanin concentration across all canopy treatments (cluster thin and leaf removal). Leaf removal reduced the shading of clusters but also reduced photosynthetic capacity, resulting in lower sugar concentrations and no differences in anthocyanins or acidity. Among canopy manipulation resulting in negative consequences, late-season and/or severe hedging was shown to reduce anthocyanins in de Chaunac grapes in British Columbia (Reynolds and Wardle, 1989c).

Crop control

Many studies have also been done on the effects of crop reduction on red wine grape composition (Weaver *et al.*, 1961; Kliewer and Weaver, 1971; Ough and Nagaoka, 1984; Reynolds *et al.*, 1994c). A few studies (Iacono *et al.*, 1994; Reynolds *et al.*, 1994c; Mazza *et al.*, 1999) have also been done on the effects of both canopy and crop manipulation on red wine grape maturity in terms of soluble solids, TA, pH, and aroma and colour compounds. Studies with *V. vinifera* red wine cultivars in which crop was adjusted by means of cluster thinning alone have found that crop reduction results in increased soluble solids, anthocyanins, total phenols, and colour intensity (Reynolds *et al.*, 1994b; Mazza *et al.*, 1999; Guidoni

Table 11.1 Concentrations of anthocyanins and phenols in Merlot wines, Niagara-on-the-Lake, Ontario, 2004, in response to four canopy management and three enological treatments. All concentrations are expressed in mg/L

Compound	Viticultural treatment				
	Control	Cluster thin (CT)	Basal leaf removal (BLR)	CT+BLR	Significance
Phenols					
Gallic acid	6.97 b	**9.79 a**	7.22 b	6.46 b	****
Catechin	40.48 a	40.91 a	40.98 a	**32.28 b**	**
Epicatechin	31.35 b	**41.29 a**	30.83 b	28.61 b	****
Caffeic acid	8.64 b	**10.12 a**	8.44 b	**4.12 c**	***
p-Coumaric acid	1.58 bc	**2.31 a**	1.69 b	1.30 c	****
Quercetin	1.01 d	**1.92 c**	**2.51 b**	**4.01 a**	****
Anthocyanins					
Delphinidin-3-monoglucoside	3.73 c	**5.16 b**	4.32 c	**7.13 a**	****
Cyanidin-3-monoglucoside	0.35 b	**0.46 a**	0.32 b	0.45 a	****
Petunidin-3-monoglucoside	4.70 c	**7.05 b**	5.39 c	**9.37 a**	****
Peonidin-3-monoglucoside	3.50 b	**5.65 a**	3.72 b	**6.37 a**	****
Malvidin-3-monoglucoside	62.96 d	**88.18 b**	**71.26 c**	**108.22 a**	****
Total non-acylated anthocyanins	75.24	106.5	85.01	131.54	
Acylated anthocyanins					
Delphinidin-3-*O*-acetylglucoside	2.68 b	**3.46 a**	2.64 b	**3.74 a**	****
Cyanidin-3-*O*-acetylglucoside	0.95 b	**1.38 a**	0.99 b	**1.40 a**	****
Petunidin-3-*O*-acetylglucoside	2.47 b	**3.33 a**	2.56 b	**3.63 a**	****
Peonidin-3-*O*-acetylglucoside	3.36 c	**5.10 b**	3.30 c	**5.59 a**	****
Malvidin-3-*O*-acetylglucoside	22.15 b	**28.25 a**	23.43 b	**30.56 a**	***
Delphinidin-3-*O*-coumarylglucoside	0.53 b	0.63 b	0.55 b	**0.78 a**	*
Cyanidin-3-*O*-coumarylglucoside	0.40 b	0.48 b	0.46 b	**0.77 a**	***
Petunidin-3-*O*-coumarylglucoside	0.41 c	0.46 bc	**0.52 b**	**0.66 a**	****
Peonidin-3-*O*-coumarylglucoside	0.24b	**0.34 a**	0.31 ab	**0 c**	*
Malvidin-3-*O*-coumarylglucoside	8.23 c	**10.15 b**	9.38 bc	**14.38 a**	****
Total acylated anthocyanins	41.42	53.58	44.14	61.51	

*,**,***,****, ns: Significant at $p < 0.05$, 0.01, 0.001, 0.0001, or not significant, respectively. Means followed by different letters are significant at $p < 0.05$, Duncan's multiple range test. Means boldfaced are significantly greater than the control, $p < 0.05$, Dunnett's *t*-test; those boldfaced and underlined are significantly less than the control, $p < 0.05$, Dunnett's *t*-test.

et al., 2002). The effectiveness of crop reduction has not always been consistent. Some have found no differences in must composition when crops were thinned (Ough and Nagaoka, 1984), whereas others have found specific fruit composition attributes to be more affected than others. Crop reduction may also have beneficial effects on individual phenolic analytes as well (Reynolds *et al.*, unpubl.; Table 11.1).

One such study with Pinot noir conducted in British Columbia and Oregon involving three canopy management treatments [vertical shoot positioning with either 10 or 20 shoots/m row; Scott Henry (vertical canopy division) with 10-shoots/m canopy; 15-shoots/m row in Oregon only] in combination with two crop levels (full crop, half crop) found that by increasing the crop level, soluble solids

were reduced substantially but colour was reduced to a lesser degree than by increasing canopy density (Reynolds *et al.*, 1994c). In the wines, TA and pH were reduced and ethanol and anthocyanins were increased in Scott Henry wines. Ethanol and wine TA increased and pH decreased with increasing shoot density. Reducing crop level increased ethanol and anthocyanins. Clove, bell pepper, and grassy aromas were least in 10-shoots/m and Scott Henry treatments. Reducing crop level increased colour, currant aroma, astringency, and intensity of finish independent of canopy treatment.

Reynolds *et al.* (2005) explored delayed shoot thinning as an alternative to canopy division in vigorous canopies. Pinot noir and Cabernet Franc vines in Ontario were subjected to six different shoot-thinning timings between Eichhorn and Lorenz phenological stages 9 to 31. An additional treatment ('double pruning'; retaining two disposable canes) was imposed on Cabernet Franc. Many of these treatments resulted in improved canopy microclimate; e.g., treatments imposed after bloom plus the double-pruned treatment produced lower leaf layer numbers and better leaf and cluster exposure than did the control and early shoot thinning in Cabernet Franc, while early shoot-thinning treatments induced higher leaf areas in both cultivars compared to later treatments. Early shoot thinning on Pinot noir increased TA and soluble solids in berries and must. Early shoot-thinning treatments on Cabernet Franc increased colour intensity in berries, and colour intensity, total phenolics, and total anthocyanins in wines. The double-prune treatment was characterized by higher soluble solids, hue/tint, colour intensity, and total phenolics overall.

Training systems and vine spacing

As with growing season canopy management, choice of a training system may have a substantial impact on leaf and cluster microclimate and, consequently, fruit composition and wine quality. These effects on red wine grape cultivars extend to phenolic analytes. Smart (1982) and Smart *et al.* (1985b) described the effects of GDC training on reduction of canopy shading in Shiraz vines, and ultimately the enhancement of ionized anthocyanins and total phenols. Carbonneau and Huglin (1982) and Carbonneau *et al.* (1978) described the positive impacts of the various iterations of the Lyre divided canopies on berry skin anthocyanins and total polyphenols in Cabernet Sauvignon, along with corresponding enhancements in wine quality.

More recently, Reynolds *et al.* (1994c) tested Scott Henry training against vertically-shoot positioned canopies (10 and 20 shoots/m row) on Pinot noir vines in British Columbia. Scott Henry-trained vines had lowest weight of cane prunings and highest crop loads, and also had lowest soluble solids, pH, anthocyanins, and juice colour, but also had lowest berry and juice TA. The reduction in colour was unexpected, since Scott Henry treatments had the least dense canopies with the lowest percentage of shaded clusters. In a follow-up study, Reynolds *et al.* (1996c) found that wine TA and pH were reduced and anthocyanins were increased by Scott Henry training, in both British Columbia and Oregon. Means of five vintages from both wine regions indicated that clove, bell pepper, and grassy aromas were

least in Scott Henry canopy treatments. Cherry and anise aromas were also highest in Scott Henry wines, but many flavours, colour, and finish were not increased by vertical canopy division.

Peterlunger *et al.* (2002) examined the effect of four training systems for Pinot noir in the Friuli hills (northeastern Italy). Simple Guyot, double Guyot, horizontal spurred cordon, and vertical spurred cordon were assessed. As has often been the case with variations to non-divided systems, the training systems affected yield but showed little or no impact on grape and wine composition including grape and wine phenolics. Sensory analysis could not show relevant differences among training systems.

Among work with French–American hybrids, horizontally-divided canopies such as GDC and Y-trellises were tested by Reynolds *et al.* (1995c, 2004a) on Chancellor grapevines in British Columbia. Yields of divided canopies (GDC and Y) averaged 42% higher than non-divided systems, but cluster weights and berries per cluster tended to be lower in the divided canopies. Crop loads (ratio of yield:weight of cane prunings) of divided canopies exceeded the presently accepted level (10–12) beyond which wine quality could be compromised. The GDC system produced fruit with lowest soluble solids, but also with lowest TA and pH, and highest anthocyanin concentration. Increased vine spacing led to smaller vine size (per m row), lower cane weights, and occasionally reduced soluble solids. Chancellor GDC wines had highest berry flavour.

Irrigation

The positive effects of limited irrigation (i.e. regulated deficit irrigation) on phenolic analytes may invariably be a consequence of reduced vine vigour and improved fruit exposure. Generally improvements in fruit exposure occur in situations where mild water deficits have been imposed (Balint and Reynolds, unpubl.; Table 11.2).

The influence of irrigation on grape and wine composition was investigated for Agiorgitiko in the Nemea appellation area in southern Greece by Koundouras *et al.* (2006). Three non-irrigated plots were studied during vintages that were very hot and devoid of summer rainfall. Vines were subjected to different water regimens as a result of the variation of soil water-holding capacity and evaporative demand. Water deficit accelerated sugar accumulation and malic acid reduction in the juice. Early water deficits during the growth period had beneficial effects on the concentration of anthocyanins and total phenolics in berry skins. A similar pattern was observed for the phenolic concentration of wines as well. Limited water availability also seemed to increase glycoconjugates of the main aromatic components of grapes. Wines produced from grapes of stressed vineyards were also preferred in tasting trials.

Chaves *et al.* (2007) assessed three irrigation strategies [deficit irrigation; partial root drying (PRD); fully irrigated] on two grapevine cultivars (Moscatel and Castelao) in Portugal. They indicated that the amount of water could be decreased by 50% (as in the case of PRD) without compromise to fruit composition. Non-irrigated and PRD vines exhibited higher concentrations of berry skin

Table 11.2 Impact of irrigation treatments on berry composition of Baco noir grapes, Lambert Farms, Niagara-on-the-Lake, ON, 2005–07

Treatment	°Brix	Titratable acidity (g/L)	pH	A520 (AU)	Anthocyanins (mg/L)	Total phenols (mg/L)
			2005			
Control	22.6 cd	12.2 b	3.36	13.5b	1472.8 cd	1935.3 bc
100ET/fruit-set	22.5 d	12.4 b	3.42	12.8b	1597.3 abc	1808.0 c
50ET/fruit-set	23.0 bc	12.3 b	3.44	12.2b	**1750.8 a**	1933.0 bc
25ET/fruit-set	22.7 cd	**11.0 c**	3.55	12.7b	**1661.9 ab**	1926.0 bc
100ET/lag phase	23.0 bc	**14.1 a**	3.40	13.1b	**1644.7 ab**	2087.3 ab
50ET/ lag phase	**23.2 b**	12.2 b	3.37	13.1b	1501.4 bcd	2091.0 ab
25ET/ lag phase	**23.7 a**	12.4 b	3.54	13.4b	**1659.5 ab**	2003.3 abc
100ET/*véraison*	22.7 cd	12.4 b	3.36	13.6b	**1705.5 a**	1989.8 abc
50ET/ *véraison*	22.6 d	12.8 b	3.34	12.0b	1367.7 d	1910.7 bc
25ET/ *véraison*	23.1 bc	**14.3 a**	3.33	**15.4a**	**1760.9 a**	**2201.0 a**
Significance	****	****	ns	***	****	*
			2006			
Control	19.3 b	16.8	3.56 ab	15.1 bcd	1738.8 bc	2717 a
100ET/fruit-set	19.7 ab	22.6	3.52 bc	16.0 abc	1773.4 abc	**2374 bc**
50ET/fruit-set	19.6 ab	18.1	3.53 bc	15.4 bcd	1816.4 ab	**2301 c**
25ET/fruit-set	19.5 ab	17.2	3.52 bc	12.6 d	**1257.7 d**	**2206 c**
100ET/lag phase	19.7 ab	16.9	3.57 ab	15.3 bcd	1775.5 abc	2672 ab
50ET/ lag phase	19.7 ab	16.0	3.59 a	16.7 abc	1857.9 ab	**2373 bc**
25ET/ lag phase	**20.0 a**	17.2	**3.49 c**	18.0 ab	**2087.0 a**	2790 a
100ET/*véraison*	19.8 ab	16.0	3.53 bc	**18.4 a**	1988.0 ab	**2305 c**
50ET/ *véraison*	19.5 ab	16.0	3.56 ab	14.4 cd	1482.0 cd	2523 abc
25ET/ *véraison*	19.7 ab	17.1	3.56 ab	12.6 d	**1181.0 d**	**2287 c**
Significance	*	ns	***	***	****	***
			2007			
Control	23.5 a	12.8 b	3.51 bc	16.2 ab	2087 abc	1897 cde
100ET/fruit-set	**22.4 bc**	13.3 b	3.48 cd	13.9 ab	**1716 de**	1796 de
50ET/fruit-set	23.0 abc	12.1 b	3.53 ab	12.5 ab	**1567 e**	1676 e
25ET/fruit-set	23.2 ab	**18.1 a**	**3.56 a**	15.8 ab	1933 bc	2063 bcd
100ET/lag phase	23.6 a	12.3 b	3.50 bcd	21.0 a	2147 a	**2712 a**
50ET/ lag phase	**21.9 c**	12.5 b	3.49 bcd	20.3 ab	1965 a	**2746 a**
25ET/ lag phase	23.1 a	13.1 b	3.51 bc	17.7 ab	2211 bc	**2370 b**
100ET/*véraison*	23.7 a	12.3 b	**3.44 e**	14.4 ab	1956 abc	**2805 a**
50ET/ *véraison*	24.1 a	12.8 b	**3.46 de**	14.4 ab	2025 a	2173 bc
25ET/ *véraison*	24.0 a	12.0 b	**3.44 e**	14.7 ab	1863 cd	**2722 a**
Significance	***	*	****	*	****	****

*,**,***,****, ns: Significant at $p \leq 0.05$, 0.01, 0.001, 0.0001, or not significant, respectively. Letters represent means separated at $p \leq 0.05$, Duncan's multiple range test. Boldfaced data indicate those values significantly greater than the control, Dunnett's *t*-test; boldfaced and underlined data are significantly less than the control.

anthocyanins and total phenols than those in deficit irrigated and fully irrigated vines. These effects on potential quality were mediated by a reduction in vigour, leading to an increase in light interception in the cluster zone. Because plant water status on most dates during the season was not different between PRD and deficit irrigated (when different, PRD even exhibited a higher leaf water potential than

deficit irrigated vines). The authors concluded that the growth inhibition in PRD was not a result of diminished water status.

Anthocyanin biosynthesis is strongly up-regulated in ripening fruit of grapevines grown under drought conditions. Castellarin *et al.* (2007) investigated the effects of long-term water deficit on the expression of genes coding for flavonoid and anthocyanin biosynthetic enzymes and related transcription factors, genes sensitive to endogenous (sugars, abscisic acid) and environmental (light) stimuli connected to drought stress, and genes developmentally regulated in ripening berries. Total anthocyanin concentration increased at harvest in water-stressed fruits by 37–57% in two consecutive years. At least 84% of the total variation in anthocyanin concentration was explained by a linear relationship between the integral of mRNA accumulation of the specific anthocyanin biosynthetic gene UDP-glucose: flavonoid 3-*O*-glucosyltransferase and metabolite concentration during a time series from *véraison* through ripening. Chalcone synthase and flavanone-3-hydroxylase (F3H) genes of the flavonoid pathway showed high correlation as well. Genes coding for flavonoid 3',5'-hydroxylase and *O*-methyltransferase were also up-regulated in berries from dehydrated plants in which anthocyanin composition was enriched in more hydroxylated and more methoxylated derivatives such as malvidin and peonidin. The induction in water-stressed plants of structural and regulatory genes of the flavonoid pathway suggested that interrelationships between developmental and environmental signalling pathways were magnified by water deficits, which actively promoted fruit maturation and, in this context, anthocyanin biosynthesis. It is also worthy of note that Downey *et al.* (2004) likewise found an up-regulation of UDP-glucose flavonoid-3-*O*-glucosyl transferase in exposed fruit post-*véraison*, and an enhancement in the activity of a gene encoding flavonol synthase in exposed fruit.

11.4 Aroma compounds; usefulness of measuring aroma compounds in this context

This section will briefly review the major groups of compounds in grapes and wines that are considered odour-active. These include esters, monoterpenes, methoxypyrazines, norisoprenoids, and thiol compounds. Traditional measurements such as soluble solids, TA, and pH can provide useful guidelines for assessing grape maturity, but provide no guarantee of superior wine grape quality, nor can they satisfactorily predict quality with any assurance. Even phenolic analytes such as anthocyanins and various phenols can provide greater insights into the level of fruit maturity, but they also do not provide the full picture. The abundance of chemical and physical changes that occur in the grape berry at, and subsequent to, *véraison* (Coombe, 1992) provides us with a plethora of objective indicators of grape composition. Some of these measurements have included texture (Lee and Bourne, 1980), lipid composition (Barron and Santa Maria, 1990), anthocyanins (Gonzalez-San Jose *et al.*, 1990), amino acids (Miguel *et al.*, 1985) and aroma compounds such as monoterpenes (Reynolds and Wardle,

1989b) and methoxypyrazines (Allen *et al.*, 1989). It is the quantitation of flavour and aroma compounds that should, theoretically, be the most definitive.

The aroma and flavour of wine are one of the main characteristics that define the differences among the vast array of wines and wine styles produced throughout the world. It is no surprise that research over the past century has focused on this; however, only in the last 20 years has there been significant advances in the chemical and sensory measurement of grape and wine flavour.

11.4.1 Esters and other aliphatic hydrocarbons

Anthranilic acid esters – occurrence, significance, biogenesis

As early as the 1920s, there were researchers looking at volatile aroma compounds in grapes. In the first successful studies, Power and Chesnut (1921, 1923) analyzed the juices of seven grape cultivars using steam distillation, and concluded that the labrusca character was due to the presence of the aromatic amino acid ester methyl anthranilate (MA; methyl-2-aminobenzoic acid). Sale and Wilson (1926) were thereafter able to find a relationship between *Vitis labrusca* flavour, typical of 'foxiness', and MA. Their studies of the labrusca or 'foxy' flavour character, associated with the northern fox grape (*V. labrusca*) and its cultivars and hybrids (e.g. Concord) were in fact among the first attempts to characterize aroma character in any detail. Sale and Wilson (1926) confirmed this conclusion in their analyses of 41 grape cultivars, and found that volatile esters and acids were associated with the labrusca character.

The advent of gas chromatography (GC) and mass spectrometry (MS) during the l950s and l960s brought increased sophistication to the problems of flavour analysis, and the labrusca flavour character was one of the first to be investigated. Stevens *et al.* (1965) identified constituents of Concord essence, which included eight alcohols and six esters; MA was not detected. Neudoerffer *et al.* (1965) again examining Concord, identified six alcohols, aldehydes, five ketones, and ten esters, as well as acetoin, acrolein, diacetyl, and ethyl chloride. MA was not identified because it did not elute from the column. Stern *et al.* (1967) identified 60 components of the essence of Concord in which a series of butenoate esters, an alkylthioester, ethyl acetate, and MA were deemed of greatest importance.

However, as early as 1955, Holley *et al.* (1955) disputed the significance of MA in the overall determination of the Concord-type aroma character. Other adherents to this notion have been Nelson *et al.* (1977) and Nelson and Acree (1978), who found no perceivable increase in 'foxiness' when MA was added to neutral-flavoured wines, and claimed MA contributed little to a wine's overall foxy character. Fuleki (1982) on the other hand, included determinations of both MA and total volatile esters concentrations in a flavour index known as the Vineland Grape Flavour Index, which was used extensively in the Ontario grape breeding program for the early screening of grape selections based on flavour character. Later work attributed the flavour character to β-damascenone (a norisoprenoid) (Acree *et al.*, 1981) or o-aminoacetophenone (Acree *et al.*, 1990).

The significance of MA in the determination of the labrusca character remains somewhat controversial to this day. Most will agree, however, that the labrusca character is a composite of many constituents and cannot be attributed to one or two compounds.

Many studies have been carried out on the development of the volatile fraction in ripening *V. labruscana* grapes. Increases in MA concentration in Concord have been documented in New York (Robinson *et al.*, 1949), Washington (Clore *et al.*, 1965), and Ontario (Fuleki, 1972). Its biogenesis has been speculated upon for many years. Kluba and Mattick (1978) suggested that it might be a by-product of tryptophan breakdown during fruit maturation. Reynolds *et al.* (1982) suggested that MA might form through a three-stage pathway from tryptophan through kynurenine and n-formylkynurenine. Recently, Wang and De Luca (2005) confirmed that anthranilic acid is methylated and hence esterified via activity of a methyltransferase.

Muscadine aroma

The muscadine aroma associated with the southern fox grape (*V. rotundifolia*) and its cultivars (e.g. Scuppernong) has also been investigated to some degree. The organoleptic perception of this aroma is quite similar to that of the labrusca flavour character. Kepner and Webb (1956) carried out the first and perhaps only comprehensive work to-date on this aroma using classical chromatographic methods. Their results indicated the presence of six alcohols, 14 aldehydes, five esters, and diacetyl. No nitrogen- or sulphur-containing compounds were present (e.g. MA). Confirmation of their results was provided by Nelson and Acree (1978) and Fuleki (1972). The esters appear to be of the greatest significance organoleptically.

Aliphatic compounds in V. vinifera *cultivars*

Many *V. vinifera* cultivars do not possess the intense aroma character usually associated with muscats, for which monoterpenes are largely responsible. The remaining types fall into one of two arbitrary categories: aromatic and non-muscat, or non-aromatic (neutral). As stressed by Wagner *et al.* (1974), a continuous gradation in the sensory perception of the aromas of grapes exists, ranging from the strongest muscat to the most neutral cultivar; the arbitrary nature of this classification thus cannot be over-emphasized.

Early attempts to characterize the volatile composition of certain *V. vinifera* cultivars include those of Ordonneau (1891), Windisch (1906), Fabre and Bremond (1933), and Semichon and Flanzy (1933). Generally, the methods were quite primitive, and only a general idea of the profile of grapes and wines was obtained. Many of the studies were basically 'fishing trips' and did not target specific aroma compounds. The methods employed generally found only esters and alcohols and therefore did not identify key (or signature) compounds of certain cultivars. Perhaps the first study was that of Haagen-Smit *et al.* (1949), who used fractional crystallization, to identify ten compounds in the volatile fraction of Zinfandel.

Flavour chemistry began to change with the advent of GC–MS. Since its conception, literally hundreds of compounds have been identified (Rapp, 1988), of

which over 680 have been identified in white wine grape cultivars (Peinado *et al.*, 2004). Originally, studies were 'fishing trips' and most did not really find anything significant. For example, when Chaudhary *et al.* (1964, 1968) looked at volatile compounds in Sauvignon blanc using GC, they were unable to detect any methoxypyrazines or any other compounds unique to the cultivar; only esters, alcohols, and other aliphatic compounds were found. Later analyses of several cultivars utilizing GC–MS included those on Grenache (Stevens *et al.*, 1967, 1969), Aligoté (Kormakova and Rodopoulo, 1974), Cabernet Sauvignon (Kepner *et al.*, 1969; Webb *et al.*, 1969; Kormakova and Rodopoulo 1974; Bayonove *et al.*, 1975), Carignane (Sakato *et al.*, 1975), Pinot noir (Brander *et al.*, 1980), and Ruby Cabernet (Kepner *et al.*, 1969). None of the authors attempted to associate specific constituents with the perceived aromas and, for the most part, reported the presence mainly of aliphatic compounds.

Biogenesis of aliphatic compounds

Much of the key information relevant to the biosynthesis of aliphatic compounds in fruit and vegetable tissue was uncovered during the 1960s and 1970s. The most likely precursors for the biogenesis of the aliphatic alcohols, aldehydes, ketones, and esters are likely the lipids. These, too, are synthesized within the fruit tissue via the well-known multi-step condensation of acetyl- and malonyl-CoA to form free fatty acids. The glycerol backbone to which the fatty acids become esterified arises from the reduction of dihydroxyacetone phosphate (DHAP). The source of the DHAP and the carbon skeletons required for the entire process is the sucrose produced via carbon fixation in the leaves during photosynthesis; this appears to be the only substance involved in aroma biogenesis that is actually translocated to the berry. It can thus be seen that maximum production of volatiles in the fruit depends on maximum photosynthesis in the leaves and maximum respiration in the fruit tissue during its development.

Research into the biogenesis of this important group of compounds has been surprisingly scanty. Reviews such as that of Forss (1973) are based primarily on phenomena recorded in dairy products, and much of this information cannot be extrapolated with confidence to fruit tissue. Others have reported on aroma synthesis in avocado (Jansen and Wallace, 1965), banana (Tressl and Jennings, 1972; Tressl and Drawert, 1973), cucumber (Grosch and Schwarz, 1972), melon (Yabumoto *et al.*, 1977), pear (Heinz and Jennings, 1966), and tomato (Yu *et al.*, 1967, 1968). Some speculative evidence for grapes has been given by Drawert and Rapp (1966) and Drawert *et al.* (1973). It appears that the many fruit species investigated possess similar pathways, so some extrapolation of results to grapes would likely constitute a relatively conservative assumption.

Biogenesis of aliphatic alcohols, ketones, aldehydes, and carboxylic acids takes place in the grape berries from lipid metabolism. The short-chain acids, alcohols, aldehydes, and ketones form first, and then the alcohols and acids esterify. Condensation of acetyl and malonyl CoA form the lipid backbone and DHAP provides the glycerol backbone of the original lipid precursors. Fatty acids are broken down in the berries to carboxylic acids, aldehydes, and alcohols via β-

oxidation by the enzyme lipoxygenase. When linoleic acid breaks down, this produces 9 and 13-carbon hydroperoxides that undergo reduction by aldehyde lyase to form hexanal plus some 9 and 12-carbon aldehydes and acids (Tressl and Drawert, 1973). These undergo further reduction to produce alcohols. The alcohols can react with the pool of carboxylic acids to produce esters. When linolenic acid breaks down, the products are *trans*-2-hexenal, a 12-carbon acid, a 9-carbon aldehyde, and a 9-carbon acid. Shorter chain alcohols and esters are created by reduction of octanoic acid, which arises through serial α-oxidation (loss of CO_2 followed by aldehyde formation at the α site) of fatty acids. An acyl thiokinase catalyzes esterification to CoA and an acyl CoA transacylase catalyzes addition of the alcohol moiety.

Biogenesis of aliphatic alcohols

Straight- and branched-chain aliphatic alcohols are common in practically all fruit tissue. Forss (1973) proposed a possible mechanism for the formation of saturated aliphatic alcohols from free fatty acids whereby a secondary (internal) hydroperoxide forms, which subsequently splits via the action of a lipoxygenase to form an aldehyde and an alkyl group. The latter may then form a primary (terminal) hydroperoxide, which then becomes oxidized to form another aldehyde, or, ultimately, reduced to form an alcohol. Tressl and Drawert (1973) proposed a similar mechanism for the creation of relatively long-chain alcohols, as well as aldehydes and carboxylic acids, from linoleic and linolenic acids in banana. In this scheme, lipoxygenase forms either a 9- (case 1) or 13- (case 2) hydroperoxy acid from linolenic acid, either of which is split by aldehyde lyase to produce, in case 1, *trans*-2-*cis*- and 9-oxononanoic acid, or in case 2, *trans*-2-hexenal and 10-*trans*-12-oxododecanoic acid. These acids and aldehydes can subsequently be reduced to produce the corresponding alcohols. This work was substantiated by Drawert *et al.* (1973) and Eriksson (1975). Shorter-chain alcohols arise via the reduction of short-chain carboxylic acids such as octanoic acid (Tressl and Drawert, 1973). An acyl thiokinase creates octanoate–CoA, which then is reacted upon by acyl–CoA reductase to produce octanal; the aldehyde may be further reduced to octanol through the action of NAD–alcohol–oxydoreductase. The origin of octanoate is claimed to be α-oxidation of fatty acids. Branched-chain alcohols such as 2-methyl-l-propanol are presumably formed through a completely different pathway to those involved in the creation of straight-chain aliphatic alcohols. Tressl and Drawert (1973) found increases in concentrations of valine and serine that correlated closely with the production of flavour components during ripening. Labelling studies involving the feeding of C^{14}-labelled amino acids subsequently revealed an equal distribution of the label in both the alcohols and the carboxylic acids. Conversion of these compounds into esters was also shown.

Aldehydes and ketones

The biogenesis of aldehydes in fruit tissue has already been partially described in the preceding discussion of alcohol formation. Aldehydes are normally of greater consequence than alcohols in grapes insofar as their organoleptic significance is

concerned. As with alcohols, aldehydes may arise directly as breakdown products of hydroperoxides formed from fatty acids by lipoxygenase (Forss, 1973). Linoleic and linolenic acids appear to be the major precursors (Grosch and Schwarz, 1972). Biosynthesis of short-chain saturated aldehydes may also occur through more traditional α-oxidation to specific carboxylic acids, which are esterified to CoA by acyl thiokinase and reduced by acyl CoA reductase (Tressl and Drawert, 1973). Branched aldehydes may occur via degradation of valine, leucine, and isoleucine (Yu *et al.*, 1968; Forss, 1973; Tressl and Drawert, 1973). Another mechanism for aldehyde formation, proposed by Grosch and Schwarz (1972), involves a dioxygenase-like reaction, in which the fatty acid precursor is acted upon by dioxygenase to eventually produce the resultant aldehydes. In this fashion, hexanal and *cis*-3-nonenal form from linoleic acid, while acetaldehyde, propanal, *cis*-3-hexenal, and *cis*-3,6-nonadienal form from linolenic acid. Other interconversions take place from this point.

Ketones occur somewhat less frequently and in less variety than do the alcohols and aldehydes. Studies on their biogenesis are, therefore, appropriately scarce. Forss (1973) and Tressl and Drawert (1973) both mentioned the likelihood that methyl ketones arise via an aborted β-oxidation of fatty acids. The latter group observed C^{14} activity in 2-pentanone after feeding labelled C^{14} octanoate. Other possible mechanisms include the formation of a 2-hydroperoxide from an alkane precursor, which is subsequently reduced to a 2-alkanol, then oxidized to the corresponding methyl ketone (Forss, 1973).

Esters

A vast array of different esters is produced by grape berry tissue. They are at least partly responsible for the labrusca flavour (q.v.), as well as the characteristic aroma of *V. rotundifolia* (Kepner and Webb, 1956), Riesling (Van Wyck *et al.*, 1967), and many other cultivars. Their occurrence in fruit appears to be universal, having been identified also in large numbers in banana (Tressl and Drawert, 1973), black currant (Nursten and Williams, 1969), citrus (Coleman and Shaw, 1971; Moshonas and Shaw, 1972), peach (Sevanants and Jennings, 1966), pear (Jennings 1961; Heinz and Jennings, 1966; Jennings and Creveling, 1966), and tomato (Buttery *et al.*, 1971).

Typical ester formation in fruit tissue is obviously dependent upon the availability of carboxylic acid and alcohol precursors. Tressl and Drawert (1973) described a typical esterification reaction in banana whereby 1-octanol formed via α-oxidation of linolenic acid is esterified to acyl–CoA via acyl–CoA alcohol transacylase. A similar enzyme was envisioned to operate in the biogenesis of all aliphatic esters whose precursors arose in this fashion. Heinz and Jennings (1966) offered a comparable conjecture in their investigation of pear volatiles. Yabumoto *et al.* (1977) presented evidence of amino acid participation in the formation of branched-chain aliphatic volatile esters in muskmelon. Their scheme involved two major pathways: (i) aldehyde formation from the triphenyl phosphate derivative of the transaminated amino acid, followed by reduction to the corresponding alcohol and, finally, esterification via acetyl–CoA; (ii) oxidation of the triphenyl phos-

phate derivative in the presence of CoA, to form the branched-chain CoA ester, which is subsequently esterified to an alcohol with the loss of CoA.

11.4.2 Monoterpenes

Occurrence

In contrast to *V. labrusca* and *V. rotundifolia*, the many *V. vinifera* cultivars possess an almost continuous gradation of aroma characters from the very neutral (Grenache, Sylvaner) through pronounced aromatic types (Chardonnay, Riesling) to the very intense muscat character (Muscat of Alexandria, Gewürztraminer). The latter aroma character, owing to its great desirability, has been investigated very extensively both from the aspect of its flavour chemistry and its inheritance.

Webb and Kepner (1957) reported numerous compounds in Muscat of Alexandria but no monoterpenes, which are the compounds that give this cultivar its unique characteristics. Although they failed to detect terpenes, they did identify five alcohols, three aldehydes, and eight esters, as well as several unidentified ethyl esters and acetals. Early investigations in Europe (Austerweil, 1946; Cordonnier, 1955, 1956) noted that high concentrations of terpenes, particularly linalool, geraniol, limonene, and α-terpineol, were associated with muscat-flavoured cultivars. Eventually, other studies found many significant compounds. Bayonove and Cordonnier (1971a), for example, further supported their previous work that terpenes were responsible for the aromas in Muscat and aromatic cultivars by quantifying linalool and other terpenes in several of these cultivars. Volatile compounds could now be targeted specifically and therefore more sense could be made out of all the 'noise' of chromatograms.

As in the case of *V. labrusca*, GC–MS has sophisticated the study of the volatile components of the muscat aroma. Stevens *et al.* (1966) identified 19 hydrocarbons, 15 alcohols, 13 esters, four aldehydes, two ketones, and six miscellaneous compounds. Linalool, geraniol, and hexanol were found to be the major components. Rodopoulo *et al.* (1974) conducted an exhaustive study into the composition of the essential oils of Muscat Frontignan, Saperavi, and two other muscat-flavoured cultivars grown in Armenia and the Crimea. Esters and terpenes were the principal components of the essence.

Attempts to answer the obvious question of what compounds are responsible for the muscat character have been made by several researchers. As mentioned previously, early work utilizing classical methods alluded to the strong possibility that terpenes were major contributors to the aroma. GC–MS studies have provided further insights. Webb *et al.* (1966), in examining the low-boiling volatile fractions of eight muscat cultivars, found that linalool concentration varied greatly between the cultivars, from very high to trace amounts. Bayonove and Cordonnier (1970b, 1971a) obtained similar results, but still felt the terpene fraction to be the major contributor to the muscat aroma, despite its oftentimes-low concentration. These same authors (1971b), however, also found large volumes of linalool in non-muscat-flavoured selections, and so concluded that linalool was 'important but not

specific' insofar as its role as an aroma constituent in muscats was concerned. Terrier *et al.* (1972a,b) substantiated this observation by the identification of several terpenes in aromatic cultivars such as Riesling; no terpenes were detected in neutral-flavoured cultivars such as Grenache. Schreier *et al.* (1976) identified another monoterpene alcohol, hotrienol (3,7-dimethyl-l,5,7-octatrien-3-ol), which they believed to have significance in the muscat aroma of cultivars such as Müller-Thurgau. In a comprehensive study of the muscat character, Ribéreau-Gayon *et al.* (1975) finally collated much of the prior work on the subject into some plausible conclusions. The threshold values of many of the terpenes, especially linalool and geraniol, were found to be extremely low (100 and 132 μg/L, respectively), and so it was felt that the contribution of these terpenes to the aroma was very significant. They also cited the phenomenon of synergism between terpenes in musts and wines, such that a greater intensity of aroma was produced.

Studies on the volatile composition of other aromatic *V. vinifera* cultivars have led to some conclusions. Drawert and Rapp (1966) and van Wyck *et al.* (1967) both conducted exhaustive studies into the constituents of Riesling aroma. The latter group concluded that the characteristic Riesling aroma could be attributed to specific concentrations of linalool, 2-phenylethyl alcohol, ethyl acetate, and 2-methyl-1-butanol. Schreier *et al.* (1976) identified 81 previously unreported compounds in Riesling, as well as six other popular German cultivars, but they failed to specify from which cultivars the various constituents were isolated. Kormokova and Rodopoulo (1974), in their study of the essential oils of sparkling wine cultivars grown in the USSR, considered geraniol to be the major component of the aroma of Rkatsiteli, a popular Russian cultivar of distinct aromatic character. Sixty-nine other constituents were also identified.

Biogenesis of monoterpenes

Mevalonic acid is the well-known precursor of all terpenoid compounds in the mevalonate pathway. Once formed, mevalonic acid may undergo two successive phosphorylations to form mevalonate-5-pyrophosphate, which is oxidized to form isopentyl pyrophosphate (1PP). IPP may then undergo isomerization to form dimethylallyl pyrophosphate (DMAPP) such that an equilibrium between the two is set up. Plants appear to maintain an active pool of DMAPP. Subsequent condensation of the two compounds with prenyltransferase yields the universal terpene precursor, geranyl pyrophosphate (GPP), which can be converted easily to most of the other major monoterpenes. These compounds can occur free, as pyrophosphates, or as glycosides; the latter were first shown in Muscat of Alexandria by Cordonnier and Bayonove (1974), who found that linalool glycosides were cleaved by β-glycosidases into the sugar and the much more organoleptically-active free terpene.

Monoterpene aroma compounds themselves are created in the berries. This is supported by the fact that when clusters of the intensely-flavoured Muscat Albardiens were grafted after fruit-set to neutral-flavoured Olivette Blanche, the characteristic muscat flavour still developed in the grafted cluster; the berries, immediately after fruit-set, acquire the enzymatic capacity inherent in the geno-

type for the conversion of translocated precursors to specific aroma compounds (Winkler *et al.*, 1974). Similar results were obtained in other more recent cluster-transfer trials involving Shiraz and Muscat of Alexandria (Gholami *et al.*, 1995).

11.4.3 Norisoprenoids

Norisoprenoids are C_{13} degradation products of carotenoids (β-carotene and lutein). The norisoprenoids accumulate during Stage III of berry growth much the same as the terpenes. Whereas the muscats and floral cultivars owe their character to terpenes and the Bordeaux types to pyrazines, the norisoprenoids are more ubiquitous and add nuances to many cultivars across a wide range of regions. They are usually found as glucosides and hence represent a pool of flavour reserves in developing grapes. Typical compounds include damascenone, vitispirane, trimethyl-dihydronaphthalene, actinidol, vomifolial, etc. Their aroma character varies from leafy, minty, and fruity through various floral characters. Many of our common wine grapes such as Chardonnay owe much of their character to groups of norisoprenoids. The typical violet aroma of Syrah is due to specific norisoprenoid compounds.

The carotenoid pigments all have squalene as an intermediate, and hence these and the terpenes share a common pathway up as far as geranyl pyrophosphate. The carotenoids form by the condensation of two GPP molecules to form geranyl-geranyl pyrophosphate, which then goes through a few steps (prephytoene→lycopersene→phytoene; followed by cyclization→β-carotene).

11.4.4 Methoxypyrazines

Methoxypyrazines are a group of heterocyclic aromatic organic compounds that are naturally present in green plant tissue, including grape berries that are associated with green, vegetal, or herbaceous characteristics. Important methoxypyrazines found in grapes include 3-isobutyl-2-methoxypyrazine (IBMP), 3-*sec*-butyl-2-methoxypyrazine (SBMP), and 3-isopropyl-2-methoxypyrazine (IPMP). Bordeaux cultivars such as Sauvignon blanc and Cabernet Sauvignon contain methoxypyrazines at significant concentrations and owe much of their distinct aroma to these potent aroma compounds (Bayonove *et al.*, 1975; Lacey *et al.*, 1991; Kotseridis *et al.*, 1998). IBMP is the most abundant methoxypyrazine found in grapes (Sala *et al.*, 2000). Bayonove *et al.* (1975) first reported IBMP in Cabernet Sauvignon, and subsequent studies (Augustyn *et al.*, 1982; Allen *et al.*, 1991, 1995; Lacey *et al.*, 1991; Kotseridis *et al.*, 1998) identified IBMP, SBMP, and IPMP in grape berries and wines. Concentrations in the fruit range from 0 to as much as 42 ng/L (Roujou de Boubée *et al.*, 2000).

One of the most significant aspects of methoxypyrazines is that their sensory thresholds are extraordinarily low. For example, the human sensory threshold for methoxypyrazines is 1–ng/L (Buttery *et al.*, 1969a,b). In red wines, IBMP and IPMP are detected at 10 ng/L (Tominaga *et al.*, 1998a,b) and 2 ng/L (Kotseridis *et al.*, 1998), respectively.

Relatively little is known about the biochemistry of methoxypyrazines. Subse-

quently, there has not been much literature published about their synthesis or degradation. Methoxypyrazine concentrations diminish greatly during the berry expansion phase and are relatively high at *véraison*, but can decrease dramatically during maturation from as high as 78 ng/L at mid-*véraison* to below 2 ng/L at harvest (Lacey *et al.*, 1991). The current belief is that sunlight and/or heat can lead to degradation of pyrazines. Allen *et al.* (1991) showed a decrease in methoxypyrazine concentrations in grapes via photodecomposition due to sunlight. Hashizume and Sumuta (1999) showed that light exposure has two opposite effects on the concentration of methoxypyrazines in grapes: (i) promoting the formation of methoxypyrazines in immature grapes; and (ii) non-enzymatically photo-decomposing the methoxypyrazines in ripening grapes. A balance between biological formation and photodegradation may determine the methoxypyrazine concentration in grapes throughout the ripening process (Roujou de Boubée *et al.*, 2000). Methoxypyrazines might form largely in the earlier stages of grape development, and photodegradation might be more important in the ripening fruits (Sala *et al.*, 2005).

Sauvignon blanc wines from cooler climates such as New Zealand have much higher concentrations than those from Australia, South Africa, or Bordeaux (Lacey *et al.*, 1991; Allen *et al.*, 1991, 1995). In Europe, Kotseridis *et al.* (1999) analyzed Merlot and Cabernet Sauvignon wines from various Bordeaux regions and wine samples from Greece (Xynomavro) for 2-methoxy-3-isobutylpyrazine concentration. Cultivar, level of maturation, and duration of maceration affected the concentration of IBMP. Higher humidity and cooler years yield higher IBMP concentration in grapes than sunnier and less humid years (Roujou de Boubée *et al.*, 2000).

Bayonove *et al.* (1975) reported that a considerable proportion of IBMP resided in grape skins. More recently Roujou de Boubée *et al.* (2002) observed that inside the berry, IBMP is found mainly in the skin (72%), and seeds (23.8%) and that the pulp contains very little (4.2%).

11.4.5 Volatile thiols

Volatile thiol compounds are currently one of the most active areas of wine flavour research. They are found in small quantities in grapes, and most certainly their precursors might be influenced by viticultural practices. They are among the most potent aroma compounds found in wine. These sulphur-containing compounds have extremely low perception thresholds, ranging from 0.8–1500 ng/kg (Tominaga *et al.*, 2000). Currently, five highly aromatic volatile thiols, 4-mercapto-4-methypentan-2-one (4MMP), 3-mercaptohexyl acetate (A3MH), 4-mercapto-4-methylpentan-2-ol (4MMPOH), 3-mercaptohexan-1-ol (3MH), and 3-mercapto-3-methylbutan-1-ol (3MMB), have been identified in grapes (Darriet *et al.*, 1995; Tominaga *et al.*, 1998a,b, 2000). 4MMP, 4MMPOH, and 3MH have aromas characteristic of box tree/broom, citrus zest, and grapefruit/passion fruit, respectively (Tominaga *et al.*, 1998b, 2000). Moreover, 3MMB and A3MH are responsible for the overtones of cooked leeks and passion fruit, respectively (Tominaga

et al., 2000). These compounds impact varietal character in certain grapes and wine. For example, they play a major role in the aroma of Sauvignon blanc (Darriet *et al.*, 1995; Tominaga *et al.*, 1998b, 2000), Cabernet Sauvignon (Tominaga *et al.*, 1998a) and Merlot (Murat *et al.*, 2001a) wines. Furthermore, Tominaga *et al.* (2000) found that these aroma compounds are also responsible for nuances of several other *V. vinifera* cultivars including Semillon, Gewürztraminer, Riesling, and Colombard.

Darriet *et al.* (1995) showed that 4MMP and 3MH exist in grapes but in the form of non-volatile, cysteine-bound conjugates. Peyrot des Gachons (2000) found that the cysteinylated precursors for 4MMP and 4MMPOH are located mainly in grape juice, while the precursor for 3MH is distributed equally between berry skin and juice. Their location does not depend on the stage of berry development (Peyrot des Gachons, 2000). The biosynthesis of these S-cysteine conjugates in grapevines is virtually unknown. It is thought that these compounds are intermediate products in the detoxification system of living organisms and formed from the breakdown of the corresponding S-glutathione conjugates (Peyrot des Gachons, 2000). Although volatile thiols are almost non-existent in grapes and must (Peyrot des Gachons *et al.*, 2000, 2005), they are released into wine from their corresponding precursors during alcoholic fermentation (Howell *et al.*, 2004; Murat *et al.*, 2001b).

Since volatile thiols are a new area of research and have only recently been identified and quantified there is little research pertaining to how these compounds can be altered in the vineyard or winery. Peyrot des Gachons *et al.* (2005) found that volatile thiol precursors were highest in vines with moderate nitrogen supply, whereas, nitrogen deficiency seemed to limit aroma potential. Furthermore, they found that volatile thiol precursors were highest in vines under mild water deficit, whereas severe water deficit stress seemed to limit aroma potential. Therefore, highest aroma potential can be achieved under mild water stress and when nitrogen status is non-limited (Peyrot des Gachons *et al.*, 2005). Powdery mildew infection on berries decreases the concentration of 3MH (Calonnec *et al.*, 2004).

As mentioned, 4MMP and 3MH (and perhaps other volatile thiols) exist in grapes in the form of non-volatile, cysteine-bound conjugates. It is suggested that the amplification of these aromas during fermentation occurs through the action of yeast carbon–sulphur lyases (Tominaga *et al.*, 1998a,b; Howell *et al.*, 2004). 4MMP, 4MMPOH, and 3MH are released into wine from their grape-derived cysteinylated precursors during alcoholic fermentation (Darriet *et al.*, 1995; Tominaga *et al.*, 1998a,b; Peyrot des Gachons *et al.*, 2000, 2005). Therefore, it appears that volatile thiols might be manipulated easily in the winery by release of these thiol compounds from their precursors. Various yeast strains can produce varying amounts of each of the compounds (Murat *et al.*, 2001b; Howell *et al.*, 2004). Fermentation temperature also has an influence on the release of thiol compounds. Howell *et al.* (2004) found that at 28 °C some yeast strains released 100-fold more 4MMP than at 18 °C. Although only small amounts (1.4–4.2%) of aroma precursors are transformed during alcoholic fermentation (Peyrot des Gachons *et al.*, 2000; Howell *et al.*, 2004), these

concentrations of volatile thiols still contribute significantly to the overall sensory profile of the resultant wine.

Following primary fermentation and malo-lactic fermentation, thiol concentrations can be considerable (relatively speaking). However, thiols can be easily oxidized after certain winemaking procedures such as racking or ageing so these compounds can decrease significantly. Blanchard *et al.* (2004) found a substantial decrease in 3MH in wines with the presence of oxygen and catechin. However, sulphur dioxide reduced these effects considerably. Anthocyanins can help stabilize volatile thiols in wine (Blanchard *et al.*, 2004). Oak ageing can be used to manipulate the composition and amount of volatile thiols present in wine; 2-furanmethanethiol, a thiol with a strong roast coffee aroma, has been identified in certain red Bordeaux wines and has been found in toasted oak staves (Tominaga *et al.*, 2000).

It is only recently that volatile thiols have been a key area of research in wine flavour chemistry. Research thus far has focused mainly on method development for identification and quantification of these compounds and their contribution in certain key cultivars such as Sauvignon blanc and Cabernet Sauvignon. There are now ongoing studies looking at ways to release volatile thiols from their cysteinylated precursors by yeast strains. Generally speaking, in the oenology and viticulture field, any aspect concerning volatile thiols is a wide-open research topic. Research is currently ongoing in areas such as New Zealand where it is felt that these compounds contribute more than just nuances to their wines. In the case that volatile thiols are in fact the most potent compounds in wines, practices in the vineyard can be further explored to maximize aroma potential from these compounds.

11.5 Effects of viticultural practices on odour-active substances in grapes and wines

11.5.1 Effects of fruit exposure on odour-active substances in grapes and wines

The effects of cluster shading on fruit composition can be quite substantial. Two seminal studies include those of Kliewer and Lider (1968) with Thompson Seedless and Koblet *et al.* (1977). Both studies found that fruit exposure increased soluble solids, reduced TA, and increased pH. Reynolds *et al.* (1986b) found a sizable (1.2 ºBrix) difference between shaded and exposed Seyval blanc clusters on vines of identical training and crop level. They also noted as much as 12 ºC difference in berry temperature between western exposed berries and shaded berries, which diminished the diurnal temperature flux of the exposed berries. Greater malate degradation also occurred in exposed clusters. Similar studies on the effect of cluster shading found higher soluble solids in clusters of Cabernet Sauvignon chosen from sun exposed regions of the canopy (Crippen and Morrison 1986a,b). The shaded berries were larger and had higher water content than the

exposed berries, effectively lowering soluble solids. Pre-harvest, anthocyanins were higher on both a berry weight and per berry basis; but by harvest there were no differences (Crippen and Morrison 1986a,b).

11.5.2 Effects of fruit exposure on monoterpenes

The rapid distillation method of Dimitriadis and Williams (1984) permitted the assessment of viticultural practices on concentrations of monoterpenes in grape berries. One of the first of these was a study in British Columbia, whereby PVT concentrations in Gewürztraminer berries were found to be highest in fully exposed clusters throughout the course of fruit maturation, but peaked at about 20 days after *véraison* (Reynolds and Wardle, 1989b) (Fig. 11.4). Partially exposed clusters (those shaded by one layer of leaves) contained a lower concentration of PVT than exposed clusters, but much more than shaded clusters. Free volatile terpenes were not as responsive to fruit exposure as PVT, but followed the same trends. By the final sampling date, soluble solids, TA, and pH were similar among the three exposure treatments, but exposure-related differences in PVT remained until after commercial maturity. Conclusions from this were that: fruit exposure enhances monoterpene concentrations in grape berries, and that FVT and PVT concentrations may not necessarily be correlated to soluble solids, TA, or pH. These results were confirmed in Arkansas by Macaulay and Morris (1993) using *V. labruscana* Golden Muscat.

Belancic *et al.* (1997) monitored the effect of sun exposure on the aromatic composition of two muscat grape cultivars, Moscatel de Alejandria and Moscatel rosada over two seasons in Chile. Fully exposed, semi-shaded (20% shaded) and fully shaded (80% shaded) clusters were sampled. Both cultivars contained similar levels of total free terpenols, but Moscatel de Alejandria was richer in total bound terpenols. The highest concentration of free terpenols was obtained from the semi-shaded treatment, although the differences between exposed and semi-shaded were negligible for Moscatel de Alejandria. Shaded grapes had the lowest concentration of terpenols, with poor muscat typicity. Linalool was the most sensitive to sun exposure. Berry temperature was considered critical for maximizing monoterpene concentrations and muscat flavour in the fruit.

11.5.3 Effects of fruit exposure on methoxypyrazines

Cultural decisions and practices that decrease shading and increase fruit exposure should be beneficial in reducing methoxypyrazine concentration in fruit. These include the use of divided canopies, basal leaf removal, cluster thinning, deficit irrigation, vine spacing and trellising, hedging, and shoot thinning. These practices can produce a more open, manageable canopy that improves fruit exposure. A few studies indicate that methoxypyrazines can be manipulated in the vineyard and within the winery. In fact, all of the studies targeting manipulation of methoxypyrazine concentration in the vineyard have been indirectly due to better fruit exposure. Since vigorous canopies that limit sunlight exposure are associated with

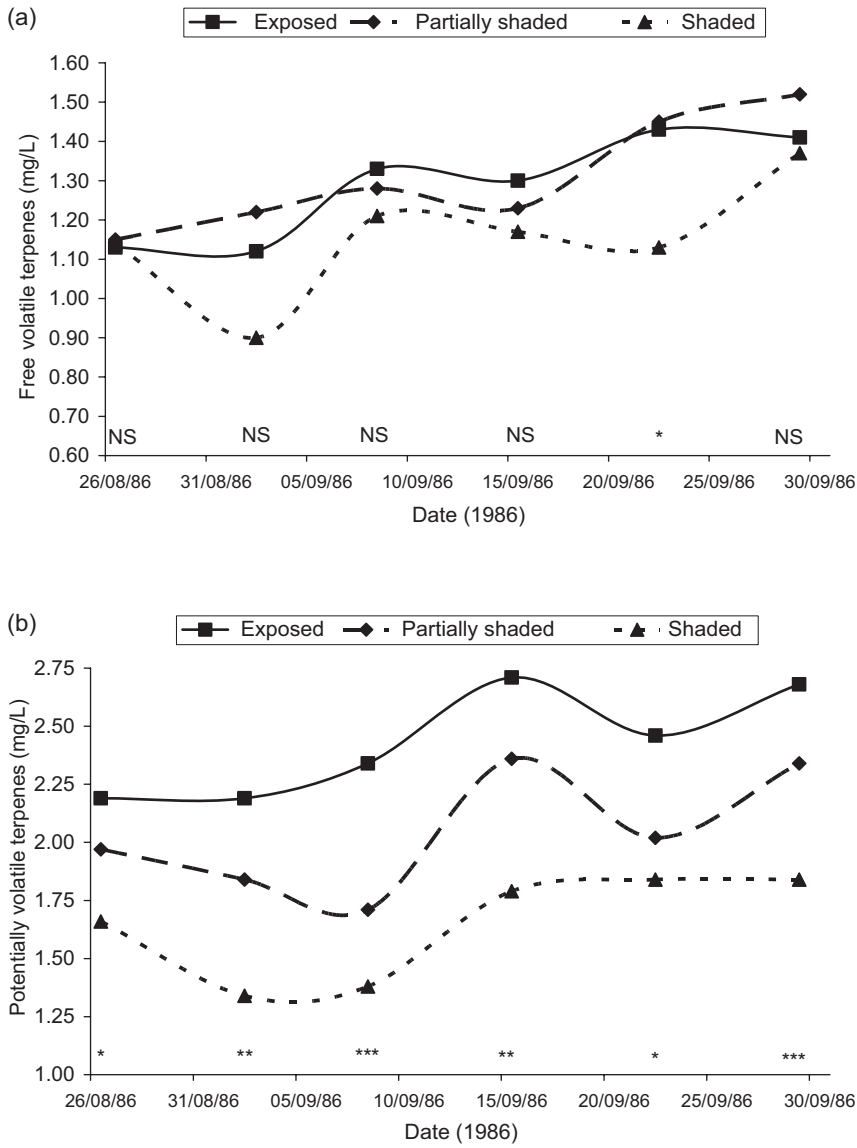

Fig. 11.4 Effects of cluster exposure on monoterpene composition of Gewürztraminer, Kaleden, British Columbia, 1986: (a), free volatile terpenes (mg/L); (b), potentially volatile terpenes (mg/L). *,**,***, ns: Significant at $p < 0.05$, 0.01, 0.001, or not significant, respectively (redrawn from Reynolds and Wardle, 1989b).

higher concentrations of methoxypyrazines, good canopy management can lower these methoxypyrazine concentrations. Since methoxypyrazines have been shown to have negative impacts on wine quality at high concentrations, studies have focused on minimizing methoxypyrazines in grapes and wine. Research seems to

indicate that conditions during grape maturation are primarily responsible for the methoxypyrazine concentration in wines (Allen *et al.*, 1991, 1995; Lacey *et al.*, 1991; Roujou de Boubée *et al.*, 2000). Shade seems to have a major effect on methoxypyrazine concentrations in grapes. Exposed clusters can have three times lower concentrations of methoxypyrazines than shaded fruits (Roujou de Boubée *et al.*, 2000). Sala *et al.* (2004), however, found that during ripening, IBMP concentrations in grapes exposed to sunshine were not different from those covered with pieces of sackcloth. Nonetheless, clusters protected from sunlight since the beginning of the *véraison* resulted in wines with a substantially lower concentration of this compound than the control samples.

11.5.4 Effects of fruit exposure on norisoprenoids

Enhanced light and temperature environments can lead to breakdown of carotenoid pigments and consequently higher concentrations of norisoprenoids. However, in many cases, the impact of light exposure and/or cluster temperature has been inconsistent at best.

Marais *et al.* (1992) were among the first to study light and temperature effects on C_{13} norisoprenoids. Norisoprenoid concentrations in Riesling and Chenin blanc were substantially higher in sun-exposed fruit than in shaded fruit. Compounds enhanced by fruit exposure included *cis*- and *trans*-vitispirane, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), and actinidol 2. Razungles *et al.* (1998) examined light and temperature effects on C_{13} norisoprenoids in Syrah. Sunlight effects on the berry carotenoid and C_{13} norisoprenoid composition was studied before and after *véraison*. Berries that were sun-exposed before *véraison* were higher in carotenoids than shaded berries. However, after *véraison*, sunlight caused the degradation of these pigments. Sunlight modified the non-epoxyxanthophyll/epoxyxanthophyll ratios. Metabolic relationships between the glycosylated C_{13} norisoprenoids and their potential precursors were tentatively established between certain C_{13} norisoprenoids and carotenoids in specific sun-exposure treatments. In addition, the sunlight increased other glycosidically-bound compounds such as monoterpenes and phenols.

Effects of the modification of whole vine or individual cluster light environment by shade cloth from berry set to maturity were studied by Bureau *et al.* (2000a) on the volatiles and glycoconjugates in Muscat of Frontignan berries. Whole vines were shaded with 50 and 70% shade-cloth, while clusters were shaded with 90% shade-cloth. The sun-exposed berries were chosen as control berries, and the berries naturally shaded under foliage were also studied. The natural shading of clusters under foliage did not decrease the concentrations of free and bound compounds compared to sun-exposed berries. The artificially shaded clusters had lower concentrations of C_{13} norisoprenoids (as well as monoterpenes) than both sun-exposed berries and berries from naturally shaded clusters. Moreover, the effect of vine shading on the aroma composition was lower compared to artificial cluster shading. Decreasing the cluster number per vine did not influence the total amounts of glycosidically bound compounds, except for monoterpene

glycoconjugates. However, the higher monoterpene glycoconjugates in these berries were likely related to their early maturity. Authors concluded that under their experimental conditions, berry aroma composition did not appear to be affected by foliage shade. In a related study using the same experimental protocol, Bureau *et al.* (2000b) looked at the effects of the modification of vine or cluster environment on glycoconjugates in Syrah berries. Vines were shaded from berry set to maturity, with black polyethylene nets of different mesh size to obtain 30 and 50% of the direct sunlight. Clusters were naturally shaded by the leaves or artificially with 90% shade bags. Sun-exposed berries were chosen as control berries. A decrease in glycoconjugates was observed in shaded clusters, particularly for phenolic and C_{13}-norisoprenoidic glycosides. In the same way, vine shading caused a decrease in the concentrations of glycosides of terpenols, phenols, and C_{13} norisoprenoids in berries, but the grape environment (microclimate) affected the berry composition more than the vine environment. A cluster thinning experiment confirmed the independence of grapes with regard to the plant for the biosynthesis of the C_{13} -norisoprenoid glycosides.

The effect of cluster exposure on the grape carotenoid profile was also investigated by Oliveira *et al.* (2004) on several Portuguese cultivars. Grape cultivar, ripeness stage, sunlight and shade exposure, altitude, and vegetative height were all the variables studied. Differences between cultivar were observed in eight different red wine grape cultivars: Touriga Brasileira, Tinta Barroca, Tinta Amarela, Souzao, Touriga Franca, Touriga Nacional, Tinta Roriz, and Tinto Cao, from the Douro region. Tinta Amarela and Touriga Brasileira produced higher concentrations of carotenoids. Carotenoids decreased during ripening. Decreases of lutein were observed until 66% of the original concentration, whereas β-carotene slowly decreased in a constant manner until the harvest date. Carotenoids were consistently higher in shaded grapes than in those exposed to direct sunlight in two white grape cultivars, Maria Gomes and Loureiro.

Lee *et al.* (2008) assessed the effects of light exposure and vine microclimate on C_{13}-norisoprenoid concentrations in Cabernet Sauvignon grapes and wines by measuring the amounts of β-damascenone (megastigma-3,5,8-trien-7-one), TDN (1,1,6-trimethyl-1,2-dihydronaphthalene), and vitispirane (6,9-epoxy-3,5(13)-megastigmadiene). The most exposed treatment (all lateral and primary leaves removed) had the highest light intensity and temperature and showed the highest concentrations of TDN and vitispirane. However, in the more shaded treatments, concentrations of all norisoprenoids were variable and dependent on the treatment conditions. When leaves were removed, C_{13}-norisoprenoid concentrations were linearly and positively correlated with increasing sunlight exposure. In contrast, in the most shaded treatments with no leaf removal there were high concentrations of norisoprenoids. β-Damascenone concentrations were highest when no leaves were removed. Grapes and corresponding wines from the south side of the vine had higher norisoprenoids than those from the north side.

Ristic *et al.* (2007) enclosed clusters of Shiraz grapes prior to flowering in boxes designed to eliminate light without altering bunch temperature and humidity. This artificial shading had little effect on berry ripening and accumulation of soluble

solids but, at harvest, the shaded clusters had smaller berries, pH, and TA. Analysis of potential flavour compounds indicated that the wines made from shaded fruit had decreased concentrations of glycosides of β-damascenone and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN). Sensory analysis of the wines indicated no difference in aroma attributes, but the wines made from shaded fruit were rated lower for astringency, fruit flavour, and flavour persistence in-mouth sensory attributes.

11.6 Effects of growing season canopy management on odour-active substances

Vertically shoot positioned canopies are typically hedged at the top and sides of the canopies to eliminate overhanging shoots, reduce canopy width, and generally reduce shade in dense canopies. Normally this is done about two weeks after fruit-set, but it can be repeated one or more times during the growing season. Basal leaf removal is generally done one week or more after hedging by removing the lower two to four leaves at the base of the shoot to expose the clusters. This can be done on both sides of the canopy, particularly in the case of red wine cultivars and during wet growing seasons; typically it is done on the least sunny side (east or north side in the Northern Hemisphere) to avoid possibility of sunburning to fruit.

Research into the potential effects of hedging and leaf removal on potential wine quality has been quite recent. Many of these studies measured standard fruit composition variables and not aroma compounds. A New Zealand study unequivocally found that basal leaf removal (either 50% or 100% of leaves in fruit zone) increased both total phenol and anthocyanin concentrations in Cabernet Sauvignon, with the greatest increase (~50% over control) occurring when the treatment was done five weeks after flowering (Smith *et al.*, 1988). Mazza *et al.* (1999) also found that leaf removal resulted in higher phenolic concentration and colour density over the control. Numerous studies on basal leaf removal have been performed on aromatic white wine cultivars; e.g. basal leaf removal on Chardonnay vines in New York increased soluble solids and lowered TA (Wolf *et al.*, 1986).

11.6.1 Effects of growing season canopy management on monoterpenes

A few studies have examined leaf removal effects on aroma compounds. Smith *et al.* (1988) were among the first to demonstrate the effectiveness of basal leaf removal on increasing both monoterpene concentration and wine sensory scores of Sauvignon blanc in New Zealand.

Work on Gewürztraminer in British Columbia indicated that FVT and PVT were responsive to leaf removal, and could also be increased by cluster thinning and hedging (Reynolds and Wardle, 1989a). Both FVT and PVT were not dependent upon soluble solids, TA, or pH. In a multi-site trial, leaf removal consistently increased berry PVT and, in one year, FVT as well, regardless of site (Reynolds *et al.*, 1996a). Must FVT and PVT were also increased by leaf removal

treatment. Increased monoterpene concentration was, in some cases, associated with lower TA, pH, and potassium, but slightly lower soluble solids as well. Tasters found more muscat aroma and flavour in both the hedged and leaf removal wines than in the control.

FVT and PVT in the berries of Pearl of Csaba, Bacchus, Schönburger, and Siegerrebe were responsive to basal leaf removal during the *véraison* to harvest period at several sites in British Columbia (Reynolds *et al.*, 1995a). Musts displayed greater treatment differences, and basal leaf removal musts usually contained higher FVT and PVT. Soluble solids and TA were largely unresponsive to basal leaf removal in the berries and must, but must pH was in many cases lower in leaf-pulled treatments. At warmer Oliver, BC sites, aroma differences occurred between control and leaf-pulled wines for two of four cultivars, and flavour differences were apparent for three of four. Tasters almost overwhelmingly indicated that the leaf-pulled treatments contained the most muscat and/or floral flavour. These distinctions could be made based on differences in PVT of 1.45, 0.10, and 0.87 mg/L for Pearl of Csaba, Schönburger, and Siegerrebe, respectively. Similar trends were apparent for a related Okanagan Riesling experiment (Reynolds *et al.*, 1995b). Also, in a multi-year experiment testing training system, vine spacing, and leaf removal, basal leaf removal consistently increased both FVT and PVT (Reynolds *et al.*, 1996b). These results suggested that berry or must monoterpene concentrations may be used as indicators of potential wine varietal character.

A multi-year project in Ontario found positive effects of basal leaf removal and cluster thinning on berry and must concentrations of FVT and PVT in Chardonnay musqué (Reynolds *et al.*, 2007b). Elevated monoterpene concentrations were measured in berries and musts from basal leaf removal and cluster thinned plots, but basal leaf removal had by far the greatest magnitude of effect. No differences were found in berry FVT across treatments, but basal leaf removal berry PVT were higher than control and thinned samples. Must FVT and PVT concentrations showed differences among treatments with basal leaf removal > cluster thinned > control in both cases. There were substantial differences in sensory profiles of the wines resulting from the viticultural practices. Cluster thinning enhanced dry fruit aroma and colour and reduced the citrus component to the aroma. Basal leaf removal enhanced citrus aroma, and lychee and dry fruit flavour. The data also showed that regardless of winery treatment (various yeasts and enzymes had also been tested), *véraison* thinning had highest dry fruit aroma.

The use of the glycosyl–glucose (G–G) assay permitted an assessment of viticultural practices whereby all glucoconjugates could be accurately measured by an enzymatic method (Abbott *et al.*, 1993). Target compounds include monoterpenes, norisoprenoids, and some phenolic compounds. Zoecklein *et al.* (1998a) found higher glycosides (measured by G–G assay) as well as higher monoterpenes and aromatic alcohols in leaf-pulled treatments of Riesling in Virginia. In a related trial, they found that both the total G–G concentration and the phenol-free fraction were highest in leaf-pulled treatments of Chardonnay and Riesling (Zoecklein *et al.*, 1998b). The concentrations of total and phenol-free

glycosides were higher in Riesling and Chardonnay fruit from leaf-pulled versus control vines on three of four harvest dates. Phenol-free glycosides averaged 80% of the total in Riesling juice and 66% of the total in Chardonnay.

11.6.2 Effects of growing season canopy management on methoxypyrazines

It has become clear that methoxypyrazines concentrations can be reduced by canopy management. Arnold and Bledsoe (1990) in California showed that severe fruit zone leaf pulling reduced vegetal sensory descriptors in Sauvignon blanc, suggesting that the leaf-pulling treatments had reduced methoxypyrazines. The canopies in question were manipulated using several leaf removal treatments. Leaves were removed on three different occasions at three severity levels, whereas control grapevines received no leaf removal. Descriptive sensory analysis showed large differences among the wines for two vegetal aromas (celery/fresh vegetable and canned green bean) as well as the vegetal-flavour-by mouth. The greatest reduction in the vegetal components was found with the middle timing/most severe leaf removal treatment. Early, severe leaf removal was nearly as effective as the middle timing treatment in reducing vegetal flavours, but late, severe leaf removal was not. Fruit composition was also altered; soluble solids were increased, and TA was reduced in the leaf-pulled treatments (Bledsoe *et al.*, 1988).

11.7 Effects of shoot density and crop level on odour-active substances

11.7.1 Effects of shoot density and crop level on monoterpenes

McCarthy and Coombe (1985), McCarthy (1986), McCarthy *et al.* (1987), and Coombe and Iland (1987) showed that PVT in Riesling berries were responsive to cluster thinning and reduced irrigation in Australia. It was apparent that these treatment differences were closely related to yield. Eschenbruch *et al.* (1987) demonstrated increases in PVT of Müller-Thurgau berries resulting from cluster thinning and shoot thinning. They concluded that PVT development in that cultivar closely paralleled soluble solids accumulation, and hence afforded no better indication of ultimate grape and wine quality. They were also unable to demonstrate a clear relationship between PVT concentration and wine quality.

In a Riesling shoot density × crop level trial, Reynolds *et al.* (1994a,b) showed that despite large yield and shade increases, increases in shoot density actually increased Riesling berry and must PVT. Reducing crop level had a minor effect on PVT concentration. Tasters found 26 shoots/m row wines equal to wines of lesser shoot densities in terms of sensory quality, despite higher yields. This may have been due to higher PVT concentration in the original berries and musts. Some monoterpenes, including linalool, linalool oxides, α-terpineol, and citronellol were associated with lower crop levels and low to moderate (16 or 26 shoots/m row) shoot densities, and also increased in concentration during ageing.

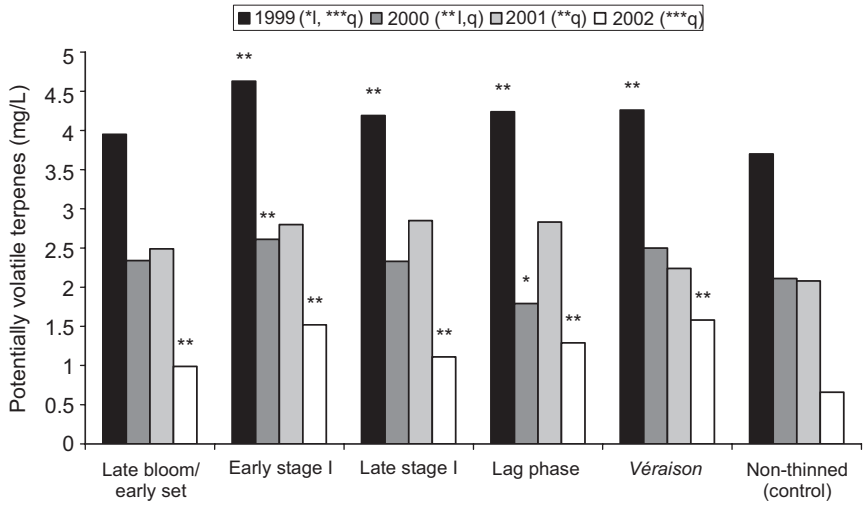

Fig. 11.5 Impact of six thinning times on Chardonnay musqué berry potentially volatile terpene concentration 1999–2001. Asterisks indicate significant difference from the control, $p < 0.05$, Dunnett's *t*-test (from Reynolds *et al.*, 2007c).

In a similar trial testing Riesling shoot density × cordon age, Reynolds *et al.* (1994c) found that increasing volume of 'old' wood increased berry and must FVT and PVT. Wines produced from vines containing more 'old' wood were higher in floral aroma and flavour, with less vegetal character. Shoot density had less of a magnitude of effect than volume of old wood.

A multi-year project in Ontario found positive effects of crop reduction on berry and must concentrations of FVT and PVT (Reynolds *et al.*, 2007c) (Fig. 11.5). The thinning treatments were carried out at five different times during fruit development (bloom; post-set; mid-Stage I; lag phase; *véraison*). Berry FVT increased 10–15% over non-thinned vines in vines thinned at bloom, set and Stage I. Berry PVT increased in all thinning treatments except bloom. Both FVT and PVT concentrations were lower in must samples than in berry samples. Must samples had lowest FVT concentrations from Stage I and lag phase-thinned vines, and lowest PVT in bloom, post-set, Stage I and *véraison*-thinned vines. Overall, it was possible to increase the concentration of both FVT and PVT through thinning. Their concentrations were increased by up to 15% and 24%, respectively. Non-thinning maximized dry fruit (raisin, fig) aroma and colour, and did not differ from all thinning treatments with respect to most other sensory descriptors. Bloom thinning maximized citrus (as did *véraison* thinning) and grassy aroma.

11.7.2 Effects of shoot density and crop level on methoxypyrazines

The effect of crop level on methoxypyrazines has heretofore not been a well-researched topic. Crop thinning (Marais 1994; Chapman *et al.*, 2004a,b) can also help decrease methoxypyrazine concentration in grapes because excessive crop to

leaf area can delay the rate of fruit maturity and therefore the degradation of methoxypyrazines.

11.8 Influence of training systems on odour-active substances in grapes and wines

Numerous training systems are used around the world for wine grapes. One of the many objectives of a successful training system is to afford optimal fruit exposure that is appropriate for the region in which the grapes are grown. Enhanced fruit exposure might lead to increased concentrations of aroma compounds. A great many studies have compared the effects of training systems on fruit composition and wine quality.

11.8.1 Influence of training systems on monoterpenes

In British Columbia, a divided canopy system (alternate double crossarm; ADC) produced yields as high as 33 t/ha, along with lower TA, and higher FVT and PVT than standard *pendelbogen* and bilateral cordon systems (Reynolds *et al.*, 1996b) (Fig. 11.6). Fruit exposure and berry temperatures were considerably higher in ADC vines than in bilateral cordon vines. However, despite significant increases in cluster exposure resulting from canopy division, basal leaf removal still reduced

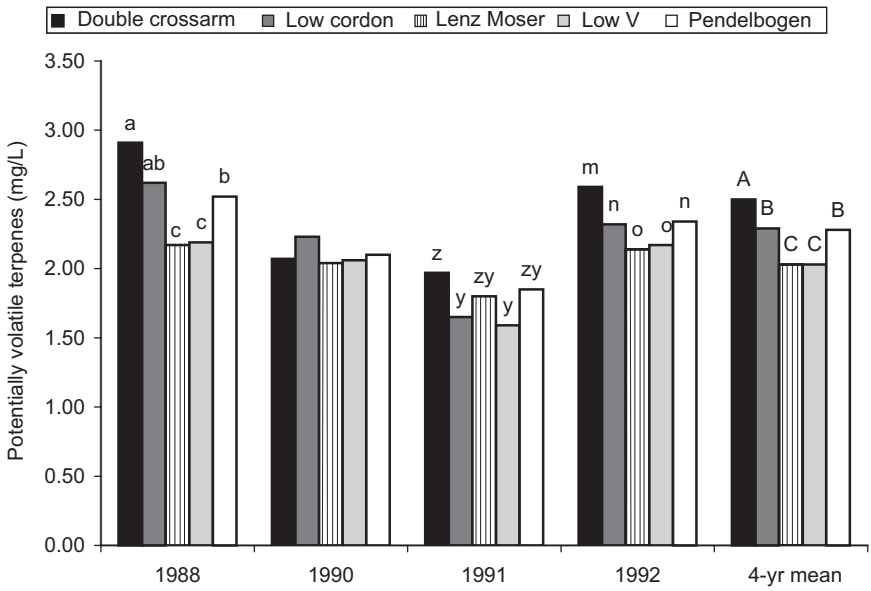

Fig. 11.6 Potentially volatile terpene concentration of Riesling berries subjected to five trellising treatments, 1988–1992. Bars within years containing different letters are significantly different at $p < 0.05$, Duncan's multiple range test (figure was redrawn from Reynolds *et al.*, 1996b).

TA and increased PVT, even in the ADC system. This suggests that natural fruit exposure can be augmented by cultural practices to increase potential wine quality.

Zoecklein *et al.* (2008) found that divided canopies generally produced wines higher in some terpenes and in overall glucoconjugates. Fruit showed consistent differences in linalool, α-terpineol, β-damascenone, and *n*-hexanol concentrations among training systems. The Smart-Dyson vertically divided system had the highest concentration of most free volatiles in both juice and wines, while GDC wines frequently had the highest concentration of phenol-free glycosides. GDC wines generally had higher fruity and floral aromas compared with the other systems.

11.8.2 Influence of training systems on methoxypyrazines

As in the case of fruit exposure and basal leaf removal, certain training systems may enhance fruit environment and potentially lead to changes in methoxypyrazine concentration. Nonetheless, Sala *et al.* (2004) found that gobelet- and bilateral cordon-trained Cabernet Sauvignon vines did not produce fruit with different berry IBMP concentrations. However, IBMP concentration of the final wines was much higher in the cordon-trained vines.

11.9 Influence of irrigation, water relations, and soil management on odour-active substances in grapes and wines

11.9.1 Influence of irrigation on monoterpenes, esters, and higher alcohols

Few irrigation studies have extended their focus to include aroma compounds. Reynolds *et al.* (2006) showed that irrigation deficits applied at *véraison* resulted in higher concentrations of FVT and PVT in Gewürztraminer than early and mid-season deficit treatments (Fig. 11.7). Related floor management treatments (clean cultivated, total herbicide, permanent sod) also produced differences, whereby FVT were highest in clean cultivated treatments but PVT were highest in sod plots.

The influence of irrigation on grape and wine composition was investigated for Agiorgitiko in the Nemea appellation area in southern Greece by Koundouras *et al.* (2006). Three non-irrigated plots were studied during vintages that were very hot and devoid of summer rainfall. Limited water availability increased glycoconjugates of the main aromatic components of grapes. Wines produced from grapes of stressed vineyards were also preferred in tasting trials.

Dos Santos *et al.* (2007) investigated the impacts of partial root-zone drying (PRD) irrigation on Moscatel vine water relations, vegetative growth, plant microclimate, berry composition (including aroma compounds), and yield components, compared to conventional deficit irrigation (50% ET_c), full irrigation (100% of ET_c), and non-irrigated vines. The PRD vines had a better microclimate in the cluster zone with higher incident photosynthetic photon flux density and higher berry temperatures than deficit irrigation and full irrigation. PRD improved berry

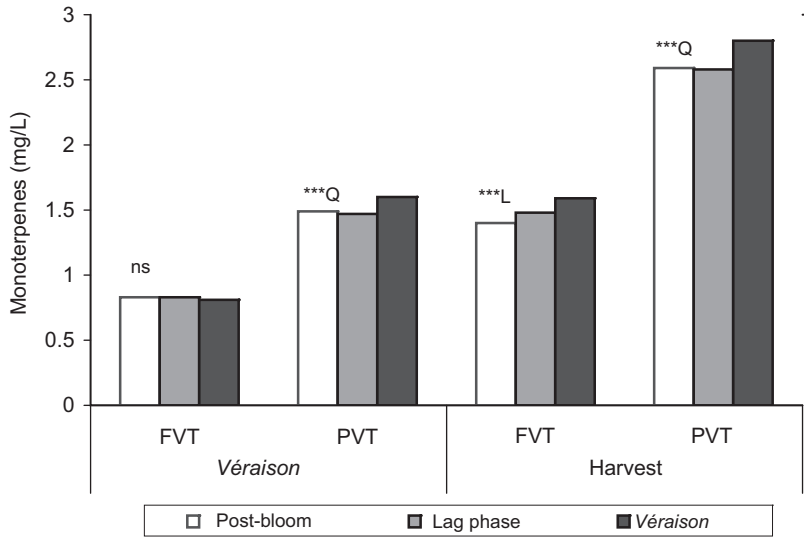

Fig. 11.7 Effect of three irrigation deficit times on free volatile terpene (FVT) and potentially volatile terpene (PVT) concentration of Gewürztraminer berries, sampled at *véraison* and harvest, 1995. Legend: postbloom, lag at $p < 0.001$ or not significant, respectively; L,Q: linear or quadratic trends, respectively. Data are pooled across three vineyards' management treatments (figure redrawn from Reynolds *et al*., 2006).

composition with higher flavour precursor concentrations, without any yield reduction compared to deficit irrigation and full irrigation.

Vineyard fertilization may have indirect effects on aroma composition. Ough and Bell (1980) in California showed that increased fertilization rate at rates between 0 and 440 kg/ha increased concentration of higher alcohols in Thompson Seedless wines. Ough and Lee (1981) showed that increased vineyard fertilization rates could likewise increase most fermentation esters such as isoamyl acetate in Thompson Seedless. Riesling vines in Washington fertilized between 0 and 224 kg/ha affected concentrations of free and bound monoterpenes in the aged wines (Webster *et al.*, 1993). Other compounds, including esters and alcohols were also impacted. Generally, most monoterpenes decreased with increasing fertilization rate, perhaps as a shade response, whereas esters and alcohols increased, as a likely effect of increased must amino nitrogen and resultant transamination.

11.9.2 Influence of irrigation on methoxypyrazines

Few studies exist that have addressed irrigation effects on methoxypyrazines. Sala *et al.* (2005) found that irrigated vines and vines planted at a higher plantation density had significantly higher concentrations of IBMP in berry samples than non-irrigated and lower plantation density vines.

11.9.3 Influence of irrigation on norisoprenoids

The limited information we have on the response of norisoprenoids to irrigation suggests that deficit irrigation might lead to increases in norisoprenoid concentration. The influence of irrigation strategy on grape berry carotenoids and C_{13}-norisoprenoid precursors was investigated for Cabernet Sauvignon by Bindon *et al.* (2007) in Australia. Two irrigation treatments were compared, one in which vines received reduced irrigation applied alternately to either side of the vine (PRD) and a second control treatment in which water was applied to both sides of the vine. The PRD vines received on average 66% of the water applied to the controls. In both irrigation treatments, the most abundant grape berry carotenoids, β-carotene and lutein, decreased post-*véraison* but, as the fruit approached maturity, the concentration of these carotenoids increased in fruit of PRD-treated vines relative to the controls, particularly for lutein. Moreover, PRD caused increases in the concentration of hydrolytically released C_{13}-norisoprenoids β-damascenone, β-ionone, and 1,1,6-trimethyl-1,2-dihydronaphthalene in fruit at harvest. During the second season of the experiment, the effect of the PRD treatment on C_{13}-norisoprenoids was greater and there was an increase in total C_{13}-norisoprenoid content per berry, suggesting that increases in C_{13}-norisoprenoids in response to PRD were independent of water deficit induced changes in berry size and concomitant altered berry surface area to volume ratios.

Linsenmeier and Löhnerz (2007) measured C_{13}-norisoprenoids in Riesling wines produced from the 1996, 1997, and 2003 vintages within the scope of a long-term nitrogen fertilization experiment (0, 60, and 150 kg N/ha). Nitrogen fertilization led to lower TDN (1,1,6-trimethyl-1,2-dihydronaphthalene) concentrations, whereas the trend was for actinidol and β-damascenone to increase with increasing fertilization, and for vitispirane to be unaffected by fertilization. Yield, which was affected by fertilization, showed negative correlations with norisoprenoids. Vitispirane, actinidol, and TDN increased with storage time. The coolest year studied, which had fewest sunshine hours, resulted in highest concentrations of β-damascenone and lowest concentrations of the norisoprenoids vitispirane, actinidol and TDN.

11.10 Impact of vineyard site on odour-active substances in grapes and wines

11.10.1 Impact of vineyard site on monoterpenes

The influence of site on fruit composition is difficult to define objectively, when site-based differences in canopy density, phenology, soil type, and cultural practices are involved. Di Stefano and Corino (1984, 1986) found only minor differences in terpene concentrations between Moscato bianco and Moscato giallo grapes grown on several sites in the Piemonte and Val d'Aosta regions in Northern Italy. Subsequent work (Corino and Di Stefano, 1988) showed that higher terpene concentrations were associated with warm sites. Likewise, Noble (1979) found

few differences between Chardonnay wines whose origins included Monterey (region I), Oakville (Napa County; region II), and Livermore (Alameda County; region III). Larrechi and Ruiz (1987) and Larrechi *et al.* (1988) used multivariate analysis to distinguish between winegrowing regions in Catalunya, Spain. Ewart (1987) found that a cool, high elevation site (High Eden, South Australia) produced Riesling fruit with highest terpene concentration, but terpene concentrations could not be linked to wine scores. Thus, although great volumes of anecdotal evidence exist for differentiating sites, very few objective studies have been carried out to quantitate these differences.

Work in British Columbia attempted to distinguish between sites based on monoterpene concentrations, by locating vineyards of similar soil type and vine vigour, and by maintaining the vines using identical cultural practices. Fruit maturation proceeded faster at Oliver, BC sites on a daily basis, and FVT and PVT were therefore usually higher in Oliver berries on any given sampling day (Reynolds *et al.*, 1995a). Cooler Kelowna, BC sites matured their fruit more quickly when expressed on a per growing degree-day basis. Oliver musts tended to be higher in FVT and PVT, although harvested at similar TA and pH. Tasters distinguished between wines from the Oliver and Kelowna sites on the basis of aroma for only one of the four cultivars studied (Reynolds *et al.*, 1995a), but the sites could be distinguished on the basis of flavour for three of the four cultivars. For Bacchus and Schönburger, the Oliver sites were clearly identified as having the more intense muscat flavour.

In a three-site experiment with Gewürztraminer, no clear pattern emerged regarding the relationship between site and FVT, but berries from both the Oliver and Kelowna sites were highest in PVT in two of five years (Reynolds *et al.*, 1996a). Must FVT and PVT were highest from the Kelowna site. The young wines from the Oliver and Kelowna sites were identified as most spicy. Aged wines from Oliver and Kelowna sites were high in citrus aroma, those from the Kaleden site were primarily vegetative, acidic, and astringent, while wines from the Oliver site were characterized by butter, cedar, and muscat flavours, as well as apricot, butter, cedar, and muscat flavour, high astringency, aftertaste, and body. These results corresponded with the PVT concentrations measured in the berry samples taken at harvest.

11.10.2 Spatial variation of aroma compounds

Recently, there has been great interest in using geomatic tools for the assessment of spatial variation in vineyards, including variation in aroma compounds. Elucidation of unique spatial patterns for aroma compounds in specific vineyard blocks could lead to the identification of sub-blocks of potentially higher value for economic exploitation. There might also be implications from this type of study for precision viticulture (Chapter 12), if spatial variability in vine vigour and yield were found to be highly correlated, and if spatial variation in yield was found to be temporally consistent within individual vineyard blocks.

A study in a 4 ha Riesling vineyard (Reynolds *et al.*, 2007d) was an attempt to

resolve the continuing question of direct and independent soil and vine vigour effects on yield components, berry, must and wine composition, and wine sensory attributes. Monoterpenes were specifically chosen as indicators of fruit maturity and as variables to associate with wine sensory attributes. Geographic information systems (GIS) delineated spatial variation in soil texture, soil and vine tissue composition, yield components, weight of cane prunings (vine size; an estimate of vine vigour), and berry composition including monoterpenes. Correlations were observed between soil texture and composition versus berry weight and PVT. However, there were no consistent soil texture or vine size effects on berry, must, or wine composition. High vine size increased the following variables: berry TA, berry PVT, and wine FVT; and decreased must pH. Sandy soil (versus clay soil) reduced wine TA and must PVT, and increased berry TA and must soluble solids. Percent sand was positively correlated in one or more years with several variables of potential significance to wine quality: berry weight, soluble solids, TA, pH, and PVT (Reynolds *et al.*, 2007d). Percent clay was also correlated in one or more seasons with soluble solids and PVT, and inversely correlated with berry weight, TA, and PVT. These results suggest that soil texture might play a role in fruit composition and varietal typicity, although non-consistent temporally. No fruit composition variables were consistently correlated with vine size, and very few correlative relationships of consequence were displayed except those between vine size and certain yield components. This suggests that vine size, at least in the range used in this study, does not play a major role in determining fruit composition.

Other soil or nutritional factors may have an impact on variables associated with varietal typicity; however, little to no work has focused on the impact of vine nutrition on concentrations of aroma compounds in grapes. Since low berry weight is frequently considered desirable from a winemaking standpoint, soil and vine nutritional factors associated with berry weight may also be determinants of the terroir effect. Soil variables found inversely correlated with berry weight (other than soil texture) included: pH, organic matter, P, K, Mg, Ca, CEC, and % base saturation as Ca; petiole variables included K, Mg, and Mn (Reynolds *et al.* 2007d). Positive soil versus monoterpene correlations included several with PVT: soil pH, P, K, Ca, Cu, B, CEC, and % base saturation as Ca; and petiole B. Few studies have found correlations between mineral nutrition and fruit composition, and direct connections between soil nutrients and aroma compounds have been difficult to determine. Webster *et al.* (1993) found an increase in PVT in Riesling with increased nitrogen fertilization, but there was little impact on FVT except where increased vegetative growth lead to greater shading (and thus lower FVT).

Vine size and soil texture did not consistently affect wine sensory attributes across vintages (Reynolds *et al.*, 2007d). However, the several sensory attributes were affected by vine size in at least one season. For example, high vine size decreased mineral aroma and citrus flavour, and increased apple attributes. Clay soil increased mineral aroma and citrus attributes, but decreased apple aroma. Vine vigour and soil texture sometimes affected composition of berries, must, and wines, and also impacted sensory perception of aroma, flavour and mouthfeel in

wine, but neither variable did so consistently (Reynolds *et al.*, 2007b). It must therefore be concluded that within the scope of this trial's conditions, that wine sensory attributes cannot be ascribed to either vine size or soil texture exclusively. Factors other than those tested apparently impacted wine sensory attributes and hence form much of the basis for so-called terroir effects (Chapter 9).

11.10.3 The influence of vine water status on spatial variation in aroma compounds

More recently, we have focused upon water status as a potential determinant of terroir. Mild 'water stress' may be beneficial to wine 'quality' but sustained water stress can have many negative consequences, including diminished winter hardiness, delayed maturity, and reduced yields, to name a few. In a trial initiated in 2005, we chose 10 vineyard blocks for each of Riesling (and Cabernet Franc, in a related study). The main objectives were: (i) to ascertain the impact of vine and soil water status on aroma compounds and wine sensory attributes; (ii) to enumerate the comparative magnitude of effects of soil texture, water status, and vine vigour; and (iii) to elucidate relationships between these variables and wine sensory quality. By meeting these objectives, we intended to elucidate the basis for terroir, by integration of several years of soil, plant nutrition, water relations, yield, fruit composition, and sensory data.

We tested the hypothesis that vine water status plays a major role in aroma compound concentration and wine sensory attributes using GPS and GIS applied to several vineyards in Niagara Peninsula, Ontario with heterogeneous soil types. Riesling data were analyzed by analysis of variance and GIS-generated maps were analyzed by spatial correlation analysis. In some instances, FVT and PVT were correlated with leaf Ψ and/or soil moisture, suggesting that mild water stress may be beneficial for wine flavour (Fig. 11.8). In most cases, sand and clay content of the soils were inversely correlated. Soil moisture content was typically higher in clay-dominated areas of the vineyards, while vine water status (leaf Ψ) also tended to be higher in clay soils. Leaf Ψ was often inversely correlated with vine size; i.e. vine water status was improved in low vine size areas. Berry weight and soluble solids were both correlated with vine water status, while TA was inversely correlated. Spatial relationships in vine water status appeared to be temporally stable, and patterns observed in one vintage appeared for the most part to be similar in the next despite different weather conditions. In addition to these reasonably good spatial correlations between soil moisture and leaf Ψ, there were some excellent spatial relationships between leaf Ψ and both vine size and soil texture. This validates our original idea that vine size and soil texture are major contributors to terroir.

Wines were subjected to sensory analysis. The results suggest that vine water status may in fact have an influence on wine sensory attributes. A sorting task was initially performed using expert judges. The judges were asked to group wines in terms of similar sensory characteristics. Statistical analysis using multi-dimensional scaling demonstrated that for the most part wines of similar water status

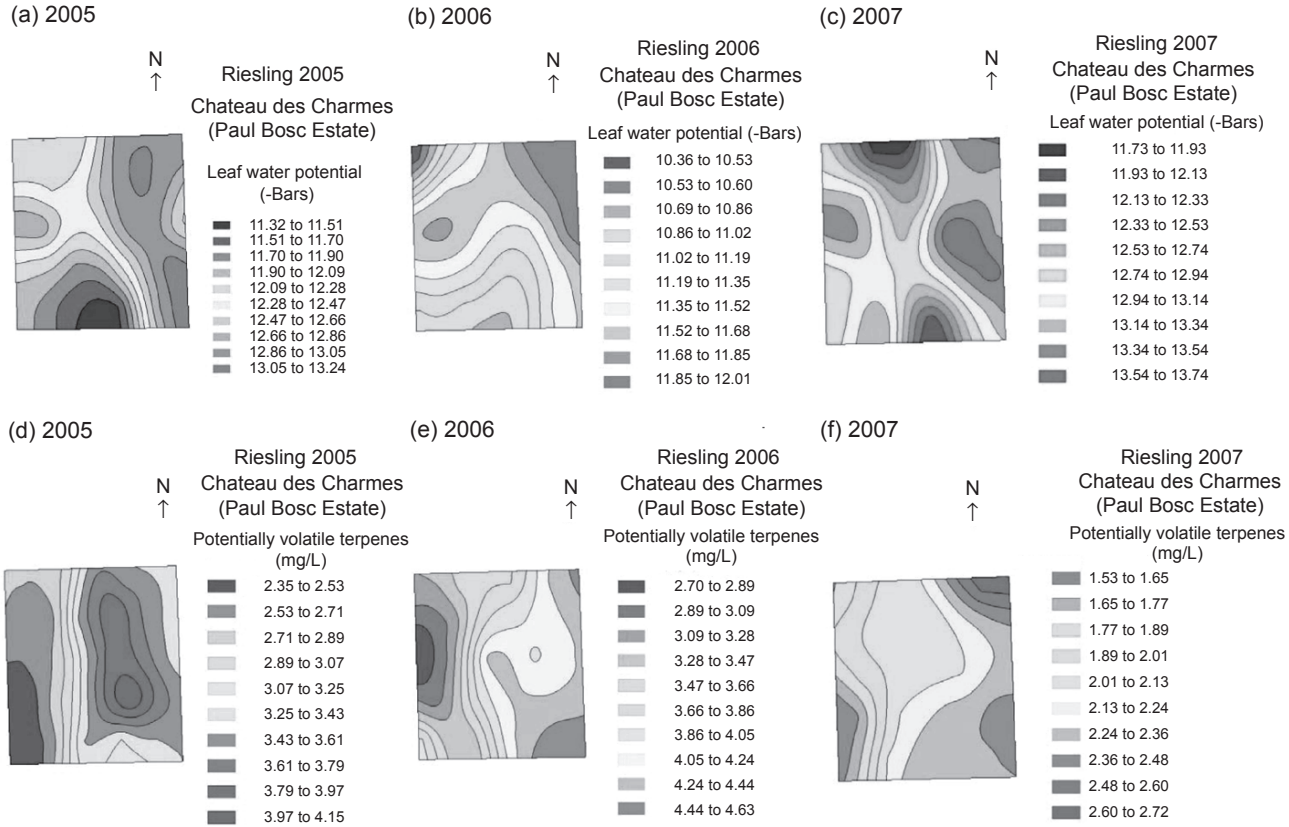

Fig. 11.8 GIS-derived maps of the Riesling block on the Paul Bosc Estate, St David's Bench, ON, 2005–2007. (a) to (c) 6 leaf water potential, 2005, 2006 and 2007, respectively; (d) to (f) potentially volatile terpenes (PVT), 2005, 2006 and 2007, respectively. Note how: (i) spatial variation in both leaf water potential and PVT are temporally stable across the three seasons; (ii) the patterns of leaf water potential and PVT are very similar spatially. These observations suggest that high flavour zones in vineyards are temporally stable and that they are related to vine water status (maps are courtesy of Graduate Research Assistant Jim Willwerth).

were grouped together. Subsequent descriptive analysis showed consistent differences in wines produced between regions within vineyards with lower and higher water status.

11.11 Impact of pre-fermentation decisions and practices

11.11.1 Impact of pre-fermentation decisions on monoterpenes

Harvest date

Many odour-active compounds clearly continue to accumulate in grape berries long after commercial maturity has been reached. Work by Hardy (1970), Bayonove and Cordonnier (1970a), and Gunata *et al.* (1985) showed that terpenes could increase in fruit long after the point of commercial maturity, while Marais and van Wyk (1986) and Marais (1987) indicated that delayed harvest of Bukettraube and Riesling, and Gewürztraminer, respectively, led to higher terpene concentrations in musts and wines. In many cases, these differences could be distinguished by sensory evaluation. Ewart (1987), on the other hand, found that wine quality was reduced in late-harvested Riesling, even though the late-harvested fruit attained the highest total terpene concentration.

In a trial by the author in British Columbia, FVT and PVT increased in three of six *V. vinifera* cultivars with delays in harvest dates between 10 and 20 days (Reynolds *et al.*, 1993). Tasters could distinguish between wines from 'early' and 'late' harvested fruit in five of six cultivars based on aroma, and three of the six based on flavour. In many cases, these tasters indicated that the late harvested treatments had either the strongest muscat and/or strongest floral character.

Pressing

Effective extraction of free terpenes, as well as liberation of free terpenes from their glycosidic precursors, may be achieved through pre-fermentation practices such as skin contact, pressing, and the use of enzymes (Cordonnier and Bayonove, 1981; Strauss *et al.*, 1986; Reynolds *et al.*, 2007b,c). Pressing treatment was shown by Cordonnier and Bayonove (1979) as well as Kinzer and Schreier (1980) to have an impact on terpene concentration in musts. In British Columbia, pressing had no effect on terpenes of Müller-Thurgau, but PVT in press juice of Muscat Ottonel and Gewürztraminer were higher than in their free run fractions (Reynolds *et al.*, 1993). FVT were not affected. Tasters could distinguish between the aromas of Müller-Thurgau and Muscat Ottonel wines made from free run and press juice. Despite the lack of difference between treatments in FVT and PVT concentration in the Müller-Thurgau, tasters indicated that the press wines had stronger floral character than the free run wines. Concentrations of FVT and PVT decreased substantially from the berry to the juice stage; losses in FVT were 52%, 41%, and 22% for Muscat Ottonel, Gewürztraminer, and Kerner, respectively, and losses in PVT were 16%, 52%, 13%, and 28% for Müller-Thurgau, Muscat Ottonel, Gewürztraminer, and Kerner, respectively.

Skin contact

Use of skin contact is controversial among some winemakers. Bayonove *et al.* (1976), Marais and van Wyk (1986), Marais (1987), and Marais and Rapp (1988) have indicated that use of and duration of skin contact can appreciably increase the concentration of specific terpenes in must and wine. Skin contact increased FVT and PVT in three of four *V. vinifera* cultivars (Reynolds *et al.*, 1993). Only Siegerrebe produced large enough aroma and flavour differences to allow tasters to distinguish between the two treatments. There was no clear indication of whether skin contact resulted in more muscat or floral character in the aroma or flavour.

11.11.2 Impact of pre-fermentation decisions on methoxypyrazines

Methoxypyrazines are very stable compounds due to their chemical nature and therefore are very difficult to remove or reduce in wines. The use of oak can reduce vegetal/herbaceous aromas/flavours in wines with high methoxypyrazine concentrations by a straight masking effect. Aiken and Noble (1984) found that 'vegetal' characteristics decreased in Cabernet Sauvignon wines after oak ageing. Pickering *et al.* (2006) found that treatment with oak chips reduced the intensity of 'lady bug taint' (Pickering *et al.*, 2004), which is a taint associated with high concentrations of IPMP. Methoxypyrazines are highly extractable in wines (Roujou de Boubée *et al.*, 2002). Since a majority of methoxypyrazines are found in grape stems and skins, limited skin contact and exclusion of stems or leaves from the winemaking process can reduce the amount of methoxypyrazines extracted into the resultant wines. Lighter pressing regimes can also reduce methoxypyrazine concentrations. Press wines contain higher concentrations of methoxypyrazines (Roujou de Boubée *et al.*, 2002). This suggests that a fraction of methoxypyrazines is extracted from the skins during rigorous pressing. Settling and clarifying white wine must decreases methoxypyrazine concentration by half (Roujou de Boubée *et al.*, 2002). They demonstrated that thermovinification could also prove to be useful by reducing IBMP from 29 to 67%. However, heating wines is not widely practised in premium wine production. Since it can be shown that winemaking procedures probably have a minimal impact on pyrazine concentrations, it is most appropriate to focus on viticulture practices for methoxypyrazine management.

11.12 Conclusions

The four basic pillars of the Cool Climate Paradigm of winegrowing are: (i) keep the fruit warm; (ii) keep the leaves exposed to sunlight; (iii) achieve vine balance; (iv) Minimize water stress. Practices that are particularly relevant to these basic pillars include hedging and basal leaf removal; training systems; vine spacing; crop control and shoot density; vineyard floor management; and irrigation. The results of many of our experiments suggest that fruit exposure, canopy manipulation, pre-fermentation practices, and vineyard site may influence monoterpene

concentration of berries and juices of several *V. vinifera* cultivars. These differences can sometimes be confirmed organoleptically in wines. A failure to find good agreement between analytical and sensory results may be due to variability among judges, but may also be ascribed in part to the confounding taster response to non-floral monoterpenes such as α-terpineol. This underscores the need to follow up work of this nature with gas chromatographic analyses of wines, to overcome problems of this nature. Our work and that of others has demonstrated that not only do vineyard and cellar practices often affect aroma compound concentration in berries and juices, but very often organoleptic evaluation may confirm these analytical results. Our specific conclusions to date are: (i) PVT are more responsive to viticultural and oenological practices than FVT; (ii) FVT and PVT are rarely correlated with soluble solids, TA, or pH, thus cannot be predicted by standard harvest indices; (iii) losses in FVT and PVT can occur between the berry and juice stages, hence the desirability of skin contact; (iv) FVT and PVT concentrations can, in some cases, be related to wine tasting results.

11.13 References

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12

Precision Viticulture: managing vineyard variability for improved quality outcomes

R. G. V. Bramley, CSIRO Sustainable Ecosystems, Australia

Abstract: Vineyards are variable. In spite of this, conventional approaches to winegrape production infer that the optimal practice is to apply uniform management strategies in vineyards on the assumption of homogeneity. The availability of a suite of technologies, which have collectively become known as Precision Viticulture, provides grapegrowers and winemakers with the means to move away from this 'one-size-fits all' approach. Instead, management may be targeted within vineyards according to variation in their inherent characteristics and particular goals in terms of grape yield and quality. Thus, better patches can be exploited whilst weaker areas may be improved. This chapter provides a summary and review of recent research into vineyard variability and the development and adoption of Precision Viticulture. A particular feature of this work has been its focus on improved fruit quality and wine flavour and aroma outcomes.

Key words: spatial variability, remote sensing, yield mapping, vineyard survey, terroir.

12.1 Introduction

Beauty is in the eye of the beholder – or so the saying goes! Much the same might be said about wine; a wine that is appealing to one person may be relatively unappealing to another. This is at least a part of the reason why many winemakers regard the uniformity of a parcel of fruit, in addition to its sensory attributes, as a key aspect of its quality. The reason for this is that it is arguably much easier to transform a uniform parcel of fruit into a wine of desired style, flavour and aroma than a parcel which, if examined carefully, might cover a range of quality 'grades'.

Furthermore, ability to meet market demand, especially for higher priced wines, is more profitable than having to sell wines that are downgraded on account of a relatively small proportion of the input fruit not having the desired attributes. Of course, one would sensibly want to keep higher quality fruit out of low price point parcels and instead process it for higher value wines. Accordingly, a key objective of modern viticultural management is to ensure not only that the fruit in the vineyard meets the specified grade, but also that within a given harvested parcel, all of the fruit meets specification. Against this background, it is not unreasonable to ask whether winemaker expectations of grapegrowers (i.e. uniform parcels of fruit) are reasonable?

Land is variable and, because of this, so too are vineyards (Fig. 12.1). Indeed, the variability of land is apparent irrespective of whether it is being examined from space, an aeroplane, the top of a hill or under one's feet. It is no surprise, therefore, that grapegrowers and winemakers have known about vineyard variability for as long as they have been growing grapes and making wine. However, without tools to either measure or manage variability, the production of winegrapes, like that of other crops, has proceeded under the assumption that the optimal strategy is to use a single uniform management practice. Indeed, all of the vineyards shown in Fig. 12.1 were under uniform management at the time that the photographs were taken (the oldest was taken in 2001) in spite of the obvious variations that they illustrate. The recent availability of the suite of tools which have become known as Precision Viticulture (PV), of which yield monitors, remote sensing, the global positioning system (GPS) and geographical information systems (GIS) are the most important, readily provides the basis for seeing that a single uniform management strategy may be far from optimal and also for targeting management in response to vineyard variation. Thus, the fundamental objective of PV is to use detailed information about the inherent biophysical characteristics and performance of a vineyard, at high spatial resolution, as the basis for viticultural management and decision making. The rationale behind this approach is that, through the use of such spatial data, any given management decision has an increased likelihood of delivering the desired or expected outcome compared to a similar decision made in the absence of such information. PV therefore seeks to promote greater control over the processes of growing grapes and making wine and, in particular, to assist grapegrowers in meeting winemaker demands.

Before such targeted management can be implemented, some key questions need to be addressed. These include: Is the magnitude of the variation large enough to warrant a change from the conventional uniform approach? Is the spatial variation temporally stable? Can the causes of this variation be identified and, if so, are they manageable? If the answer to each of these questions is 'yes', exactly how should management be targeted in response to the variation? In other words, is our viticultural knowledge sufficient to support a decision to change management and, if not, how might such knowledge be acquired? Finally, even if all the tools and knowledge needed for implementation of targeted management are available, is such implementation going to be economically (and/or environmentally) beneficial?

Fig. 12.1 Vineyard variability can readily be seen when comparing different vineyards such as these in (a) Saint Emilion, France, (b) Stellenbosch, South Africa, (c) Marlborough, New Zealand, (d) New South Wales, Australia, (e) Lake County, California and (f) Languedoc, France. Within-vineyard variability is also evident in each of these vineyards. All of them exhibit topographic variation; sometimes subtle (c), but often much less so (e). Variation is evident in vine vigour, whether in well-established (a, f) or younger (b) vineyards. The vineyard in (d) is highly frost prone in the low-lying area, but is unaffected elsewhere, while Trought *et al.* (2008) have quantified marked differences in vine performance between vines growing in Marlborough's predominant gravelly soils and the silty hollows that dissect them (c). Given the steep slope in (e), one might wonder at the basis for the uniform irrigation management that has been implemented.

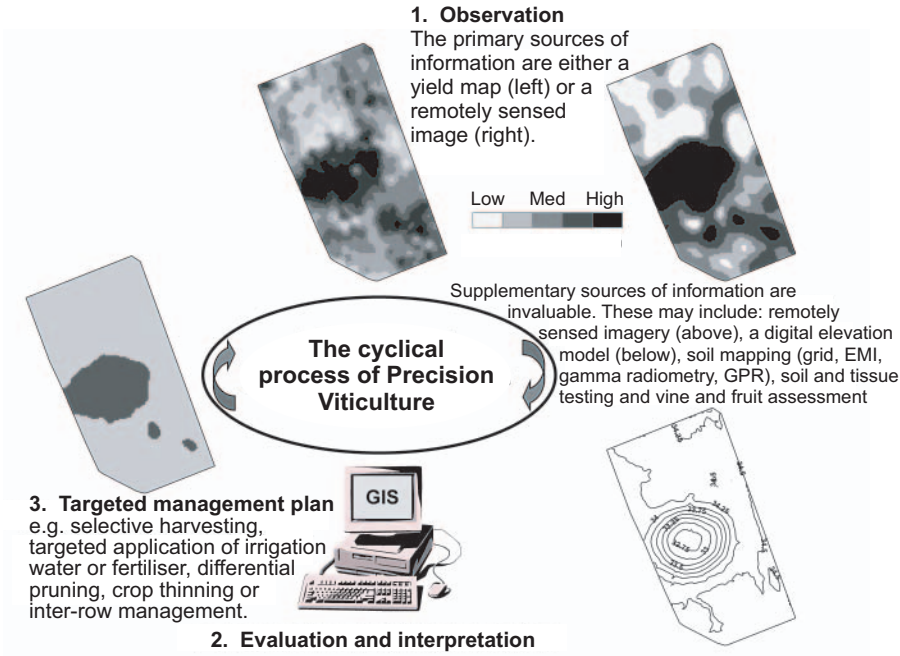


Fig. 12.2 The cyclical process of Precision Viticulture. In this example from a 4.3 ha Shiraz vineyard in the Padthaway region of South Australia, understanding of observed variation in yield is promoted by access to remotely sensed imagery and an elevation model. Coupled with appropriately targeted sampling of fruit, this led to the development of a selective harvesting strategy and, subsequently, differential management of the mid-row to reduce vine vigour in the 'hollow'. Bramley and Hamilton (2007a) provide further details. EMI = electromagnetic induction; GPR = ground penetrating radar.

Since the tools of PV are well understood, and in many cases have been commercially available for several years, we will not dwell on their mode of operation here; readers interested in these aspects are referred to Proffitt *et al.* (2006) and Tisseyre *et al.* (2007) amongst numerous other references. Rather, the focus here is on recent research that has sought to address the questions posed above; to understand and quantify variability in vineyards; and to provide a basis for the commercial adoption of PV following the schema shown in Fig. 12.2. This schema reflects the fact that the implementation of PV is a continuous cyclical process of *observation*, *evaluation and interpretation*, leading to implementation of a *targeted management plan* followed by further observation…. Implicit in Fig. 12.2 is the idea that information collected at time *A* has at least some predictive utility in respect of later decisions made at times *B* and *C*, an important aspect of PV that is discussed more fully in Section 12.2.3.

Because much of the research targeted at vineyard variability has been undertaken in Australia, this chapter has a necessarily Australian flavour. However, it is important for readers to understand that vineyard variability is not a peculiarly Australian problem (Fig. 12.1). Indeed, work conducted in France (e.g. Tisseyre *et*

al. 2001), Spain (Arnó *et al.*, 2005), Chile (Ortega and Esser, 2003), New Zealand (Trought *et al.*, 2008), Canada (Reynolds *et al.*, 2007) and the USA (Johnson *et al.*, 2003a; Cortell *et al.*, 2005) confirms this, as does the comparative analysis of yield variation in Australia and Europe reported by Taylor *et al.* (2005). Readers are therefore encouraged to approach this chapter with a view to considering *how* an understanding of vineyard variability might assist with wine production in their vineyards or regions, rather than thinking about *whether* vineyard variability is something that they should be concerned about – it is!

12.2 Spatial variation in grape yield and vine vigour

Access to accurate estimates of yield prior to harvest are valuable to both grapegrowers and winemakers, whether operating in so-called 'New World' or 'Old World' countries. In the former, the scheduling of winery intake, sometimes from many separate growers, is a critical part of vintage planning and management. In the latter, strict rules may govern the yields that may be produced if the resultant wines are to be certified, such as under the French *Appellation d'Origine Controllée* (AOC) system (e.g. Goode, 2005). In both cases, the assumption of a yield–quality interaction is a key driver, even though this interaction may not be well founded (Trought, 2005). Considerable effort has therefore gone into understanding yield variability and the prediction and regulation of yield (e.g. May, 1972; Clingeleffer *et al.*, 1997; Dunn *et al.*, 2001, 2004; Dunn and Martin, 2003, 2004; Serrano *et al.*, 2005; Trought, 2005). However, the main focus of this work has been on understanding, accommodating and regulating seasonal (i.e. temporal) variation. Almost without exception, this work has not considered the importance of within-vineyard (i.e. spatial) variation – which, of course, one might expect to impact significantly on the estimation of yield (see Section 12.5.3). Understanding the nature and extent of within-vineyard variation is the key aspect of PV and, in the context of promoting uniformity in parcels of fruit delivered to wineries, is one area where PV can deliver significant commercial benefits (e.g. Bramley *et al.*, 2005b).

As indicated above, yield estimation is by no means the sole reason for interest in measuring and monitoring within-vineyard variation. However, of the tools currently available for assessment of variation in vine performance, as opposed to other biophysical attributes of vineyards (soils, landform, etc.), remote sensing of vine vigour and yield monitoring are the most readily available. They also provide key data layers for understanding how vineyard variability might be managed.

12.2.1 Remote sensing of vine vigour

'Wine is a product of sunlight' (Smart and Robinson, 1991) – something that was probably recognised hundreds of years ago. In spite of this, it has only been within the last 15–20 years that early work which demonstrated the potential utility of remotely sensing the reflectance of sunlight by vegetation (e.g. Birth and McVey,

1968; Jordan, 1969; Rouse *et al.*, 1973; Tucker, 1979) has been applied to agricultural management (see Lamb, 2000 and references therein). The thinking behind this application is based on the idea that plants integrate the effects of their biophysical environment (e.g. climate, soil, the incidence of pests and diseases) and express them through their canopy characteristics. Thus, canopies that are small as a consequence of some aspect of the plant's biophysical environment – perhaps low soil water availability, for example – reflect less light than larger, less constrained canopies. Consistent with this idea was the early application of remote sensing in viticulture, which was initially focussed on identification of vineyard areas in California affected by phylloxera (*Daktulosphaira vitifoliae*; Johnson *et al.*, 1996). Thus, vines affected by phylloxera tend to have smaller, more stressed canopies and so areas in imagery identified as being of low apparent vigour are those worth inspecting first on the ground for the presence of phylloxera. The Phylloxera and Grape Industry Board of South Australia has recently extended this idea and uses remote sensing as a key part of its biosecurity activities (Edwards *et al.*, 2004). Hall *et al.* (2002) provide a review of other potential applications of remote sensing to viticultural management.

Much of the early work on vineyard remote sensing (e.g. Johnson *et al.*, 1996) made use of the normalised differential vegetation index (NDVI; Rouse *et al.*, 1973), which is commonly used in other agricultural remote sensing applications (e.g. Lamb, 2000; Hall *et al.*, 2002). NDVI is calculated from measures of the reflectance of sunlight at wavelengths which correspond to the red (R) and infrared (IR) parts of the electromagnetic spectrum (NDVI = [IR–R]/[IR+R]). More recently, and especially in Australia, the main focus has been on the use of the so-called *plant cell density* (PCD) or *simple index* (PCD = IR/R) as an indicator of variability in vine vigour and, thus, as the basis for a targeted management strategies (Fig. 12.2) such as selective harvesting (e.g. Bramley *et al.*, 2005b) or differential pruning (Proffitt and Malcolm, 2005). Both NDVI and PCD provide a measure of the photosynthetically active biomass (PAB) and are therefore correlated with the size and health or absence of stress (i.e. vigour) of vine canopies.

Although commercial remote sensing of vineyards is available in Chile (Ortega and Esser, 2005), South Africa (Strever, 2007) and the USA (Johnson *et al.*, 1996, 2003a), and exploration of its potential has occurred in France (Tisseyre *et al.*, 2007), the use of remote sensing in commercial viticulture is arguably more widespread in Australia than in other winegrowing countries. Typically in commercial Australian applications, PCD is provided as a qualitative measure and is derived from the use of an airborne multispectral digital video system, as described by Lamb (2000) and Proffitt *et al.* (2006), with an on-ground resolution (i.e. pixel size) of 50 cm. Further to the work of Lamb *et al.* (2004), such imagery is acquired at *véraison* ± 2 weeks. The relatively high (50 cm) resolution is used to facilitate removal of non-vine signals from the imagery. However, Lamb *et al.* (2004) have argued that coarser imagery (3 m pixels) comprising mixed pixels (sometimes referred to as 'mixels') of vine and non-vine signals is at least as good for predicting grape phenolics and colour at harvest as the finer resolution imagery. Irrespective of the image resolution, anecdotal evidence suggests that vineyard

managers prefer to see imagery presented as a 'smoothed' map surface as illustrated by the example shown in Fig. 12.2. This was produced from data acquired at 50 cm resolution, the non-vine signal was removed following a proprietary classification algorithm, and the resultant vine-only pixels locally averaged to give a smooth surface comprising 2 m pixels – the same resolution used in yield mapping (Bramley and Williams, 2001). As Fig. 12.2 clearly illustrates, variation in vine vigour (and yield) shows marked spatial structure; that is, the variation is not random but, rather, there are clearly identifiable areas of lower and higher vigour (and yield).

Commercial PCD images tend to be qualitative because they do not include targets of known reflectance. As a consequence, such imagery expresses variation in PAB relative to the total range in any individual image with this range normalised to 256, 1024 or 65 536 digital numbers (i.e. colours) depending on whether the imagery is 8, 10 or 16 bit. Because of this, and also the fact that variation in PCD may be driven by variation in a range of biophysical factors, remotely sensed imagery must be 'ground-truthed' against meaningful measures of vine performance (Proffitt *et al.*, 2006), even if these are simple qualitative inspections of the size of the vine and/or its canopy. However, Proffitt and Malcolm (2005) have correlated PCD with absolute measures of canopy surface area in the Margaret River region of Australia and used this as a basis for identifying zones for the purposes of differential irrigation. Similarly, Johnson *et al.* (2003b) correlated NDVI with measures of leaf area index (LAI – in this case measured as m^2 leaf area m^{-2} ground area and then converted to a per vine basis knowing the planting density) in the Napa Valley. Meanwhile, Johnson (2003) demonstrated that, over the course of a single season, increases in LAI (m^2 m^{-2}) were tracked by increases in NDVI. This work enabled Johnson *et al.* (2003a) to use Ikonos satellite imagery to derive estimates of LAI as an input to an irrigation scheduling tool. Hall *et al.* (2008) have since shown that the reason that this works is the strong correlation between NDVI and planimetric canopy area (defined as the canopy width multiplied by the discrete cordon length along the trellis wire). The ability of remotely sensed imagery to predict LAI is presumably therefore a function of canopy architecture. Interestingly, both Johnson *et al.* (2003a,b) and Proffitt and Malcolm (2005) were working with vertically shoot positioned (VSP) canopies, as were Dobrowski *et al.* (2003) who correlated PCD with dormant pruning weights measured post-harvest (and therefore post-image acquisition). Because they are generally confined, the planimetric area of VSP canopies, and corresponding NDVI or PCD values, can be expected to be relatively low. This is important because as Hall *et al.* (2008) point out, the curvilinear relationship between LAI and NDVI or PCD means that, for relatively large, sprawling canopies (high values of NDVI), small differences in high NDVI values may correspond to larger differences in LAI. Thus, while Dobrowski *et al.* (2002) have shown the PCD relationship with LAI to be considerably less curvilinear than that for NDVI, the use of such imagery for quantitative prediction of vine attributes, without ground-truthing, should be treated with caution.

Lamb *et al.* (2004) demonstrated the potential utility of remote sensing as a

predictor of fruit quality, although the relationships between NDVI and their measures of colour and phenolics at harvest (R^2 = 0.19–0.35) have not proved strong enough to warrant commercial use of remote sensing for quantitative prediction of fruit quality. Stamatiadis *et al.* (2006) fared little better with a ground-based sensor which also measured NDVI. Nevertheless, accompanied by on-ground assessment of fruit, remotely sensed imagery is commonly used as a simple basis for vintage decision making. It has proven to be a cheap source of information which may promote financial benefits which greatly outweigh its cost of acquisition (Bramley *et al.*, 2003, 2005b).

While remote sensing of vine vigour is without doubt a useful and cost-effective tool, the fact is that absolute values of the various indices (PCD, NDVI, etc.) are rarely supplied by commercial providers. In the absence of the use of 'calibration squares' of known reflectance and subsequent adjustment of the data to accommodate seasonal and atmospheric effects, the same 'value' or colour in images obtained in two different years may mean quite different things. Furthermore, robust interpretation of the data is dependent on it being 'ground-truthed' against measured vine attributes. With respect to implementation of the schema shown in Fig. 12.2, there are some scenarios where these possible limitations are outweighed by a lack of alternative means of acquiring high-resolution spatial data describing vineyard performance; hand harvested vineyards are one example. However, in mechanically harvested vineyards, remote sensing is more appropriately regarded as a supplementary source of such information (Fig. 12.2) to complement the data provided by a yield monitor. An important reason for this is that yield monitor data do not need ground-truthing.

12.2.2 Monitoring and mapping of within-vineyard yield variation

The first published winegrape yield map was obtained during the Australian vintage of 1999 (Bramley and Proffitt, 1999), although Wample *et al.* (1999) had earlier used the same yield monitoring system in juice grapes. Like the example shown in Fig. 12.2 (and those from around the world referred to in Section 12.2.3), yield variation in the map of Bramley and Proffitt (1999) shows marked spatial structure; the range of variation in such yield maps obtained in blocks under uniform management is typically ten-fold (Bramley and Hamilton, 2004, 2005). Recently, other yield monitoring systems have become available and have been used for several years in both research and commercial application (e.g. Bramley and Hamilton, 2004, 2007a; Bramley *et al.*, 2005b; Proffitt *et al.*, 2006). Proffitt *et al.* (2006) provide brief descriptions of how these yield monitors work, and a protocol detailing how the data derived from them should be transformed into useful maps was detailed by Bramley and Williams (2001). Refinements to this mapping procedure were provided by Bramley (2005b) and Bramley *et al.* (2008b). In brief, yield is measured 'on-the-go' as the harvest progresses, with the measurements geo-referenced through the use of a differential global positioning system (dGPS; accurate to approximately ±50 cm in Australia). The data are then interpolated onto a smooth map surface using a geostatistical method known as

'local block kriging' (e.g. Isaaks and Srivastava, 1989; Webster and Oliver, 2007). An example of such a map is given in Fig. 12.2 along with an indication of how yield maps may form the basis for the identification of zones of characteristic vineyard performance (see Section 12.2.3); numerous other examples are provided by Proffitt *et al.* (2006).

Given the very low additional cost of yield monitoring over and above the cost of harvesting (Bramley, 2007a), and that having some information about vineyard variability is clearly much better than having none, one might take the view that since the crop has to be harvested, it may as well be yield monitored at the same time. Furthermore, and as indicated earlier, an important feature of yield monitor data is that it does not need to be ground-truthed. Indeed, whereas the total yield recorded by a yield monitor should be adjusted to the tonnage recorded at the winery weighbridge (Bramley and Williams, 2001), it cannot be ground-truthed in the same way that remotely sensed imagery ought to be, since once the crop has been harvested, it is no longer available for re-measurement by whatever means!

12.2.3 Temporal stability in patterns of within-vineyard variation and the delineation of management zones

Figure 12.2 seeks to illustrate how the implementation of PV is a continual cyclical process in which observations (remote sensing, yield monitoring, etc.) are used to inform the targeting of management followed by further observation which enables evaluation and, if necessary, refinement of the targeted management strategy. It follows, therefore, that the value of the spatial information highlighted in Fig. 12.2 is considerably enhanced if it has a predictive value; that is, if it contributes towards a future management decision. Indeed, such data are probably not worth collecting at all in the absence of any predictive value, and this is the basis for the (uninformed) criticism of yield mapping – that by the time a grower has a yield map, it is too late for him or her to do anything with it. The question as to whether the patterns of within vineyard variation are stable in time is therefore a critical one, because the answer to it governs the predictive utility of data collected this year for decisions made in later years. It also cuts to the heart of the merits of using such data as the basis for 'zonal viticulture' (Bramley and Hamilton, 2004; 2005, 2007a).

A significant constraint to the adoption of Precision Agriculture (PA) in broadacre cereal cropping has been a perception that, irrespective of any economic benefit that may accrue in a given year, investment in PA may not be cost-effective because the magnitude of inter-annual variation in crop yields may be greater than the range of intra-annual (i.e. within field) variation. Inter-annual variation in crop yield is driven primarily by seasonal variations in climate and the use of rotation of crops with differing responses to such climate variations. Thus, Whelan and McBratney (2000) posed the 'null hypothesis of precision agriculture' which states that 'given the large temporal variation evident in crop yield relative to the scale of a single field, then the optimal risk aversion strategy is uniform management.'

Bramley and Hamilton (2004) used yield maps collected over two to four vintages in the Sunraysia, Coonawarra and Clare Valley districts of Australia as a basis for testing two approaches to assessing temporal stability in patterns of yield variation. In Coonawarra, the first of these, which is based on *k*-means clustering of yield map data (Cuppitt and Whelan, 2001), gave similar results to the second probabilistic approach. The latter is based on the method of Diker *et al.* (2004), and involves assessing yield obtained in any given year against a target for that year, and using the results from several years to construct a yield probability surface. In the case of the Clare Valley data of Bramley and Hamilton (2004), this method was shown to offer the advantage of enabling incorporation of expert knowledge – in this case of a cold flowering period in one of the years of the study. However, providing the yield maps have been produced using kriging, the method based on *k*-means clustering has the advantage of enabling tests of the statistical significance of differences in cluster means based on the mean kriging variance (Bramley and Hamilton, 2004). This is also the same as the method which allows other data layers such as high-resolution soil maps (see Section 12.4.1) to be clustered with yield maps and remotely sensed imagery for the purpose of identifying vineyard management zones (e.g. Proffitt *et al.*, 2006) as a basis for 'zonal viticulture' (Bramley and Hamilton, 2005). The same methodology (albeit with a small modification in which the *median* kriging variance is used for tests of significant differences between cluster means) has recently been promoted for general application in PA (Taylor *et al.*, 2007).

Irrespective of the methodology used to assess temporal stability in the patterns of spatial variation, the results of Bramley and Hamilton (2004) from contrasting vineyards (3–7 ha; Merlot, Cabernet Sauvignon, Ruby Cabernet), each under uniform management, provide strong evidence in support of the view that, in the absence of targeted intervention, within-vineyard patterns of yield variation are temporally stable. Thus, in Coonawarra for example, Bramley and Hamilton (2004) clustered yield data from three seasons and thereby identified low, medium and high yielding zones.[1] Importantly, the low yielding zone was always low yielding and the medium zone always medium yielding, even though the mean yield for the block varied substantially due to climatic variation between the seasons. Similarly, results obtained from a second Sunraysia vineyard (8 ha; Cabernet Sauvignon) over three years (Bramley and Hamilton, 2007a), from another in the Padthaway region (4.5 ha; Shiraz; Fig. 12.2) over seven years (Bramley, 2007b; Bramley and Hamilton, 2007a) and from a large (44.5 ha; Shiraz, Gewürztraminer, Riesling) Eden Valley vineyard (Bramley and Williams, 2007), provide additional strong evidence of temporal stability in patterns of variation in yield and vigour. Further evidence of stability in patterns of yield variation is provided by work conducted by other workers in an Australian vineyard on the Mornington Peninsula (7 ha; Pinot Noir) over three years (Oke *et*

[1]Here, and throughout this chapter, 'low', 'medium' and 'high' are used as relative terms only. Thus, a low value in one block, could be of similar magnitude to a high value in another. Typically in a 3 zone solution, the mean yield in the medium zone will be approximately equal to the mean block yield.

al., 2007), a Spanish vineyard (5 ha; Pinot noir) over three seasons (Arno *et al.*, 2005) and in a small (1.2 ha; Shiraz) French vineyard over seven seasons (Tisseyre *et al.*, 2008). The fact that the results from this last study in a non-irrigated vineyard are so similar to those conducted elsewhere in irrigated blocks is significant given the presumption (e.g. Ojeda *et al.*, 2005) that the use of irrigation might be expected to smooth out vineyard variation, and in view of the importance attached to soil water availability in 'Old-World' studies of terroir (see Section 12.6) conducted in non-irrigated vineyards.

In contrast to these other studies, Reynolds *et al.* (2007) found that spatial variation in yield in a Canadian vineyard (4 ha; Riesling) 'changed substantially' over four years even though spatial variation in 'vine size', as determined by measures of pruning weight, was relatively stable; the reasons for these results were unclear. Note, however, that in this Canadian work, assessment of temporal stability was apparently based on inspection of maps (which do indeed appear variable) rather than the type of spatial analysis used by Bramley and Hamilton (2004) or Tisseyre *et al.* (2008). It is also worth pointing out that, while the ratios of yields obtained in low, medium and high yielding zones in the Coonawarra study of Bramley and Hamilton (2004) were approximately constant over three years, Oke *et al.* (2007) calculated a 'temporal coefficient of variation' and found that the magnitude of yield variation within their zones varied across seasons. They therefore noted that temporal stability in spatial patterns of yield variation did not infer a spatially uniform magnitude of between-season variation; the magnitude of between-season variation was greater in some areas which tended to lie within the low yielding zones. Bramley and Hamilton (2004) obtained somewhat similar results at their Clare Valley site, and ascribed these to the influence of extreme temperatures at flowering exerting greater impact on some parts of this vineyard than others. Nevertheless, the overwhelming evidence from contrasting vineyards from around the world is that, even though the range of within-vineyard variation is typically of the order of ten-fold (e.g. 2–20 t ha^{-1}), within-vineyard patterns of spatial variation in grape yield and vine vigour are temporally stable. It is on this basis that, with respect to winegrape production, the null hypothesis of PA (Whelan and McBratney, 2000) may be rejected. It is also the basis on which 'zonal viticulture', that is, the targeting of management to zones within vineyards (Fig. 12.2), may be justified (Bramley and Hamilton, 2004, 2005).

12.3 Spatial variation in fruit and wine quality

Delineating zones of characteristic vineyard performance on the basis of measures of grape yield and vine vigour is all very well but, as inferred in Section 12.1, winemakers are likely to be more interested in the identification of zones based on the sensory attributes of the fruit. Further, both grapegrowers and winemakers would want to avoid a situation where the targeting of management based on variation in yield causes either a deleterious effect on fruit and wine quality *per se*, or on their ability to manage it. Two key questions therefore are: Do patterns of

spatial variation in fruit quality match those for variation in yield, and are these therefore also temporally stable?

A significant issue in addressing these questions is that, at the time of writing, there is still no commercially available on-the-go sensor of any index of fruit quality. One consequence of this is that studies of spatial variation in fruit quality attributes have been dependent on manual sampling and subsequent laboratory analysis. As a result, production of maps of attributes of fruit quality and other spatial analysis has relied on fewer data, or a lower sample *support* (e.g. Webster and Oliver, 2007), than are available for studies of yield variation using yield monitors, or vigour variation using high-resolution remote sensing. Nevertheless, several studies from around the world have demonstrated that indices of fruit quality measured at vintage are indeed spatially variable (Johnstone, 1999; Bramley, 2001, 2005a; Ortega and Esser, 2003; Bramley and Hamilton, 2005, 2007a,b; Cortell *et al.*, 2005; Bramley and Williams, 2007; Reynolds *et al.*, 2007; Tisseyre *et al.*, 2008; Trought *et al.*, 2008), albeit with a lower range of variation than in the case of yield. Of these, the studies of Bramley and Hamilton (2007a), Cortell *et al.* (2005) and Reynolds *et al.* (2007) were the only ones to also consider impacts on wine quality, although Bramley *et al.* (2005b) provided compelling commercial examples of the impact of vineyard variability on wine style and price point. The only studies to assess multi-year data were those of Bramley (2005a), Bramley and Hamilton (2007a), Bramley and Williams (2007), Bramley *et al.* (2005b) and Tisseyre *et al.* (2008).

Bramley (2005a) examined spatial variation in a range of fruit quality attributes in the Coonawarra and Sunraysia vineyards studied by Bramley and Hamilton (2004) using a sampling intensity of approximately 26 vines ha^{-1}. Variation in sugars (in this case defined in terms of baumé), titratable acidity and juice pH measured immediately prior to vintage was not as strongly spatially structured as yield and the total range of variation was considerably less; Tisseyre *et al.* (2008) report very similar results. In terms of the range of variation reported, these results were also similar to earlier non-spatial work on fruit quality variation (e.g. Krstic *et al.*, 2002). A plausible reason for the lower range of total variation is that in a mature crop, sugar accumulation, for example, will tend towards a maximum, and as a consequence, spatial variations may therefore be 'smoothed out', a suggestion that is supported by the observation of a large *nugget effect* (e.g. Webster and Oliver, 2007) in the fruit quality data of Bramley (2005a) relative to the total variation. However, Bramley (2005a) found that measures of colour and phenolics were more strongly spatially structured and, along with berry weight (a surrogate measure of skin surface area), closely followed the patterns of variation in yield; clustered maps of the full range of quality indices studied also had a spatial structure similar to that for yield. Bramley (2005a) therefore concluded that the patterns of variation in fruit quality attributes were, for all practical purposes, the same as those for variation in yield. This is an important finding for two reasons. First, it demonstrates that variation in fruit quality attributes is not random, as is implicit in the work of Krstic *et al.* (2002); this has significant implications for vineyard sampling and crop assessment (Section 12.5.3). Second, it suggests that

the delineation of management zones can proceed using yield/vigour sensors, even when the sole objective is to define a basis for selective harvesting (e.g. Bramley *et al.*, 2005b) – a finding with particular significance given the lack of an on-the-go fruit quality sensor, and the understandable reluctance of commercial practitioners to engage in large and expensive sampling campaigns. Tisseyre *et al.* (2008) reached a similar conclusion, albeit based more on the reduced range of variation in fruit quality attributes compared to yield or vine size. However, although it seems reasonable to assume that the *patterns* of spatial variation in quality attributes follow those for yield, the *rank order* of the zones with respect to fruit quality may not be constant (Bramley, 2005a). Thus, whereas the medium yielding zone is always the medium yielding zone, in some years it may be of medium quality, but in other years higher quality and, in yet others, lower quality. It is therefore essential that even when zones have been robustly delineated for the implementation of a selective harvesting strategy, a thorough pre-harvest assessment of the fruit is undertaken to ensure that individual parcels are consigned to the appropriate product stream.

Cortell *et al.* (2005) delineated 'vigour zones' in two Willamette Valley (Oregon) vineyards on the basis of vine growth during the period between budbreak and *véraison* as measured by hand sampling at an intensity of around 220 vines ha^{-1}. They then examined the effects of vigour on berry composition based on samples collected at approximately 25 samples ha^{-1} and also investigated the possible role of soil properties in causing the vigour differences. Although this is a similar sampling intensity to that used by Bramley and Hamilton (2004) and Bramley (2005a), because the two vineyards studied by Cortell *et al.* (2005) were small (1.28 and 0.21 ha), the total number of soil and berry samples collected was below that considered necessary (e.g. Webster and Oliver, 2007) for the geostatistical analysis subsequently employed in this work. Nevertheless, Cortell *et al.* (2005) inferred an effect of vine vigour on berry chemistry based on zone means, with lower vigour zones being associated with berries with higher concentrations of proanthocyanidins in skins and wines into which larger proportions of tannins were extracted. Their results are therefore consistent with much of the other work in which measures of vine vigour have formed at least a part of the basis of delineation of vineyard zones, with subsequent identification of between-zone differences in fruit attributes (e.g. Bramley *et al.*, 2005b; Bramley and Hamilton, 2007a; Tisseyre *et al.*, 2008).

At contrasting sites in the Sunraysia and Padthaway regions of Australia, Bramley and Hamilton (2007a) found marked differences in the quality of fruit and wine produced in zones delineated using a variety of spatial data collected over several years. Unlike the Coonawarra work (Bramley, 2005a), these quality differences were temporally stable in both pattern and rank order and, as with the Coonawarra study, the variation appeared attributable to variation in the land underlying the vineyard (see Sections 12.4 and 12.6).

In contrast to the results obtained in the aforementioned studies, Reynolds *et al.* (2007) found that, although various indices of fruit quality were spatially variable, there appeared to be no relationship between patterns of fruit quality or yield

variation; there was no suggestion of temporal stability in either. Thus, the apparent impact of a range of vineyard attributes (e.g. clay content of the soil) was not temporally stable, leading Reynolds *et al.* (2007) to conclude that vintage and wine age (i.e. time in the cellar) had a greater impact on sensory attributes than vine size or soil texture. The reason for the marked difference in the results of Reynolds *et al.* (2007) compared to other workers is unclear, although it is noteworthy that this Canadian work was conducted in a much colder climate than that conducted elsewhere. It is possible that the effects of winter vine damage, which is common in some cold-climate winegrowing regions, may exert effects which are greater than those driven by soil and landform variation (see Section 12.4 below). Examination of this issue would be valuable since it would inform the extent to which adoption of PV in such climates is worthwhile; Reynolds *et al.* (2007) concluded that it was not.

Overall, one can conclude that indices of fruit and wine quality are spatially variable with the patterns of variation tending to follow those for variation in yield and vigour, although perhaps not in very cold winegrowing regions. However, while such knowledge can inform commercial decision making such as the implementation of selective harvesting, temporal instability in fruit quality ranking means that yield–quality interactions remain poorly founded (Trought, 2005). Therefore it is hoped that sensors of fruit quality are soon available which permit the mapping of quality attributes with the same sample support as is possible with yield mapping. In addition to promoting targeted management, such sensors would also promote greatly improved understanding of the factors that control fruit and wine quality, and therefore their management.

12.4 The drivers of vineyard variation

As noted in the Section 12.1, land is variable and so too is its productivity; Figs 12.1 and 12.2 provide viticultural illustrations of this. Runge and Hons (1999) have ascribed variability in broadacre crop production to the strong influence of soil properties, rooting depth, nutrition, agronomic management and the interaction of these factors with climate. Other workers (Moore and Tyndale-Biscoe, 1999; Machado *et al.*, 2002), also working predominantly in rain-fed broadacre cereal cropping systems, have also identified plant available stored water and seasonal rainfall as having the greatest effect on yield, and certainly a greater effect than a variable supply of nitrogen (N). Mallarino *et al.* (1999) showed that the correlation coefficients between yield of grain crops in the mid-west of the USA and indices of soil fertility were highly skewed towards the low end of a range between 0 and 0.77. Machado *et al.* (2002) therefore advocated that 'seasonally stable factors' such as soil texture should provide the basis for identification of management zones in which targeted management of 'seasonally unstable factors' such as N availability and the incidence of pests and disease should be practised. Other studies have highlighted the importance of topographic variation in addition to variable water supply in a range of annual cropping systems from around the world

(Kaspar *et al.*, 2003; Grove *et al.*, 2005; Reuter *et al.*, 2005; Persson *et al.*, 2005). It should therefore be no surprise that variation in soil and topography should have a substantial impact on variation in vineyard performance (Figs 12.1 and 12.2), especially since in perennial systems, a measure of variation in performance made at any given time may be a reflection of the integration of effects exerted over several seasons. Trunk circumference, for example, provides an indication of vine vigour over the life of the vine (potentially many years), whereas pruning weight is a measure of vigour during the preceding season. In a vineyard in which a spatially variable soil constraint exists, similar patterns of spatial variation in trunk circumference might be expected (e.g. Trought *et al.*, 2008).

Bramley (2001, 2003a) and Bramley and Lanyon (2002) have demonstrated how variation in soil depth drives yield variation in the Coonawarra vineyard studied by Bramley and Hamilton (2004). Variation in soil depth in this vineyard is in turn controlled by variation in topography (Bramley, 2003a). The soil in this vineyard is known as a *terra rossa* and comprises an undifferentiated light red clay over limestone. Consistently low yielding areas occur on 'ridges' where the soil is shallow and plant available water is consequently low, whereas consistently higher yielding areas occur in 'hollows' where the soil is deeper (Fig. 12.3). However, 'ridges' and 'hollows' are used simply as relative terms here since the total range in elevation in this vineyard is only 1.2 m; for all intents and purposes, most people would regard it as flat! Similarly, Bramley and Hamilton (2007a) demonstrate the effect of variation in soil and topography on the yield and quality of fruit and wines in contrasting vineyards in Sunraysia and Padthaway (Fig. 12.2) with ranges in elevation of 2.2 and 4.6 m. The yield and quality variation measured in another Sunraysia vineyard (Bramley and Hamilton, 2004; Bramley, 2005a) may also be associated with soil and topographic variation – in this case, the depth and texture of a predominantly sandy topsoil in a strongly duplex soil with a heavy clay sodic subsoil. In the latter example, shallow topsoils and therefore enhanced exposure to the hostile subsoil occurred in the lower-lying parts of the vineyard (elevation range of 17.3 m). Meanwhile, in a Clare Valley vineyard (elevation range of 13 m), Bramley (2003b) demonstrated how yield was being impacted by soil and groundwater salinity which in turn was controlled by proximity to a surface water dam and/or the natural drainage lines.

In a somewhat similar study, albeit confined to eight rows of a Sauvignon Blanc vineyard in Marlborough, New Zealand, Trought *et al.* (2008) demonstrated a strong association between trunk circumference (i.e. vine vigour) and soil properties. The Marlborough wine region is predominantly associated with the active floodplain of the Wairau River which is dominated by gravelly soils dissected by thin 'strips' of much siltier soil (Fig. 12.1c). Vines growing in the silty soils were more vigorous than those in the gravels and, presumably as a consequence of this, vine phenology was more advanced (by 11 days at harvest) where vines were growing in gravels compared to silty soils.

In the aforementioned examples, the role of varying topography in driving vineyard variation was predominantly associated with its effect on soils. However, in a much more steeply sloping 44 ha vineyard planted to mixed varieties in

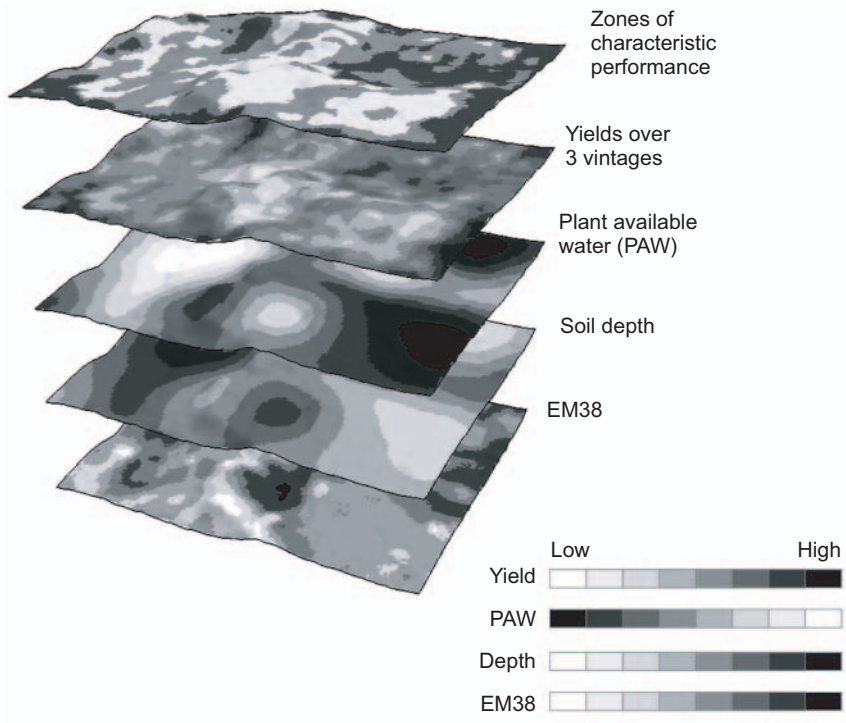


Fig. 12.3 An illustration of the use of high-resolution soil survey and elevation modelling to inform understanding of vineyard variability and delineation of management zones in a 7 ha vineyard in the Coonawarra region of Australia. EM38 data were shown to correlate closely with soil depth (Bramley, 2003a). Along with measures of clay content (Bramley and Janik, 2005), these data were used to estimate plant available water (PAW). When incorporated with yield maps and the elevation model, it can be seen that higher ground is associated with shallow soils, leading to lower PAW (smaller volume of soil in the root-zone) and, thus, lower yield. Clustering of these data promotes identification of zones in which targeting of irrigation may be appropriate, or perhaps selective harvesting of fruit (Bramley and Hamilton, 2005).

Australia's Eden Valley (range in elevation of approximately 100 m), Bramley and Williams (2007) demonstrated a more direct effect of topography on vine performance. Aspect was shown to control potential photosynthesis (incident irradiation) and, through interactions with slope and landscape position, to also control temperature to the extent that the range of variation in modelled season degree days (base of 10 °C) varied by approximately 185. If this vineyard were planted to a single variety, such a range in heat accumulation could be expected to equate to approximately 10 days in terms of harvest date. Further analysis of these data (Bramley, Ouzman and Boss, unpublished) suggests that in the 22 ha of this vineyard that is planted to Riesling, the higher priced (i.e. better quality) wines come from southeast facing slopes in the warmer eastern end of the block.

Very little work on soil impacts on within-vineyard variation has been pub-

lished outside of Australasia, with the conclusions derived from much of this being equivocal by comparison with the Australasian work. An exception is the work of Olivier and Conradie (2008) who examined the effects of soil type and irrigation on the sensory attributes of wines produced from vineyards in the coastal (Stellenbosch) and inland (Breede River) regions of South Africa. This work was conducted in vineyards in which distinct soil types could be identified with the dominant differences being in terms of soil water holding capacity and drainage. Marked soil type-dependent differences in the sensory attributes of both Sauvignon Blanc and Cabernet Sauvignon were noted in both regions with these being moderated, but not eliminated (cf Ojeda *et al.*, 2005) by the use of 'scientifically scheduled' irrigation. In contrast, Ortega and Esser (2003) looked at variation in several indices of soil fertility in Chilean vineyards but did not relate these to variation in yield or vigour. Davenport *et al.* (2003) found that the use of variable rate fertiliser management based on soil and tissue testing did not reduce yield variation in juice grapes in Washington State. Cortell *et al.* (2005) assessed variation in available water holding capacity in their Oregon vineyards and inferred that it was driving the variation in vine vigour that they measured, although this was apparently based on visual interpretation of maps rather than rigorous spatial analysis. Meanwhile Reynolds *et al.* (2007) attempted to relate a range of soil properties to fruit, vine and wine characteristics in an Ontario Riesling vineyard but found few strong correlations. They also failed to find consistent soil texture effects on berry, must or wine composition, although clay soils were reported to increase mineral aroma. Again, it is possible that the contrast between the results of Reynolds *et al.* (2007) and those of others may be due to the cold temperatures experienced in Ontario and the possibility of vine damage during the dormant winter period.

12.4.1 Tools for vineyard survey at high spatial resolution

Just as commercial grapegrowers and winemakers are unlikely to be willing to sample fruit and vines with the intensity employed in much of the vineyard variability research, neither they nor their contractors are likely to be willing to survey their soils at high resolution using conventional techniques such as digging pits. However, as Bramley (2003a) and Bramley and Janik (2005) have illustrated, conventional approaches to soil (and plant tissue) testing are inadequate tools if the aim of the survey is to understand variable vineyard performance or to develop a basis for targeted management. Thus, the 75 m regular grid-based soil sampling which was the industry standard for Australian vineyard soil survey for many years (e.g. McKenzie, 2000) is inadequate as a tool for identification and understanding within-vineyard variation. Taylor and Minasny (2006) developed a protocol for converting such grid survey data into continuous maps which adds considerably to the utility of such grid-based survey data, especially at the whole farm scale. However, with respect to within-vineyard variation, just as there is demand for yield monitors, remote sensing and the elusive on-the-go quality sensor, so too is there a need for tools for surveying vineyard soils at high spatial resolution.

All of the above-mentioned Australasian studies have employed the use of high-resolution soil survey using electromagnetic induction and the EM38 instrument in particular. In the studies where topographic variation was also assessed, digital elevation models derived from the use of real-time kinematic GPS (accurate to 2–3 cm in the *x*, *y* and *z* planes) were produced. Proffitt *et al.* (2006) and Tisseyre *et al.* (2007) discuss the application of these tools to vineyard assessment and their mode of operation in more detail, along with other tools such as gamma ray spectrometry and ground penetrating radar (e.g. Hubbard *et al.*, 2002; Lunt *et al.*, 2005; Pracilio *et al.*, 2006). These are less commonly used in viticulture although in some situations where induction methods are ineffective, such as vineyards with trellises supported by steel posts (Lamb *et al.*, 2005), they may provide a useful alternative to induction-based methods. Corwin and Plant (2005 and references therein) provide an extensive review of the application of electromagnetic soil sensing to broadacre agriculture; a recent review of high resolution on-the-go soil sensors is provided by Adamchuck and Viscarra-Rossell (2009). Lanyon *et al.* (2007) have highlighted the possibility of integrating several such tools with sensors of vine canopy condition in a single survey platform; Fig. 12.3 illustrates how the data derived using them might be integrated as a basis for delineating vineyard management zones.

12.5 Options for targeting management within vineyards

Early adopters of PA in broadacre cereal systems have overwhelmingly used yield mapping and other tools, such as high-resolution soil survey and elevation modelling, to promote the variable rate application (VRA) of inputs to the production system; the most common use of VRA has been for nitrogen (N) fertiliser application (e.g. Lowenberg-DeBoer, 2003; Griffin and Lowenberg-DeBoer, 2005; Godwin *et al.*, 2003). In contrast, the early adopters of PV have placed much greater focus on the use of remotely sensed imagery, with or without yield mapping, as a basis for 'selective harvesting' (Bramley *et al.*, 2005b). One reason for the difference between the focus of wine grape producers compared to their broadacre counterparts producing bread wheats, for example, has been the stronger quality imperative which drives the wine industry; to most people, a sliced white loaf is simply that. Another is the typically higher rate of use of inputs such as fertilisers in broadacre systems. A third is the uncomfortable realisation that hits many farmers and advisers presented with yield maps or remotely sensed imagery – that our viticultural understanding may not be as good as we thought it was, with many so-called 'best-management practices' being less than optimal. Figure 12.4 provides a good illustration of this. It shows a map of the gross margin derived from grapegrowing in a 33 ha Chardonnay vineyard near Griffith in New South Wales. This was produced from a yield map and knowledge of the costs of production for this particular block; the yield map was obtained as part of a PV extension project involving a group of growers from the region. As can be seen, a significant part of the vineyard was either operating at a loss as a grapegrowing

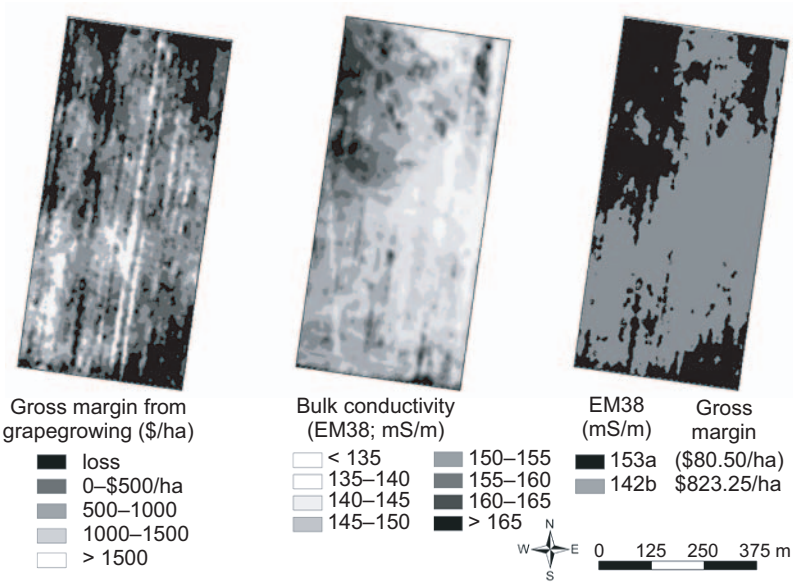


Fig. 12.4 Variation in the gross margin obtained from grape growing in a 33 ha Chardonnay vineyard near Griffith, New South Wales, and the possible association between poor returns and a soil constraint. The fact that simple comparison between the gross margin and EM38 maps is not immediately suggestive of similar patterns of variation indicates that careful ground truthing of the EM38 map would be essential to understanding the cause of the variation in vineyard performance. On the other hand, *k*-means clustering of these maps does suggest that poor returns are associated with a soil constraint. As an alternative to the focus on gross margins, the data underlying these maps suggest that the effective costs of production vary markedly. Thus, whilst the average cost of production (± $15/t; Aus$ used throughout) leads to gross margins in the $500–1000 range, the effective cost of production in areas returninga loss was more than $45/t higher than the average for the block as a whole. Areas returning more than $1500/t had effective costs of production that were more than $45/t below the average.

entity, or was generating returns that were considerably less than what was required of the vineyard. Also shown is a map of apparent electrical soil conductivity obtained through the use of an EM38 sensor. When the gross margin and EM38 maps are clustered together, the poorly performing areas of this vineyard appear to be those in which the values recorded by the EM38 are significantly higher ($p < 0.05$) than in the remainder of the block. Although this simple analysis does not prove that a soil constraint is limiting production, it is strongly suggestive of the idea that closer investigation of vineyard soil properties and consideration of a targeted soil amelioration strategy may be highly beneficial. Unsurprisingly, the information contained in Fig. 12.4 gave the vineyard manager considerable food for thought! Thus, acquisition of high-resolution soil survey data may provide a powerful impetus to examining opportunities for ameliorating constraints to production through targeted management and, thus, for addressing poor and/or variable financial performance in vineyards.

12.5.1 Selective harvesting

Selective harvesting is defined as the split-picking of grapes at harvest according to different yield/quality criteria with consignment to different product streams in order to exploit the observed variation in vineyard performance (Bramley and Hamilton, 2005). Several commercial examples exist in which very large increases in profitability have been achieved (e.g. Bramley *et al.*, 2005b), perhaps the best known example being from a Cabernet Sauvignon vineyard in Western Australia. In this case, an increase in the retail value of production of more than $40 000/ha was achieved simply by running two chaser bins next to the harvester after using remotely sensed imagery, with limited analysis of fruit quality attributes by way of ground-truthing, to identify two harvesting zones (Bramley *et al.*, 2003). Through use of this strategy, rather than consigning all the fruit to a product selling for $19/bottle, a significant tonnage was allocated to a product worth $30/bottle.

Of course, the use of selective harvesting is by no means confined to vineyards that use mechanical harvesting. As illustrated by an example from the Clare Valley (Bramley *et al.*, 2005b), selective harvesting is just as possible when harvesting is done by hand. Indeed, many Old World producers might argue that they have been selectively harvesting for centuries, albeit without aids such as high-resolution remotely sensed imagery or hard data to support their management decisions; I recently met a winemaker in the Mosel Valley, Germany, who produced 15 different Rieslings from a total area of just over 7 ha with the assignment of fruit to product stream based solely on a (presumed) intimate knowledge of the various small blocks which made up his vineyard, coupled with intensive sensory assessment of fruit prior to harvest.

It is also important to point out that selective harvesting may not be an appropriate strategy in all cases. It is only going to offer advantage when the commercial opportunity exists to parcel fruit from the same vineyard into separate products – whether on the basis of price point or wine style – or in such a way that gives the winemaker greater control over the winemaking process, such as when blending different ferments. Thus, selective harvesting is not a strategy that should be pursued without close cooperation between grower and winemaker. Whether or not such differentiation is supported in the intended target markets is also a key consideration. Scollary *et al.* (2008) discuss these issues in more detail. Bramley and Hamilton (2007a) also provide an example in which a clear case for selective harvesting existed in a Sunraysia vineyard, but in which winery logistics (a single crusher and large fermentation tanks) mitigated against implementation of such a strategy. Many grapegrowers and winemakers faced with this predicament have concluded that selective harvesting will not work for them. However, this view is based almost entirely on the notion that entire vineyard blocks need to be harvested at once. On the contrary, parcelling like sections of different blocks offers operations with large fermentors the opportunity to take advantage of selective harvesting. It is therefore to be hoped that further research and refinement of winery supply chain logistics and harvest scheduling will incorporate spatial data at the intra-block scale rather than regarding the problem of harvest scheduling as one of deciding the order in which whole blocks are harvested, as is currently the case in much of the Australian industry.

12.5.2 Other targeted management

Leaving aside the fact that targeted management strategies other than selective harvesting are relatively uncommon, the opportunity nevertheless exists for the inputs to viticultural production to be targeted, whether these be fertilisers, soil amendments, sprays, irrigation water or even labour. Indeed, some wineries do not wish to pursue selective harvesting, perhaps because such a strategy does not fit well with their product profile or due to logistical constraints such as limited processing capacity (e.g. Bramley and Hamilton, 2007a). In the example shown in Fig. 12.4, production was directed at a successful commercial table wine and the vineyard manager and winemaker had little interest in pursuing selective harvesting with a view to allocating some of the fruit to higher (or lower) value products. On the contrary, their objective was to maximise the tonnage of fruit produced at a quality appropriate for the intended product. In this example, targeted amelioration of a soil constraint, perhaps informed by whole-of-block experimentation (see Section 12.5.4) in addition to soil analysis and the information shown in Fig. 12.4 may therefore be appropriate.

Proffitt and Malcolm (2005) provide an example from Margaret River in which, having identified vineyard management zones using remotely sensed imagery, irrigation water was applied differentially with the aim of managing vine vigour. Based on a reduced need for leaf plucking and shoot thinning, this strategy was considered highly cost-effective. In another example from the same authors, remotely sensed imagery was used as the basis for allocating labour in a vineyard that was being cane-pruned, in such a way that all staff ended up pruning a similar number of vines of similar degree of difficulty (i.e. vigour), thereby earning a similar amount. This strategy helped maintain morale amongst staff, kept truancy low and, when its cost was compared to the estimated costs of pruning had the vineyard been treated as a single uniform entity, a saving of nearly 12% was reported. Herein lies another potential reason as to why growers may wish to consider the targeted management of inputs – reducing or stabilising the costs of production against a background of generally increasing costs and/or declining terms of trade.

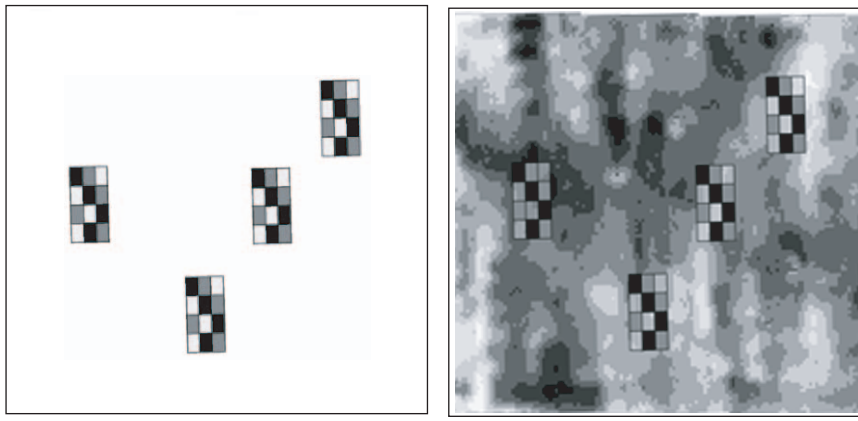
12.5.3 Implications for vineyard sampling and crop assessment

Intuitively, if a vineyard can be divided into zones of characteristic performance with statistically significant differences in zone means for measures such as yield (e.g. Bramley and Hamilton, 2004) or trunk circumference (Trought *et al.*, 2008), and these zones are not of equal area, then a better (i.e. more precise) estimate of the mean for the whole block should be obtained by weighting the estimate for the whole block on the basis of the proportional area of the zones. Pragmatically, this may mean that if 30 samples are to be taken to estimate yield in a two zone vineyard, for example, the number of samples allocated to each zone should be weighted according to the proportional area of the zones. Proffitt and Malcolm (2005) and Proffitt *et al.* (2006) provide an example from the McLaren Vale region of South Australia which strongly supports this suggestion. Bramley (2001)

provided another example in relation to assessment of fruit suitability for harvest based on measurement of maturity (assessed in terms of baumé (Bé) in this example) in the Sunraysia vineyard studied by Bramley and Hamilton (2004) and Bramley (2005a). The mean baumé (vintage 2000) obtained from 120 target vines was 12.1 °Bé with a range of 10.9–15.7 °Bé. When the sample was taken from four randomly chosen vines (at this time, the recommended procedure was to take five bunches from each of four vines), the estimated block mean was 12.4 °Bé, this difference presumably reflecting the reduced precision of a smaller number of samples. However, when the four samples were allocated separately to areas corresponding to the two zones subsequently identified by Bramley and Hamilton (2004), estimates of 13.7 and 12.5 °Bé were obtained which, in respect of a decision about the timing of harvest, corresponds to roughly one week. In similar work conducted in Cabernet Sauvignon and Zinfandel vineyards in the Napa Valley, Greenspan and O'Donnell (2001) obtained very similar results and concluded that, whereas knowledge of vineyard variability did not necessarily have any impact on sampling effort, targeting the sampling to zones certainly led to improved understanding of vineyard performance. Overall, whereas Krstic *et al.* (2002) found no difference between a range of sampling strategies based on random sampling (i.e. ignoring spatial variation), these examples illustrate that spatial variability can have a marked impact on vineyard sampling activities. Many viticulturists use the same target vines for regular assessment of vineyard status. Although this strategy certainly supports understanding of the production system, it is suggested that the location of such target vines should be chosen carefully.

12.5.4 Whole-of-block experimentation

An obvious question facing growers like the manager of the block shown in Fig. 12.4 is: how much should their management be modified when applying differential strategies to different vineyard zones? In situations where more than one option appears viable, how should they select the most appropriate one? One response to this question is that they should do some experiments in their own vineyards. However, if an experiment were to be conducted with the aim of guiding management of any of the vineyards shown in Fig. 12.1, where ought it to be located? This question is somewhat similar to the question addressed in the previous section as to where in the vineyard target vines should be located for the purposes of phenological monitoring, yield estimation or fruit assessment. In Coonawarra (Fig. 12.3) for example, should a trial be implemented in the low, medium or high yielding zone? At Griffith (Fig. 12.4), should a trial be established in the areas operating at a loss, the more profitable areas, or in both? Whatever the answers to these questions, on what basis should the results obtained be confidently translated to locations beyond the experimental area, whether these be other parts of the same vineyards, other blocks in other parts of Coonawarra or the Griffith region, or in vineyards even further afield? These sorts of questions were the inspiration for early work in broadacre cereal production that explored the idea of conducting experiments with highly replicated designs over whole management units (Adams



(a) (b)

Fig. 12.5 Possible locations for a plot-based viticultural experiment (a) in the absence of any knowledge of vineyard variability and (b) when a yield map is available. It is easy to see that in (b) the results of the experiment may be compromised by whether the plots are located in a lower (light grey) or higher (dark grey) yielding area. Equally, the range of yield variation in the area under any group of plots is considerably less than for the block as a whole. If this variation was being driven by a covariate such as clay content, extrapolation of the results from one of the possible experimental locations based on clay content would be problematic since it would only be possible to assess the effect of clay content on yield over a restricted range of clay contents.

and Cook, 1997; Cook *et al.*, 1999). A major driver behind the development of this approach was the realisation that the traditional small plot approach to experimentation may be compromised by inherent variation underlying the experimental area (Bramley *et al.*, 2005a; Panten and Bramley, 2006; Panten *et al.*, 2008, 2009; Fig. 12.5). Another was the somewhat paradoxical realisation that the range of variation in covariates that may be useful in understanding the response to experimental treatments may be too small in a traditional plot experiment to allow the extrapolation of results beyond the experimental area.

Recent work (Bramley *et al.*, 2005a; Panten and Bramley, 2006; Panten *et al.*, 2008, 2009) has sought to address these issues by applying the whole of block approach of Adams and Cook (1997) and Cook *et al.* (1999) to vineyards. An example is shown in Fig. 12.6 in which different mid-row treatments were applied to a 4.8 ha Merlot vineyard in the Clare Valley. The vineyard manager was concerned that vine performance in this organically managed block was being constrained by inadequate nutrition and/or competition for soil water from the inter-row sward. The highly replicated design was readily implemented by vineyard staff, and the manager was very positive about the opportunity it afforded him to see how response to the treatments varied across the block.

Analysis of such experiments is dependent on some complex geostatistics (Bishop and Lark, 2006) but as Fig. 12.6 shows, it promotes robust evaluation of the significance of differences between possible management strategies over the

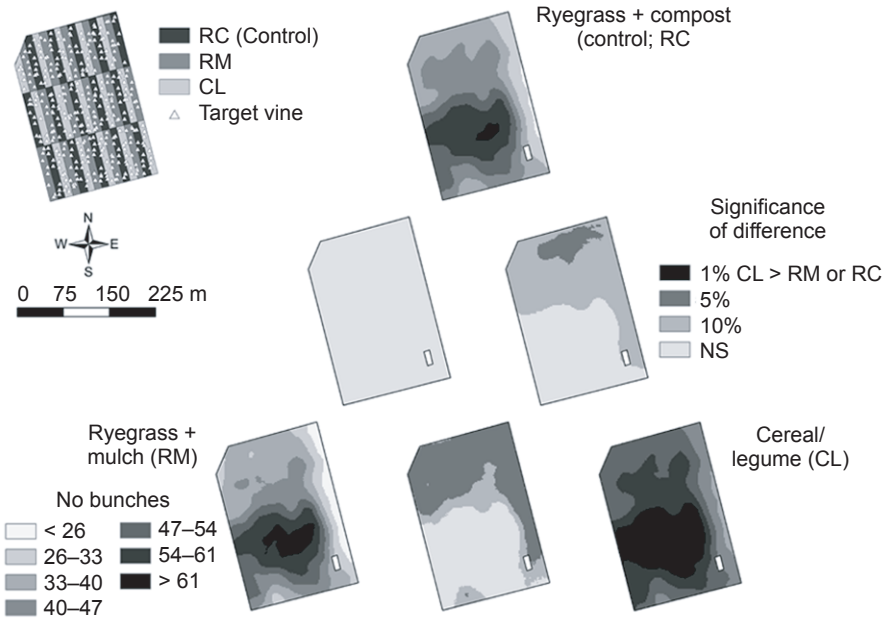


Fig. 12.6 A whole-of-block experiment conducted in South Australia (Panten *et al.*, 2009) in which a highly replicated design (top left) was applied in 2004 over an entire 4.8 ha vineyard to assess the merits of three mid-row management strategies. The method of Bishop and Lark (2006) was used to analyse treatment effects. Treatment-specific responses, in this case assessed in terms of the number of bunches per m of row measured for 378 target vines at vintage in 2006, are shown in the maps in the corners of the triangle, with the significance of the difference between these shown in the maps positioned between them; the same legends apply to each bunch number or difference map. Although there is no significant difference between the RM and RC treatments, the benefits delivered by the CL treatment are markedly spatially variable.

entire management unit in which they may be applied. It therefore overcomes criticism of a lack of statistical rigour in the simple map algebra approach used by Bramley *et al.* (2005a). The spray experiment discussed by Bramley *et al.* (2007) and Bramley (2007b) provides a further example of what is possible using this method. In this example from Tasmania, the whole of block approach enabled identification of the fact that disease pressure (powdery mildew) and the relative merits of two alternative spray programs, are impacted by position in the landscape, a finding which is most unlikely to have been identified had a small plot design been used.

12.6 Precision Viticulture and terroir

As stated in Section 12.1, grapegrowers and winemakers have known about vineyard variability for as long as grapes have been grown and wine made. In 'Old World' countries, this knowledge, combined with a typically small block size

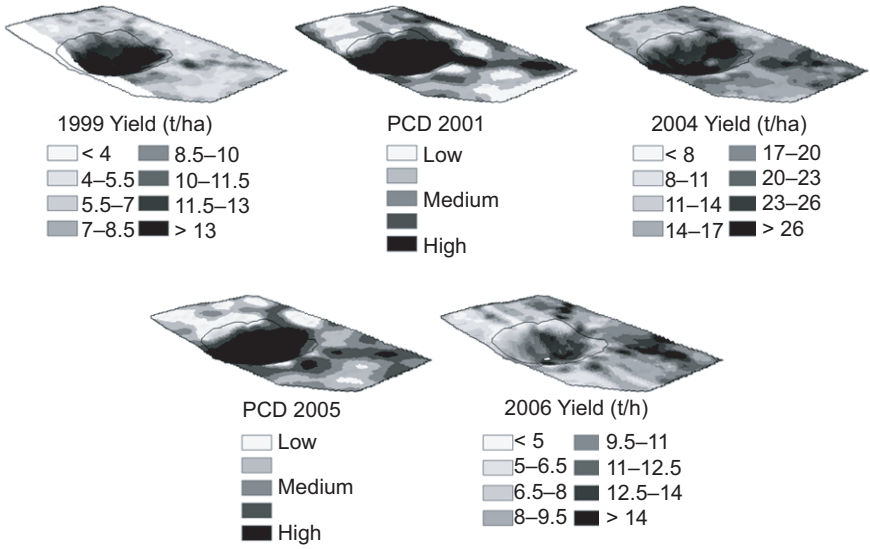


Fig. 12.7 Managing *terroir* in Padthaway. Lucerne was planted in the mid rows in the 'hollow' during 2004. By 2006, it was well established and helping to reduce vine-available soil water. Thus, the area of higher yielding vines producing lower quality fruit than the remainder of the block was considerably reduced compared to previous years (Bramley and Hamilton, 2007a,b).

(often less than 1 ha) has promoted the concept of terroir – the almost mythical connection between a wine and the biophysical environment of the vineyard from which it is derived (e.g. Goode, 2005). There is something of a paradox therefore, in the idea that wines express the *region* from which they come, and in the fact that the French *Appellation d'Origine Controllée* (AOC) system, and others like it in other countries, have *regionally* based rules which prescribe the varieties to be grown, at what yields they may be produced, and which prohibit the blending of fruit sourced from different denominations along with certain practices such as irrigation.

Recognising that the traditional view of terroir being reflective of soil and land attributes (Seguin, 1986; Laville, 1990; van Leeuwen *et al.*, 2004) is appropriate, Bramley and Hamilton (2007b) posed the question as to the scale at which the terroir concept is appropriate? By way of example, they asked whether wine produced from the vineyard shown in Fig. 12.1c is most reflective of the terroir of that particular vineyard, or of either the region (Marlborough) or country (New Zealand) in which it is located? One could ask similar questions about any of the vineyards shown in Fig. 12.1. Thus, irrespective of the differences *between* the vineyards shown in Fig. 12.1, it is not unreasonable to ask whether ignoring the differences *within* them is sensible?

For example, the Riesling vineyard studied by Bramley and Williams (2007) produces fruit that is consigned to various products at three different price points even though it is under uniform management. It should be no surprise therefore that

Bramley and Hamilton (2007a) demonstrated that different sections of vineyards under uniform management produce markedly different wines. However, in the case of the Padthaway vineyard shown in Fig. 12.2, they also suggested that at least some elements of terroir may be manageable. In an update to this work, Bramley and Hamilton (2007b) demonstrated that they are (Fig. 12.7).

As all the vineyard variability work referred to in this chapter demonstrates, the performance of vineyards is variable whether yield, fruit quality, wine quality, wine style or value is the measure of interest. It is therefore ironic that, in the main, the impacts of variability have only been considered at regional scales (e.g. Laville, 1990). An important consequence of this is that few cause and effect relationships between soil and land attributes and wine characteristics have been established. Indeed, the regional focus may be one reason for the lack of importance attached to soil fertility and vine nutrition with respect to fruit and wine quality (Seguin, 1986) while the prohibition of irrigation in many Old World regions may explain the pre-occupation with the effect of soil hydrological properties on wine style and quality (Seguin, 1986; van Leeuwen *et al.*, 2004). Nevertheless, White (2003) has noted the 'scant attention' paid to soil and its complex interaction with wine grapes in the New World, even though there is a more liberal approach to adoption of new technologies in countries such as Australia and Chile. As White (2003) suggests, the true influence of terroir can only be satisfactorily studied for small areas mapped at large scale, an idea that is strongly supported by the vineyard variability research referred to above. Indeed, as the work of Taylor (2004) suggests, the use of PV may promote development of more robust *digital* terroir functions than the regionally derived *site index* of Tesic *et al.* (2002). Thus, although Bramley and Hamilton (2007a,b) have raised questions about the utility of the concept of terroir at regional scales, it is clear that PV has much to offer in promoting robust understanding of the impacts of soil and land attributes on grape and wine production and, coupled to appropriate experimental approaches, how management practices might be modified to gain greater control over fruit and wine quality and, indeed, over at least some of the aspects of terroir.

12.7 Future directions

A important consequence of the cyclical nature of PV (Fig. 12.2) is that it lends itself to incremental as opposed to immediate adoption (Cook and Bramley, 1998). Clearly, having at least some information about the production system is better than having none, but having access to every available technology is not a prerequisite to starting down the PA or PV path. Proffitt *et al.* (2006) provide a useful decision framework for the adoption of PV. Incremental adoption and acquisition of data also mean that, as new sources of data become available, they can complement existing information and give rise to new opportunities.

12.7.1 New sensors

As has been alluded to already, a major opportunity exists for the development of

on-the-go sensors for various indices of fruit quality. Tisseyre *et al.* (2001) noted the development of a prototype sensor for soluble solids (Brix), pH and titratable acidity but, to date, no such sensor has become available. Producers of red wines would no doubt like access to an on-the-go sensor for juice colour (anthocyanins) given the importance of colour as a quality index, and the acknowledged relationship between juice colour and wine quality (Francis *et al.* 1999; Gishen *et al.* 2002). NIR spectroscopy offers one area of opportunity in this regard (Dambergs *et al.* 2003; Gishen *et al.*, 2002), and such an approach may also be suitable for sensing of Brix. One problem facing developers of such technologies is how the sample should be presented to the sensor. In the case of gravimetric yield monitors, this is not a problem given the ready availability of cheap load cells, but the engineering of a suitable solution to sample presentation may be a key aspect of developing a fruit quality sensor. The recent success of Agati *et al.* (2007) and Cerovic *et al.* (2008) in developing a non-invasive method of measuring grape anthocyanins and phenolics is therefore significant. While there is potential for an array of such sensors to be integrated via wireless sensor networks (e.g. Morais *et al.*, 2008) and used for monitoring spatially variable development of fruit maturity, it is to be hoped that a harvester-mounted version of the Agati/Cerovic sensor will be developed soon; a hand-held version has recently been commercialised. Note that the likely time delay between the point at which fruit is harvested and where a measure of fruit quality is obtained will mean that careful attention will need to be paid to the issue of convolution of on-the-go fruit quality data. An appropriate time/position shift will therefore have to be applied to the data as is recommended in the case of yield monitor data (e.g. Bramley *et al.*, 2008b).

Other, as yet uncommercialised, opportunities for proximal on-the-go sensing of vine performance include the use of digital photography for yield prediction (Dunn and Martin, 2004), canopy porosity and juice quality (Praat and Irie, 2003) and infrared thermometry for the detection of stress (Grant *et al.*, 2006; Guisard and Tesic, 2007; Loveys *et al.*, 2007). Such sensors lend themselves to integration onto a common sensing platform (e.g. Lanyon *et al.*, 2007) and/or for deployment simultaneously with routine vineyard operations such as shoot trimming or slashing of the mid-row.

12.7.2 Improved natural resource management, environmental accreditation and product tracking

In broadacre agriculture, where the major focus in PA has been on the targeted application of inputs, there have been claims made in support of the environmental credentials of adopters of PA (e.g. Wrigley and Moore, 2006) based on the intuitive appeal that, by maximising the efficiency of use of inputs such as fertiliser, the opportunity for their export off-farm via run-off or leaching to groundwater is minimised. In Europe, where the use of agrochemicals such as fertilisers and pesticides is increasingly regulated, PA offers a means of demonstrating that best practice has been followed (Stoorvogel and Bouma, 2005). However, demonstrating that PA delivers an environmental benefit with hard data

is difficult given that agricultural activities are generally diffuse sources of pollutants rather than point sources. However, Stoorvogel and Bouma (2005), Wong *et al.* (2006) and Bramley *et al.* (2008a) provide illustrations from a range of agricultural systems of the potential utility of PA approaches to environmental stewardship. To date, there have been no such published examples from the wine industry, no doubt due to the relatively low rate of use of inputs such as nitrogen fertiliser by wine grape growers. However, grapegrowers have a comparatively high rate of usage of pesticides and herbicides and variable rate application of such products can be expected to deliver benefits. Gil *et al.* (2007) describe a system which permits such products to be applied at variable rates based on the ultrasonic detection of canopy size. In view of the increasing regulation of chemical use by agriculture, further development and commercialisation of such systems are likely to be an area of considerable endeavour in coming years.

Likewise, given the increasing scarcity of critical natural resources such as irrigation water, the use of PA/PV approaches to optimise water use, both to minimise its wastage and also reduce the risk of soil salinisation, is an important area of future work. Hedley *et al.* (2005) have described the use of EM38 to optimise the use of irrigation water in pastoral soils, an approach that could readily be translated to viticultural production systems. Such approaches can also contribute to improving decisions as to the location of key infrastructure such as farm dams (irrigation ponds). A major consequence of the work of Bramley (2003b) in quantifying the yield penalty caused by soil salinisation in the vicinity of a dam in a Clare Valley vineyard was that the dam was subsequently maintained at as low a level as possible, with water pumped to storages higher in the landscape with reduced risk of localised salinisation. This decision was made purely in support of a strong desire to maintain the vineyard soil resource and, thus, the long-term sustainability of this Clare Valley property.

Food safety and quality assurance are also gaining increasing prominence in the international wine industry with European consumers, in particular, demanding assurances as to the sustainability of practices used in the production of their food. Recent controversies such as 'food miles' also suggest that perceptions of unsustainable practices may be used as non-tariff trade barriers by competing wine-producing nations. Use of the tools of PV in concert with technologies such as bar coding and advanced supply chain management may offer the means to both monitor and manage such quality assurance issues (Bollen *et al.*, 2001; Praat *et al.*, 2001).

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13

Fungal contaminants in the vineyard and wine quality

E. S. Scott, The University of Adelaide, Australia; R. G. Dambergs, The Australian Wine Research Institute, Australia; and B. E. Stummer, CSIRO Entomology, Australia

Abstract: Fungal diseases such as powdery mildew and bunch rots reduce yield and quality of grapes and affect chemical and sensory properties of wine. The effects of powdery mildew, botrytis bunch rot and ripe rot on grape and wine quality are reviewed. Approaches to detection and quantification of fungal contamination are examined, including enzyme, immunological and DNA assays and near infrared reflectance spectroscopy. Such approaches offer efficient and objective means of quantifying fungal contamination at the winery to facilitate quality control and decisions about use of grapes. Alternatives to conventional fungicides for disease management and their effects on quality are also examined.

Key words: botrytis bunch rot, grey mould, grapevine powdery mildew, grape and wine quality, DNA-based detection, near infrared reflectance spectroscopy, Botrytis laccase.

13.1 Introduction

Diseases caused by fungi have the potential to cause serious crop loss and spoilage of grapes and wine. Disease can affect ripening and reduce yield and quality of grapes, and wines made from fruit contaminated with powdery mildew and bunch rot fungi can exhibit altered chemical composition, resulting in changes in colour, mouthfeel and aroma attributes (Ough and Berg, 1979; Pool *et al.*, 1984; Gadoury *et al.*, 2001b, 2007; Stummer *et al.*, 2003, 2005; Calonnec *et al.*, 2004; La Guerche

et al., 2006; Ribéreau-Gayon *et al.*, 2006a; Meunier and Steel, 2009). The effects of botrytis bunch rot are well documented whereas those due to powdery mildew are less well defined.

Diseases such as powdery mildew and bunch rots can be detected and incidence or severity estimated in the vineyard based on visual assessment of symptoms and signs. Consignments of grapes may then be assessed visually for fungal contamination at the winery to determine if specifications have been met. Both symptom recognition and quantification of fungal contamination in large consignments of machine-harvested fruit are difficult, especially where infection is moderate or slight. Furthermore, tolerance limits for disease vary according to winery specifications, the intended wine style (Godden, 2000; Iland *et al.*, 2004) and grape supply and demand. Enzyme assays, immunological assays, DNA analysis and near infrared reflectance spectroscopy offer objective approaches for measurement of fungal contamination at the winery to improve efficiency and allow better-informed decisions to be made about the use of fruit and must.

Contamination of grapes by fungal pathogens in the vineyard is generally controlled by a combination of cultural practices and fungicide application, sometimes in an integrated program with biological control agents. There is increasing interest in reducing inputs of conventional chemical fungicides and in the adoption of organic and biodynamic viticultural practices. Rigorous scientific evaluation of the effect of such practices on fungal contamination and on grape and wine quality is, therefore, required.

In this chapter, the effects of fungal contamination on grape and wine quality and new approaches for detecting and quantifying contamination are discussed. Recent research on alternatives to conventional fungicides for the control of powdery mildew and bunch rot and their impact on quality is briefly discussed also.

13.2 Common fungal diseases that affect grape and wine quality

Various fungal diseases can affect foliage and bunches in the vineyard, leading to considerable crop loss if conditions are conducive for disease development and control measures inadequate. Disease results from the interaction of the host (the grapevine), the pathogen and the environment, particularly weather (Pearson and Goheen, 1988; Agrios, 2005). Rain or wet conditions are favourable for diseases such as downy mildew and bunch rots, whereas powdery mildew is most severe during mild, overcast weather and does not require rain (Pearson and Goheen, 1988; Emmett *et al.*, 1992; Flaherty *et al.*, 1992; Nicholas *et al.*, 1994).

Powdery mildew is an economically important disease of grapevine worldwide. The disease, caused by *Erysiphe necator* (formerly *Uncinula necator*, sometimes called *Oidium tuckeri*), is recognised by grey-white powdery growth on green tissues of the vine (Fig. 13.1). This superficial growth is composed of hyphae and conidia (asexual spores) of the fungus. The fungus penetrates the epidermal cells

Fig. 13.1 Powdery mildew on berries of Chardonnay.

only and draws nutrients from them; it is an obligate biotroph, meaning that it grows only on living grapevine tissues (Pearson and Gadoury, 1992). Clusters are most susceptible between flowering and berry set (Emmett *et al.*, 1992; Gadoury *et al.*, 2001a, 2003) and berry susceptibility decreases with maturity (Gadoury *et al.*, 2001a; Ficke *et al.*, 2002, 2003). While it does not rot infected berries, severe powdery mildew may inhibit expansion of the epidermis, resulting in splitting and allowing secondary infection by bunch rotting fungi (Pearson 1982, 1988; Emmett *et al.*, 1992). Recently, Gadoury and co-workers (Gadoury *et al.*, 2007) described an inconspicuous form of powdery mildew on berries, termed diffuse powdery mildew, characterised by limited mycelial development without sporulation which results in inconspicuous colonies invisible to the naked eye. Diffuse powdery mildew developed when berries become infected just as ontogenic resistance began to be strongly expressed, about two to three weeks post-bloom in New York State. Berries with diffuse powdery mildew were subject to increased losses due to botrytis bunch rot and other spoilage microorganisms (Gadoury *et al.*, 2007; see Section 13.3.3).

Downy mildew, caused by the fungus-like pathogen *Plasmopara viticola*, also affects green tissue of the vine. The pathogen infects during mild, wet conditions

Fig. 13.2 Botrytis bunch rot on Merlot following inoculation with suspension of *Botrytis cinerea* spores.

in spring, grows within the tissue and sporulates via stomata in warm, wet weather to produce a downy white growth. Berries of susceptible cultivars may be infected until approximately two weeks after bloom (Kennelly *et al.*, 2005), about the time that stomata are converted to lenticels. Once infected, berries raisin and discolour as they mature, then drop (Lafon and Clerjeau, 1988). Although downy mildew causes yield loss through defoliation and loss of flower clusters and berries, negative effects on quality of juice, must or wine have not been documented.

Botrytis bunch rot or grey mould is caused by the ubiquitous necrotrophic fungus *Botrytis cinerea*. This fungus can infect foliage and flowers, and causes mid-season rot of immature berries and grey mould on ripe berries following rain or in humid conditions. Elmer and Michailides (2004) summarised knowledge of pathways of infection of grapes, including colonisation of flowers and necrotic tissue. Recent microscopic studies by Viret *et al.* (2004) have shown that the fungus colonises the receptacle and, to a lesser extent, the stigma and style, of the flower. It then remains quiescent, or latent, in the developing berry until *véraison* (McClellan and Hewitt, 1973; Viret *et al.*, 2004), when increasing sugar content and decreasing concentration of resveratrol and other antifungal compounds may facilitate resumption of fungal growth and disease expression (Jeandet *et al.*, 1995, 2002). Also, colonisation of withered flower parts trapped in bunches may lead to infection of the pedicel or rachis, resulting in necrosis of clusters (Bulit and Dubos, 1988; Nair *et al.*, 1995; Holz *et al.*, 2003; Viret *et al.*, 2004). In wet conditions, *B.*

cinerea may infect berries directly from *véraison* onwards, particularly where tight clusters result in splitting or grapes are damaged by other fungi, infested with insects or damaged by hail or birds. Following rainfall, *B. cinerea* sporulates at the surface of mature, infected berries to produce olive-brown or grey conidiophores with masses of conidia (Fig. 13.2) (Bulit and Dubos, 1988; Emmett *et al.*, 1992, 1994; Nicholas *et al.*, 1994; Elmer and Michailides, 2004).

Disease risk increases with the use of cultivars and management systems that promote compact clusters with frequent berry to berry contact and slow drying (Marois *et al.*, 1986; Vail and Marois, 1991). Other factors associated with susceptibility include the number of pores and lenticels on the berry surface, the amount of wax and thickness of the cuticle, and thickness and number of epidermal and subepidermal layers (Marois *et al.*, 1986; Eibach, 1994; Gabler *et al.*, 2003). In certain cultivars and environmental conditions, particularly humid nights and mornings interspersed with sunny and windy afternoons, intact infected berries become dehydrated and the high sugar concentration arrests development of *B. cinerea*, resulting in noble rot. The characteristics of noble rot and the production of aromatic, botrytised wines are described elsewhere (e.g. Donèche, 1992; Ribéreau-Gayon *et al.*, 2006a) and will not be discussed in detail here.

Various other filamentous fungi may also cause bunch rot, often in combination with *B. cinerea*, particularly after rain or hail damage near harvest. These include *Aspergillus*, *Alternaria*, *Cladosporium*, *Penicillium* and *Rhizopus* species (Hewitt, 1988; Flaherty *et al.*, 1992; Hall and Emmett, 2000). The interaction of *Penicillium* species and *B. cinerea* is discussed in Section 13.3.1. *Aspergillus* and *Penicillium* species, which may produce ochratoxin A and other mycotoxins (Battilani *et al.*, 2006; Leong *et al.*, 2006), are discussed in Chapters 4 and 14. *Colletotrichum acutatum* and *Greeneria uvicola* cause ripe rot and bitter rot, respectively, and combinations of various filamentous fungi, yeasts and bacteria cause sour rot. Phomopsis cane and leaf blight (*Phomopsis viticola*), anthracnose or black spot (*Elsinoë ampelina*), black rot (*Guignardia bidwellii*), black measles or esca (*Phaeomoniella chlamydospora*, *Fomitiporia mediterranea* and related fungi), eutypa dieback (*Eutypa lata*) and botryosphaeria rot and canker (*Botryosphaeria* spp.) can also damage berries (Pearson and Goheen, 1988; Emmett *et al.*, 1992, 1994; Flaherty *et al.*, 1992) but their effects on wine quality are not well documented.

13.3 Effects of fungal diseases on grape and wine quality

Wines made from grapes affected by powdery mildew, botrytis bunch rot and ripe rot, with or without secondary fungi, show reduced quality and/or negative sensory attributes. The effects of these diseases on grapes and wine are discussed below, beginning with botrytis bunch rot.

13.3.1 Botrytis bunch rot

Grey mould or grey rot, and noble rot, caused by *Botrytis cinerea*, have been the

focus of much research, and the effects of the fungus on grape and wine quality are well documented (e.g. Canal-Llaubères, 1992; Donèche, 1992; Ribéreau-Gayon *et al.*, 2006a). Grey mould can cause significant yield loss (Bulit and Dubos 1988; Emmett *et al.* 1992; Flaherty *et al.*, 1992) and results in oxidation, detrimental changes to colour and aroma attributes, and difficulties in filtration and clarification of wines (Iland *et al.*, 2004; Ribéreau-Gayon *et al.*, 2006a,b). Iland *et al.* (2004) recommended that infection (disease severity) of grapes destined for winemaking be less than 5% and preferably zero; some Australian wineries set a maximum tolerance of 3% (Viti-Notes, 2005) or impose a price penalty at 3–6% and reject fruit at 10–12% (Godden, 2000; Edwards *et al.*, 2009).

Effects of botrytis bunch rot on composition of grapes and wine

Grapes infected by *B. cinerea* exhibit altered chemical composition. The fungus oxidises glucose to produce gluconic acid and glycerol, the relative concentrations varying according to growth phase and whether infection develops as grey mould or noble rot. Grey mould is characterised by high concentrations of gluconic acid. Tartaric and malic acids are degraded, as are grape proteins and amino acids (Ribéreau-Gayon *et al.*, 2006a).

As a necrotrophic fungus, *B. cinerea* secretes a variety of lytic enzymes, toxins and other substances which kill plant tissue. Pectinases (pectin methylesterases, polygalacturonases and pectin and pectate lyases), cellulases and hemicellulases break down components of the plant cell walls, causing tissue disintegration and providing carbohydrates as a food source for the fungus (Kars and van Kan, 2004). Several aspartic proteases are also produced, although their role is unclear (Movahedi and Heale, 1990; Kars and van Kan, 2004; ten Have *et al.*, 2004). *B. cinerea* also secretes laccase (*p*-diphenol oxidoreductase, EC 1.10.3.2) which, at the pH of grape juice and wine, oxidises phenolic compounds (anthocyanins, flavanols and hydoxycinnamic acids) to quinones, which then polymerise to form brown compounds (Dubernet *et al.*, 1977; Slomczynski *et al.*, 1995). Laccase activity, therefore, results in browning of white wines and loss of red colour in red wines, and produces an oxidative precipitate (Canal-Llaubères, 1992; Iland *et al.*, 2004; Ribéreau-Gayon *et al.*, 2006a; Claus, 2009).

B. cinerea produces an extracellular glucose polymer called β-glucan (β-(1,3)(1,6)-D-glucan) or cinerean. In liquid culture with glucose, about 60% of this high molecular weight polysaccharide is attached to the hyphae as a capsule and 40% remains in solution (Stahmann *et al.*, 1992). During vinification, β-glucan aggregates in the presence of ethanol and blocks filters (Dubourdieu and Ribéreau-Gayon, 1981; Iland *et al.*, 2004; Ribéreau-Gayon *et al.*, 2006a,b). β-glucan may be broken down by addition of β-glucanase just after fermentation (Villettaz *et al.*, 1984; Canal-Llaubères, 1992; Ribéreau-Gayon *et al.*, 2006b; Claus 2009; see Vol. 2, Chapter 5). As *B. cinerea* is able to metabolise β-glucan when the glucose supply is exhausted (Stahmann *et al.*, 1992), it may function as a food reserve as well as in adhesion to surfaces and water storage (Tenberge, 2004).

Infection of grapes by *B. cinerea* has been associated with increased content of pathogenesis-related proteins (Renault *et al.*, 1996; Bézier *et al.*, 2002), which may

contribute to haze and deposits during storage (Waters *et al.*, 1996). Conversely, Girbau *et al.* (2004) reported decreased content of chitinases and thaumatin-like proteins, as well as an unidentified pathogenesis-related protein, in naturally infected Chardonnay and Semillon grapes and artificially inoculated Chardonnay and Merlot grapes compared with uninfected controls.

While *B. cinerea* is not known to produce mycotoxins harmful to human health, infection can predispose grapes to secondary invaders such as *Penicillium*, *Aspergillus* and *Trichothecium* species which can produce mycotoxins (Serra *et al.*, 2005; see Chapter 4).

Effects of botrytis bunch rot on sensory attributes of grapes and wine

Bunch rot has negative effects on organoleptic qualities of grapes and wine (Pallotta *et al.*, 1998; Darriet *et al.*, 2000; Ribéreau-Gayon *et al.*, 2006a) and may impart mushroom and earthy aromas. The mushroom aroma is associated mainly with 1-octen-3-one and 1-octen-3-ol, whereas the earthy aroma is attributed to (–)-geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) and an earthy–camphor aroma to 2-methylisoborneol. These odorants have been detected in juice of various white and red grapes at concentrations above the perception threshold (Darriet *et al.*, 2000; La Guerche *et al.*, 2006; see Vol. 2, Chapter 4), although 1-octen-3-one and 2-methylisoborneol did not persist through fermentation (La Guerche *et al.*, 2006). The majority of *B. cinerea* isolates tested produced mushroom or earthy–camphor aromas (La Guerche *et al.*, 2006), but none produced detectable geosmin (La Guerche *et al.*, 2007). Geosmin was produced by *Penicillium expansum* on grape juice only after prior culture with 34 of the 156 strains of *B. cinerea* tested, suggesting that the earthy odour arises from dual infection by particular strains of these two fungi (La Guerche *et al.*, 2007).

In addition to production of undesirable aroma compounds, desirable grape aroma compounds are negatively affected. For example, quinones produced as a result of laccase activity react with thiol compounds to diminish the characteristic aroma of Sauvignon blanc wines, and *B. cinerea* has been reported to degrade the monoterpenes linalool, geraniol and nerol of Muscat grapes to less fragrant compounds (Boidron and Torres, 1978; Rapp *et al.*, 1986; Ribéreau-Gayon *et al.*, 2006a). Furthermore, esterases produced by *B. cinerea* can diminish desirable fermentation aromas (Ribéreau-Gayon *et al.*, 2006a).

13.3.2 Powdery mildew

The effects of powdery mildew on grape and wine quality are less clearly defined than those of botrytis bunch rot, although some wineries use a tolerance threshold of 5%, or even 3%, of bunches affected (Viti-Notes, 2005). From the following review of the literature concerning the effects of powdery mildew on yield and quality of grapes and wine, it appears that some adverse effects are not readily detected at such low thresholds.

Powdery mildew affects vegetative growth of the vine; however, the relationship among disease, yield and quality is not clearly defined. Likewise, *Vitis* species

and interspecific hybrids vary in response to infection. Foliar infection reduces photosynthetic efficiency and water use efficiency (Lakso *et al.*, 1982; Pearson and Gadoury, 1992) and has been reported to reduce yield by up to 65%, as well as pruning weight and winter-hardiness (Pool *et al.*, 1984; Pearson and Gadoury, 1992). However, Gadoury *et al.* (2001b), in a four-year study of Concord vines (*V. labruscana*) in New York State subjected to one of three cropping intensities with or without powdery mildew, found that the number of nodes that developed periderm was reduced whereas the number of shoots per node and yield, in terms of weight per vine, per cluster and per berry, did not differ between untreated and sprayed plots. The absence of yield reduction was attributed to the compensatory effect of shoot production on the remaining nodes. Likewise, Stummer *et al.* (2003) showed that cluster and berry weight were generally unaffected. However, Stummer *et al.* (2005) subsequently reported that Chardonnay grapes with severe powdery mildew tended to ripen earlier (by one to two weeks) than healthy grapes and, when harvested at a standard grape sugar ripeness, fruit with powdery mildew severity of >60% exhibited reduced cluster and berry weight (by up to 62 and 40% respectively). Likewise, cluster and berry weight of Cabernet Sauvignon decreased with increasing disease severity in two vintages in France (Calonnec *et al.*, 2004), with the total loss of yield due to powdery mildew in untreated vines estimated to be almost 24%. Ultimately, because the fungus is biotrophic and is generally controlled in viticulture, disease severity rarely approaches levels that would produce detectable effects upon yield.

Effects of powdery mildew on composition of juice and wine

Reports of effects of powdery mildew on quality attributes of grapes and wine also are variable. Total soluble solids content (°Brix or Baumé), a key indicator of quality, increased (Calonnec *et al.*, 2004; McFadden-Smith and Pickering, 2006), remained largely unchanged (Pool *et al.*, 1984; Ewart *et al.*, 1992; Stummer *et al.*, 2003; Calonnec *et al.*, 2004) or decreased (Piva *et al.*, 1997; Gadoury *et al.*, 2001b; Stummer *et al.*, 2003) in grapes with powdery mildew. This variation may reflect, in part, the competing effects of disease in decreasing photosynthesis and in predisposing berries to desiccation, as well as environmental conditions, secondary infection by other microorganisms and crop phenology. Effects on juice pH also have been somewhat variable, but only occasionally has the pH of juice from severely diseased grapes exceeded the threshold of 3.6 (Stummer *et al.*, 2003; McFadden-Smith and Pickering, 2005), above which spoilage is considered to be more likely (Jackson and Lombard, 1993). Titratable acidity (TA) of juice extracted from severely diseased berries is generally greater than that from healthy berries (Pool *et al.*, 1984; Amati *et al.*, 1996; Piva *et al.*, 1997; Gadoury *et al.*, 2001b; Stummer *et al.*, 2003, 2005; Calonnec *et al.*, 2004; McFadden-Smith and Pickering, 2006). Ethanol concentration in wine generally reflects total soluble solids content at harvest (Piermattei *et al.*, 1999; Stummer *et al.*, 2003, 2005) and TA in wine that of TA in grapes (Stummer *et al.*, 2003, 2005). As before, most of the above effects would not be expected at commercially-relevant incidence and severity of powdery mildew.

Colour of infected red and white grapes and the resulting wines can be adversely affected. Gadoury *et al.* (2001b) reported decrease in red colour (absorbance (*A*) at 520 nm) and increase in browning (A_{520}:A_{430}) of juice from Concord grapes with powdery mildew compared with healthy controls. Reduced colour and phenolic content in must from Sangiovese (*V. vinifera*) grapes has also been reported (Amati *et al.*, 1996; Piermattei *et al.*, 1999). Total and extractable anthocyanins decreased with increasing proportion of diseased berries added to batches of healthy berries of Cabernet Sauvignon (Calonnec *et al.*, 2004). Likewise, red wines made from grapes affected by powdery mildew show decreased anthocyanin concentration (Amati *et al.*, 1996; Piermattei *et al.*, 1999; Stummer *et al.*, 2003; Calonnec *et al.*, 2004), with consequent negative effects on colour intensity. In particular, concentrations of delphinidin-3-glucoside, petunidin-3-glucoside and malvidin-3-glucoside in Sangiovese wines decreased, whereas those of caftaric acid and *trans*-resveratrol increased compared with healthy controls (Amati *et al.*, 1996; Piermattei *et al.*, 1999). Similarly, *trans*-resveratrol and piceid (both isomers) increased with powdery mildew severity in red Cariñena grapes (Romero-Pérez *et al.*, 2001). Conversely, for juice of Chardonnay grapes and the resulting wines, total phenolics, hydroxycinnamates and flavonoids increased with increasing disease severity (Stummer *et al.*, 2003, 2005) and brown pigments also tended to increase (Stummer *et al.*, 2003, 2005; McFadden-Smith and Pickering 2006).

The concentration of pathogenesis-related (PR) proteins increases in response to powdery mildew. Infected berries exhibited increased activity of chitinase, β-1,3-glucanase (Jacobs *et al.*, 1999), osmotin and thaumatin-like proteins (Monteiro *et al.*, 2003). Girbau *et al.* (2004) reported increased concentration of chitinases and the thaumatin-like protein, VvTL2, in juice from Chardonnay grapes with severe powdery mildew (> 30%), but differences were not significant at lesser disease severity. These pathogenesis-related proteins persisted through winemaking and increased heat-induced haze. As such, wines made from severely affected grapes may have greater potential to develop haze and deposits during storage (Girbau *et al.*, 2004).

Where grapes have been sorted according to disease severity before analysis and before vinification, the above characters have, in general, tended to vary with severity (Stummer *et al.*, 2003, 2005; Calonnec *et al.*, 2004; Girbau *et al.*, 2004; McFadden-Smith and Pickering, 2006). Differences between slightly diseased grapes and the corresponding healthy controls were generally variable and not statistically significant. Likewise, diffuse powdery mildew had no significant effect on the composition of Pinot noir grapes in terms of sugar concentration, pH, titratable acidity, tartaric or malic acid concentration (Gadoury *et al.*, 2007).

E. necator does not produce mycotoxins harmful to human health; however, splitting due to restricted expansion of the epidermis can predispose grapes to secondary fungal invaders, some of which can produce mycotoxins.

Effects of powdery mildew on sensory attributes of juice and wine

Grapes with powdery mildew have a distinctive musty aroma. Extracts from

Cabernet Sauvignon grapes exhibiting 'powdery mildew aroma' yielded 22 odorants when analysed by gas chromatography with olfactometry and gas chromatography with mass spectrometry (Darriet *et al.*, 2002). Using aroma extraction dilution analysis, Darriet *et al.* (2002) identified the most potent odorants as 1-octen-3-one (mushroom odour) and (Z)-1,5-octadien-3-one (geranium leaf), along with an unidentified odorous zone (fishy–mushroom odour). Likewise, Stummer *et al.* (2005) reported that a trained sensory panel perceived a mushroom aroma from juices obtained from Chardonnay bunches with as little as 1–5% of the surface affected by powdery mildew and a dusty aroma when disease severity was > 30%.

Negative sensory attributes, such as off-flavours (Ough and Berg, 1979; Pool *et al.*, 1984) and 'mildew-like' and hydrogen sulphide aromas (Pool *et al.*, 1984), have been reported for wines made from Thompson Seedless, Carignane, Ribier and Rosette grapes with as little as 3% berries (by weight) with powdery mildew. In contrast, Ewart *et al.* (1992) reported increased bitterness but no mouldy characters in wine made from Verdelho grapes with 80–100% powdery mildew.

Recent studies using batches of naturally infected grapes sorted into disease severity categories before vinification have suggested that some changes in organoleptic properties are discerned only when the proportion of diseased fruit used to make the wine is large (Stummer *et al.*, 2003, 2005; Calonnec *et al.*, 2004). This accords with the report by Darriet *et al.* (2002) that the compounds contributing mushroom, geranium leaf and fishy–mushroom odours were not detected or

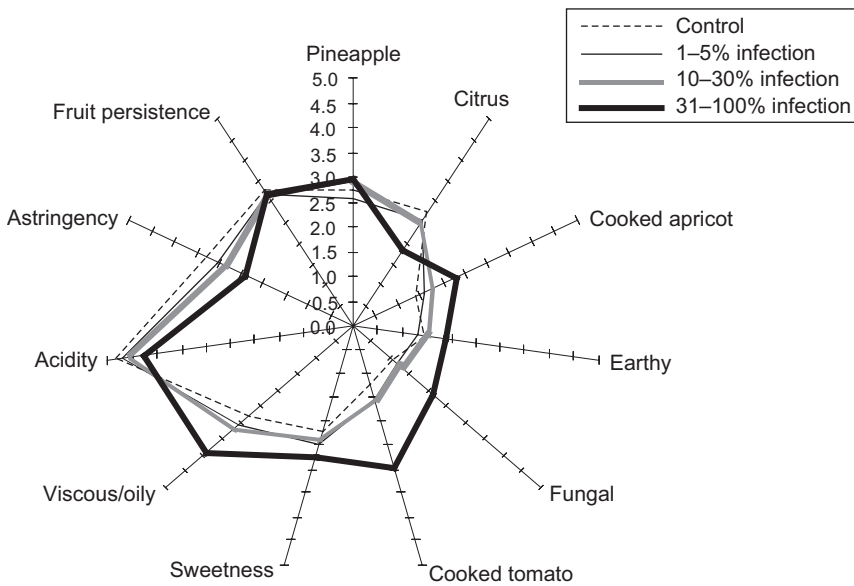

Fig. 13.3 Mean ratings for sensory attributes for Chardonnay wines (2002 vintage) made from grapes assigned to powdery mildew severity categories. Each value is the mean score from duplicate fermentation replicate wines that were presented to 16 assessors in two replicate sessions (Stummer *et al.*, 2005, *Australian Journal of Grape and Wine Research*, Wiley-Blackwell, used with permission).

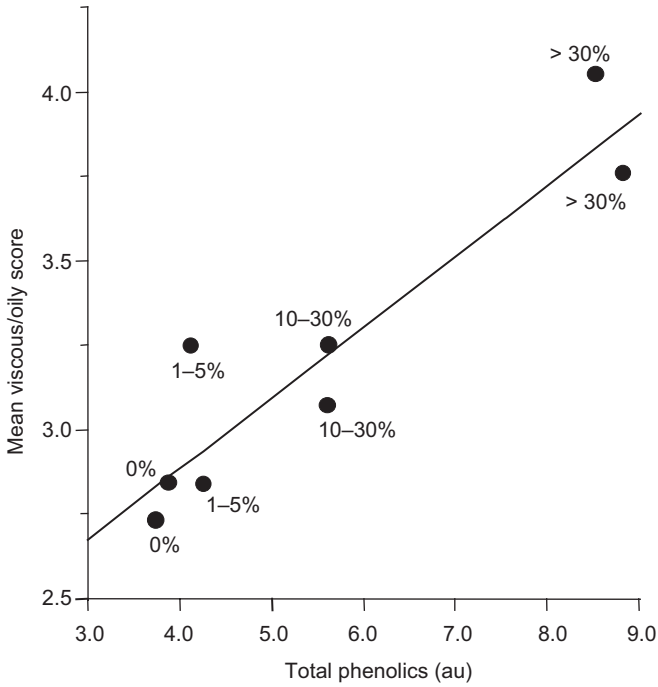

Fig. 13.4 The relationship between total phenolics measured in eight Chardonnay wines and a viscous/oily attribute rated by a trained sensory panel (correlation coefficient 0.93 (P = 0.001)). Values next to the symbols indicate the severity of powdery mildew on grapes used to make each wine (adapted from Stummer *et al.*, 2005, *Australian Journal of Grape and Wine Research*, Wiley-Blackwell, used with permission).

were much less intense in wines made from diseased berries than in the corresponding juice or grapes, due to conversion to much less odorous compounds (such as 3-octanone) by *Saccharomyces cerevisiae* during fermentation. Only when the proportion of mildew-affected Cabernet Sauvignon grapes exceeded 25% did professional tasters in France distinguish such wines, whereas the threshold for non-experts was 50% (Calonnec *et al.*, 2004). In the same study, the detection threshold for Sauvignon blanc wines was 50%, even though the concentration of 3-mercaptohexanol, which contributes to the characteristic aroma of Sauvignon blanc wines, decreased with increasing severity of powdery mildew (Calonnec *et al.*, 2004). In Australian research, a trained tasting panel detected pronounced fungal, earthy, cooked apricot and cooked tomato aromas in wines made from Chardonnay grapes with > 30% infection (Fig, 13.3; Stummer *et al.*, 2005). However, a consistent effect of powdery mildew on Chardonnay wine was a pronounced viscous/oily mouthfeel character compared with the disease-free control, when as little at 1–5% of the bunch surface was infected. This character was correlated with total phenolic concentration of the wine (Fig. 13.4; Stummer *et al.*, 2005). Furthermore, Stummer *et al.* (2005) noted that the imposition of skin contact during processing might increase the influence of powdery mildew on wine quality.

Diffuse powdery mildew did not affect the intensity of various organoleptic characters of Pinot noir wines; however, negative descriptors were applied more commonly to wines made from grapes with diffuse powdery mildew than to those made from disease-free grapes. This may reflect increased populations of secondary spoilage microorganisms (Gadoury *et al.*, 2007).

13.3.3 Association of powdery mildew with other microbes and insects

Powdery mildew has been associated with increased populations of other microorganisms on berries, some of which have the potential to spoil wine. Total microbial populations isolated from the surface and from juice of Chardonnay berries with 31–100% of the cluster affected by powdery mildew were larger than those from healthy berries or those less severely affected in South Australian studies (Stummer *et al.*, 2003, 2005). However, few of these organisms were recovered from clarified juice or from early stages of fermentation. *B. cinerea* and spoilage yeasts were not isolated; however, yeast-selective media were not used. In contrast, Gadoury *et al.* (2007) recovered large populations of yeasts and bacteria, including the potential spoilage yeasts *Hanseniospora* and *Metschnikowia*, from Pinot noir grapes with diffuse powdery mildew in New York State. Furthermore, berries with diffuse powdery mildew released more volatile ethyl acetate, ethanol and acetic acid than healthy berries and infestation by sap beetles, ants and wasps (yellow jackets) was greatly increased in the former. As noted above, there was a strong association between diffuse powdery mildew and botrytis bunch rot (Gadoury *et al.*, 2007), which the authors suggested might reflect infection by *B. cinerea* via wounds inflicted by insects feeding. In comparing these studies, it should also be noted that conditions between *véraison* and harvest are generally considerably wetter, and therefore more conducive to bunch rot, in New York State than in South Australia.

13.3.4 Ripe rot

Ripe rot may damage grapes in subtropical regions where conditions during ripening are warm and humid, for example, on the east coast of Australia and southern USA (Daykin and Milholland, 1984; Melksham *et al.*, 2002; Meunier and Steel, 2009). Affected berries taste bitter and may ooze pink/orange spores of *Colletotrichum acutatum*, then shrivel and drop. Meunier and Steel (2009) reported effects on juice and wine quality of Cabernet Sauvignon comprising healthy, 1.5% and 3% ripe rot-affected grapes. Total soluble solids and titratable acidity of juice tended to increase with increasing infection, whereas wines made from affected grapes exhibited increased pH, alcohol, gluconic acid, glycerol, residual sugar and volatile acidity but decreased titratable acidity. Wine made from affected grapes was browner by hue than that from healthy grapes, which was attributed to polyphenoloxidases, such as laccase, and acid modulation. Affected grapes had a musty, hessian sack-like aroma. A sensory panel distinguished wine made from 3% diseased grapes from other treatments and described it as more

bitter and harsh on the palate, but with less red colour and fruit intensity than wine from healthy grapes (Meunier and Steel, 2009).

13.4 Detection and quantification of fungal contamination of grapes, juice and wine

Assessment of disease requires expertise in recognition of symptoms. Disease on grapes is generally assessed as incidence (proportion of vines or bunches that show any symptoms) or severity (proportion of surface area of foliage or bunches diseased) in the vineyard before harvest (Waller *et al.*, 2002). Disease severity is often determined using standard disease assessment scales or keys (e.g. Horsfall and Barratt, 1945; Pearson and Gadoury, 1992; Emmett *et al.*, 1997; Wicks *et al.*, 1999; Nutter *et al.*, 2006) and bunches can be collected for confirmation of the causal pathogen in the laboratory. However, assessors vary in their interpretation of disease severity, in part due to experience and training, and also due to their perception of visual stimuli (Emmett *et al.*, 1997; Nutter *et al.*, 2006). This was illustrated by a recent study in which batches of grapes assigned to the same disease severity categories, using standard disease area assessment charts, by industry personnel in two wine regions of South Australia differed in amount of *E. necator* DNA detected (Dambergs *et al.*, 2008).

Assessment of grape consignments at the winery typically involves visual inspection of samples sorted by hand to estimate infection as incidence or on a weight for weight basis (Marois *et al.*, 1993; Dewey *et al.*, 2008). However, assessment at the winery is likely to under-estimate fungal contamination, especially for machine-harvested grapes, due to sloughing off of superficial fungal structures (Stummer *et al.*, 2006) and disintegration of rotten berries (Dewey and Meyer, 2004). Furthermore, diffuse powdery mildew is invisible to the naked eye. As these assessments may determine acceptability, value or subsequent processing of consignments, objective measurements would enhance decision making.

13.4.1 Methods for assessing fungal contamination

Techniques for detecting and quantifying fungal pathogens or their activities in grapes, juice and wine include: (i) observation, including microscopy, of fungal structures on diseased grapes or on nutrient medium following isolation of pathogens in the laboratory; (ii) biochemical assays for secreted fungal enzymes; (iii) immunological assays that use specific antibodies to detect fungal components (antigens); (iv) DNA hybridisation and polymerase chain reaction (PCR) assays for the detection of pathogen-specific DNA sequences; and (v) spectroscopic analysis to assess differences in composition of grape material. In this section, the application of DNA-based diagnostic methods in grapevine pathology and spectroscopic methods for analysis of food and beverages is reviewed briefly, followed by discussion of recent advances in detection and quantification of botrytis and powdery mildew in grapes. Some general resources are given in section 13.8.

Molecular diagnostics based on DNA hybridisation using cloned fragments of pathogen DNA and PCR assays to amplify pathogen-specific DNA sequences have been developed as research tools for the detection of various diseases of grapevine, including eutypa dieback (Lecomte *et al.*, 2000; Lardner *et al.*, 2005), phomopsis cane and leaf blight (Melanson *et al.*, 2002), powdery mildew (Evans *et al.*, 1996; Stummer *et al.*, 2006) and botrytis bunch rot (Suarez *et al.*, 2005; Cadle-Davidson, 2008). Furthermore, PCR using primers based on sequence characterised amplified regions (SCARs) and internal transcribed spacer (ITS) sequences of DNA have been used to distinguish *E. necator* isolates belonging to specific phenetic groups (Délye *et al.*, 1997; Stummer *et al.*, 2000) and biotypes (Hajjeh *et al.*, 2005). In addition, PCR assays using primers based on the 14α-demethylase and β-tubulin genes have been developed to detect fungicide-resistant genotypes of *E. necator* (Délye *et al.*, 1999) and *B. cinerea*, respectively (Luck and Gillings, 1995). PCR using primers based on the ITS region has recently been used in combination with air sampling to detect and estimate numbers of airborne spores of *E. necator* (Falacy *et al.*, 2007), an approach that has the potential to predict the risk of outbreaks of airborne diseases (West *et al.*, 2009).

Techniques based on spectroscopy and chemometrics exploit the fact that all organic compounds have a spectral fingerprint due to molecular bond vibrations. The primary vibration arises from the mid-infrared (MIR) wavelength region and weaker overtones of those vibrations occur in the near infrared (NIR) wavelength region. The strong signal in the mid-infrared region has the advantage of increased sensitivity, but this strong signal can make sample presentation difficult, in that very small path-lengths are required. Consequently, MIR has gained favour for analysis of liquids, such as wine and juice, whereas NIR is more commonly used for analysis of soil, grapes and vine tissue (Gishen *et al.*, 2005). The fingerprint of organic compounds is contained within the NIR and MIR spectra; the secret to unlocking the power of spectroscopy has been the development of algorithms for analysis of chemometric data and the evolution of readily accessible computing power (Dambergs *et al.*, 2004; Gishen *et al.*, 2005; Cozzolino *et al.*, 2009). As a result, spectroscopic techniques are well recognised as rapid, non-destructive methods and have been widely applied in the food and agricultural industries (Osborne *et al.*, 1986; Schenk and Westerhaus, 1993; Batten, 1998; Dambergs *et al.*, 2004; see Chapter 5).

In the wine industry, the predominant use of NIR methods to date has been in the analysis of wine ethanol (Dambergs *et al.*, 2004), and it is also applicable in the analysis of methanol in wine fortifying spirit (Dambergs *et al.*, 2002). Methods for simultaneous analysis of grape quality parameters such as total soluble solids, pH and anthocyanin content have also been developed (Gishen and Dambergs, 1998; Dambergs *et al.*, 2003, 2006; Cozzolino *et al.*, 2004). Some large wine companies use this technology to measure grape anthocyanin content on a routine basis (Kennedy, 2002) and it may be possible to use the same methods to monitor the degree of mould contamination simultaneously.

The use of NIR for detecting fungal contamination has been demonstrated for powdery mildew and rust in grain (Asher *et al.*, 1982) and for mould contamination

of tomato purée (Davies *et al.*, 1987). Short wave NIR is very penetrating and has been applied in both reflectance and transmittance mode to detect single, mould-affected corn kernels (Pearson *et al.*, 2001), with a view to using automated high-speed sorting. Similarly, NIR spectroscopy has been used to detect mould damage and associated toxins in single wheat kernels (Dowell *et al.*, 1999). The high sensitivity of the MIR wavelength region can be harnessed for use on solid samples by using attenuated total reflectance (ATR), as applied to detect contamination of corn by *Fusarium* species (Kos *et al.*, 2002, 2004) and to classify dried grapes according to ochratoxin A concentration (Galvis-Sánchez *et al.*, 2007). Reports of spectroscopy and chemometrics to quantify small concentrations of compounds, such as fungal toxins, must be tempered by the fact that such compounds probably are not measured directly but their concentrations may correlate with other fungus-related changes in the sample matrix, thus the calibrations may be matrix-dependent (Dambergs *et al.*, 2006). Other, more abundant, fungal metabolites may have a unique spectral signal and occur in a concentration range that can be detected directly by spectroscopy; for example, fungal melanins in soil have distinct infrared profiles (Paim *et al.*, 1990). Lack of specificity may confound the use of correlative methods to classify fungal infection of food crops, for example, the presence of fungal components, such as chitin, or damage to the host plant may be common to different fungal species or biotypes. Nevertheless, NIR spectroscopy was reported to discriminate among soybean seeds damaged by *Phomopsis* sp. (seed decay), *Cercospora kikuchi* (purple seed stain) and *Peronospora manshurica* (downy mildew) (Wang *et al.*, 2004), and to differentiate between three morphologically similar species of *Epichloë* which are endophytes of grasses (Petisco *et al.*, 2008).

13.4.2 Botrytis bunch rot

Contamination of grapes can be quantified routinely as fungal colonisation or laccase activity. However, laccase activity does not necessarily indicate degree of contamination by *B. cinerea*, because strains of *B. cinerea* vary in amount of laccase produced (Cotoras and Silva, 2005) and bunch rot-associated fungi other than *B. cinerea* produce laccase. Estimating laccase activity allows corrective action, such as additional sulphur dioxide, pasteurisation or avoiding exposure to air (Iland *et al.*, 2004; Claus, 2009), to minimise negative effects of the enzyme on wine.

Laccase activity can be determined by the oxidation of the colourless orthodiphenol syringaldazine to a purple quinone (Grassin and Dubourdieu, 1989). Iland *et al.* (2004) provide a detailed procedure for estimating laccase activity, as units/ml, in juice or wine, preferably without prior addition of SO_2 or ascorbic acid, based on measuring absorbance at 530 nm. They recommend fermenting a representative 1 kg sample of the grapes in accelerated laboratory fermentation and assaying the resulting wine. Subsequently, DeScenzo *et al.* (2005) and Dewey *et al.* (2008) described a high-throughput SO_2-tolerant assay for laccase activity based on oxidation of 2,2'-azino-bis(3-ethyl-benzothiazoline-6-

sulfonic acid) (ABTS) in a glycine-HCl buffer following filtration of 1 ml samples of juice and wine through quartz sand plus polyvinylpolypyrrolidone in 96-well plates. Tests based on oxidation of syringaldazine (Grassin and Dubourdieu, 1989) or on the method of DeScenzo (DeScenzo *et al.*, 2005; Dewey *et al.*, 2008) are available commercially (e.g. www.dolmar.com.es; www.tizwine.com; www.etslabs.com).

Iland *et al.* (2004) advise that guidelines for tolerance of laccase should be based on experience and the desired wine style, whereas Ribéreau-Gayon *et al.* (2006a) recommend tolerance limits of one and three units/mL for white and red grapes, respectively.

Colonisation of plant tissue by *B. cinerea* may be quantified by immunological or DNA-based assays. Dewey and co-workers have reported immunological assays for *B. cinerea* in grape juice, in plate-trapped antigen–enzyme-linked immunosorbent assay (PTA–ELISA) format (Dewey *et al.*, 2000; Dewey and Yohalem, 2004) and in a quicker and more robust but less sensitive tube assay format (Dewey and Meyer, 2004). More recently, Dewey *et al.* (2008) reported a *Botrytis*-lateral flow device (B-LFD) that quantifies contamination of juice as % botrytis rot (w/w) in 10 minutes. However, there was little correlation between % infection of berries measured by B-LFD and laccase activity measured with the high-throughput assay mentioned above (Dewey *et al.*, 2008). Immunological test kits available for detection of *B. cinerea* in plant materials have been applied to grape berries. For example, Celik *et al.* (2009) described the use of a BOTR00083

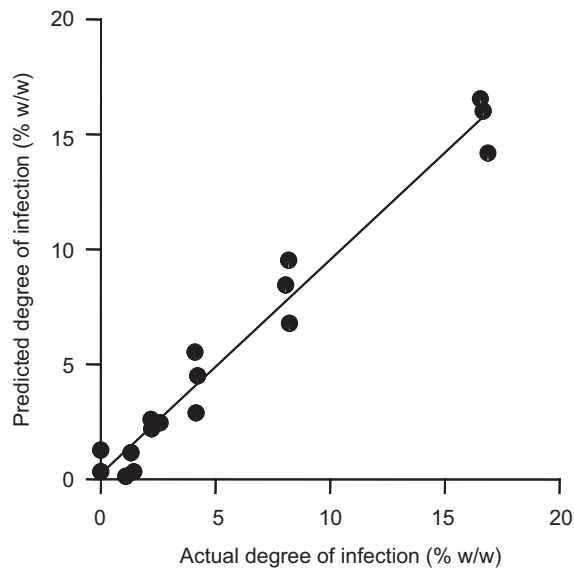

Fig. 13.5 The relationship between the actual degree of infection by *Botrytis cinerea* (disease severity) and the values predicted from spectral scans of grape homogenates using partial least squares regression. The calibration used a 600–1800 nm wavelength range and 1-out cross-validation.

Table 13.1 Calibration statistics for the prediction of infection by *Botrytis cinerea* from spectral scans of grape homogenates

Wavelength (nm)	R^2	SECV (% w/w)	SDref/SECV
400–2500	0.97	1.07	5.4
600–1800	0.95	1.26	4.6
600–1000	0.96	1.21	4.8

Note: SECV refers to standard error of cross-validation; SDref refers to standard deviation of reference data.

Pocket Diagnostic™ lateral flow antibody kit (see www.pocketdiagnostic.com), and the QuickStix™ Kit for Botrytis in Wine Grape Juice (EnviroLogix, USA) is being used in epidemiological studies of botrytis bunch rot in Tasmania, Australia (Edwards *et al.*, 2009; K. Evans, pers. comm., 2009). The latter test, when positive, results in a colour reaction with a signal intensity proportional to the amount of *B. cinerea* antigens in the juice (www.envirologix.com).

DNA-based PCR assays have recently been developed to detect and quantify *B. cinerea* in plant tissue (Suarez *et al.*, 2005), including grape berries (Cadle-Davidson, 2008; Celik *et al.*, 2009). Based on the quantitative or Real-Time PCR TaqMan® system, these assays have been developed to study disease development, including latent or quiescent infection, and rot of table grapes in storage. As quantitative PCR currently requires specialist equipment and technical expertise, it is more likely to be offered as a commercial laboratory service than as a test for use in the winery.

Changes in grape compositional profiles, such as the increase in glycerol and gluconic acid associated with infection by *B. cinerea*, have been monitored by MIR spectroscopy (Versari *et al.*, 2008), but this does not imply specificity. Preliminary research using NIR spectroscopy of grape homogenates has shown potential for rapid quantification of infection by *B. cinerea* (Gishen *et al.*, 2005). Figure 13.5 shows the calibration performance for a partial least squares regression calibration for the prediction of the degree of infection by *B. cinerea* using NIR reflectance spectra of homogenates prepared from grapes inoculated with the fungus (Dambergs and Scott, unpublished data). The calibration statistics using a variety of wavelength ranges are shown in Table 13.1. Robust calibrations could be obtained with a narrow, short-wavelength NIR range that is achievable with cheap silicone diode array sensors, which would offer rapid scanning (Osborne *et al.*, 1986).

13.4.3 Powdery mildew on grape clusters

Immunological approaches for detection of powdery mildew have received less attention than botrytis diseases. Two monoclonal antibodies were identified for detection of powdery mildew in grapes and must (Markovic *et al.*, 2002), but specificity to antigens tightly bound to conidia confounded further development.

DNA assays have been developed to quantify powdery mildew and can detect

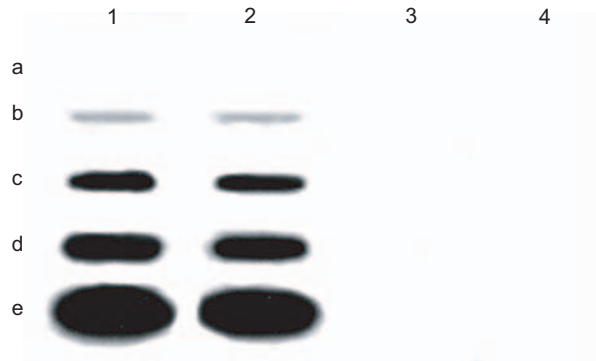

Fig. 13.6 Slot-blot hybridisation of probe pEnA1 to DNA extracted from conidia of *E. necator*, various grapevine-associated fungi and grapevine DNA supplemented with *E. necator* DNA. The effect of grapevine DNA on the detection of *E. necator* was assessed by comparing the signal intensities from *E. necator* DNA to those from a mixture of grapevine and *E. necator* DNA. Slots 1a to e contain increasing concentrations of genomic DNA from a single-spore isolate of *E. necator*, 0 (sterile water), 0.05, 0.5, 1.0 and 5.0 ng, respectively; slot 2a, grapevine DNA (100 ng); slots 2b to e, grapevine DNA (100 ng) plus *E. necator* DNA at 0.05, 0.5, 1.0 and 5.0 ng, respectively; slots 3a to e and 4a to e, DNA from other grapevine-associated fungi (no hybridisation) (Stummer *et al.*, 2006, *Mycological Research*, Elsevier, used with permission).

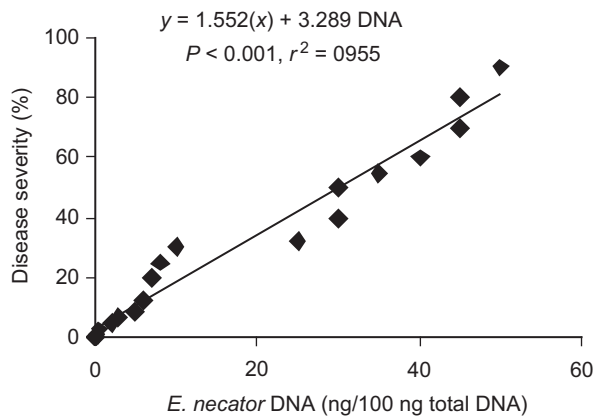

Fig. 13.7 Relationship between powdery mildew severity (% of bunch surface affected) and *E. necator* DNA content, estimated by slot-blot hybridisation to probe pEnA1 (Stummer *et al.*, 2006, *Mycological Research*, Elsevier, used with permission).

contamination of grapes and must equivalent to less than 5% of the grape cluster infected, a level that might be missed by visual inspection alone (Stummer *et al.,* 2006). *E. necator* DNA was not detected in clarified juice and wine, suggesting that the fungus, which colonises only the epidermis of the grape berry, settles out with skins and other solids after the addition of pectinase during juice clarification.

A DNA hybridisation assay using an *E. necator*-specific cloned DNA sequence

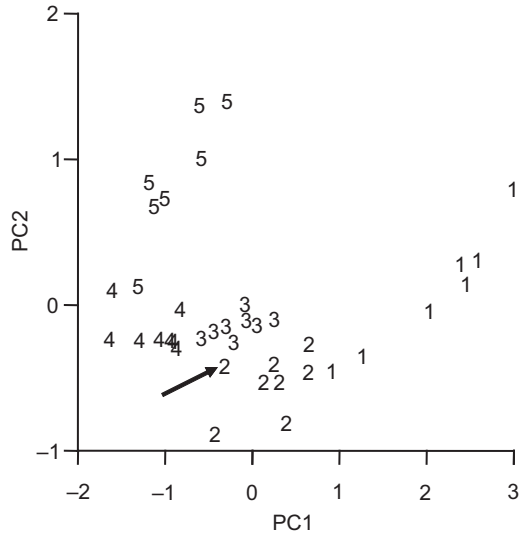

Fig. 13.8 A plot of the first two principal components (PC1, PC2) from principal component analysis of spectral data (400–2500 nm) prepared from homogenates of grapes with varying severity of powdery mildew. Samples were visually classified as < 1% infected (1), 1–10% infected (2), 11–30% infected (3), 31–60% infected (4) and > 60% infected (5), and confirmed by DNA analysis. The arrow indicates the only sample that was incorrectly classified using linear discriminant analysis (see Table 13.2).

Table 13.2 A classification matrix for prediction of powdery mildew severity with linear discriminant analysis (LDA) using the first three scores from principal component analysis of raw spectra (400–2500 nm) as the input data

	Level 1	Level 2	Level 3	Level 4	Level 5	% correct
Level 1	7					100
Level 2		7	1			88
Level 3			8			100
Level 4				8		100
Level 5					7	100
Total	7	7	9	8	7	97

Note: For the reference data, samples were visually classified as < 1% infected (level 1), 1–10% infected (level 2), 11–30% infected (level 3), 31–60% infected (level 4) and > 60% infected (level 5). One-out cross validation was used for the LDA. Actual disease severities (levels 1–5) are in rows and predicted values are in columns. The single level 2 sample that was incorrectly classified as level 3 is indicated with an arrow in the principal component analysis plot (see Fig. 13.8).

(pEnA1) provided a strong correlation between disease severity, expressed as the percentage surface area of a cluster with powdery mildew, and *E. necator* DNA content (Figs 13.6 and 13.7; Stummer *et al.*, 2006). While useful as a research tool, the DNA hybridisation assay is slow (up to one week), labour-intensive and unsuitable for routine use in industry. A PCR assay using primers derived from this

clone was 50 times more sensitive for detecting *E. necator* DNA in grape homogenate (threshold approximately 1 pg) than the hybridisation assay, although not quantitative (Stummer *et al.*, 2006). Quantitative PCR offers potential for estimating the degree of contamination (see Section 13.7).

Powdery mildew can also be detected with NIR spectroscopy (Dambergs *et al.*, 2005, 2008; Gishen *et al.*, 2005; Dambergs and Stummer, 2007). Principal component analysis (PCA) of spectra collected from homogenates of grapes with varying severity of powdery mildew (assessed visually and confirmed with DNA analysis) showed distinct clustering related to the degree of infection (Fig. 13.8). Linear discriminant analysis using the PCA scores as the input data showed a high classification rate, with the only sample incorrectly classified spanning an adjacent infection category (Table 13.2).

13.5 Alternatives to conventional fungicides for control of powdery mildew and botrytis and their effects on wine quality

Concerns about the health of vineyard staff, pesticide residues, effects on fermentation, environmental pollution and fungicide resistance have prompted research on alternatives to conventional fungicides. Mineral and botanical oils, inorganic salts, surfactants, emulsions of seaweed, fish or crustacean shells, milk, compost extracts and antagonistic microorganisms have been assessed as alternatives to conventional fungicides for control of powdery mildew and bunch rots. With the exception of mineral oils, some of these materials have been adopted for routine use in organic and biodynamic systems. Recent research on the use of alternative materials is discussed below, with emphasis on studies in which effects on grape and wine quality have been considered.

Mineral or petroleum oils exhibit protective and curative activity against powdery mildew (Northover and Schneider, 1996). Dell *et al.* (1998) reported JMS Stylet-Oil (2%) to be as effective as a demethylation inhibiting (DMI) fungicide against powdery mildew, but less effective than the fungicide iprodione in reducing the severity of botrytis bunch rot. Northover and Homeyer (1998) reported similar responses, whereas Wicks *et al.* (1999) found even the most effective mineral oil treatment tested (1% v/v in water at 1000 L/ha) to be inferior to a DMI in protecting bunches from powdery mildew. Although addition of JMS Stylet-Oil to Chardonnay juice before inoculation with yeast had little effect on fermentation (Dell *et al.*, 1998), repeated applications of mineral oils (JMS Stylet-Oil, Sun Spray Ultra-Fine oil) and soybean oil to run-off reduced net assimilation rates and yield and delayed accumulation of soluble solids in Chardonnay and Cabernet Sauvignon berries (Finger *et al.*, 2002). In another study, Stylet-Oil reduced total soluble solids by up to 3.5 °Brix but had little effect on yield, titratable acidity or pH in several cultivars (Northover and Homeyer, 1998). Canola oil (Northover *et al.*, 1993; Reynolds, 2004) and tea tree oil (Reuveni *et al.*, 2006) have controlled powdery mildew in some situations and have been formulated as commercial

products, for example Synertrol Horti® Oil (Organic Crop Protectants, Australia) and Timorex. Plant-derived jojoba wax (1% v/v) was as effective as sulphur in controlling powdery mildew and reduced the severity of botrytis bunch rot, as did canola oil, with little overall effect on yield or quality (Reynolds, 2004).

Inorganic salts, such as potassium phosphates (Reuveni and Reuveni, 1995), potassium silicate (Reynolds *et al.*, 1996) and potassium bicarbonate (Crisp *et al.*, 2006c,d) have controlled powdery mildew as well as conventional fungicides in some conditions, with no obvious detrimental effects on yield or grape and wine quality where examined.

Bovine milk (1 in 10 dilution) and whey (25–45 g/L), used alone or in rotation with a canola oil-based product (Synertrol Horti® Oil) and/or potassium bicarbonate (Ecocarb®, Organic Crop Protectants), controlled powdery mildew as effectively as sulphur or a DMI fungicide on some cultivars in conditions of moderate disease pressure, provided spray coverage was thorough. These treatments had no obvious effect on soluble solids, pH and titratable acidity of grapes (Crisp *et al.*, 2006c), nor on quality of juice (Crisp *et al.*, 2006b) or wine (Scott, 2007; Crisp *et al.*, 2008). In particular, the milk components lactoferrin and lactoperoxidase exhibit antimicrobial activity. Lactoferrin caused hyphae of *E. necator* to collapse and conidia to rupture (Crisp *et al.* 2006e). Lactoperoxidase in formulations with iodine and/or thiocyanate and hydrogen peroxide (LP-system), sometimes with oil as an adjuvant, has potential for control of powdery mildews (Boots and Floris, 2006) and is the subject of a patent application (Ravensberg *et al.*, 2002 cited in Boots and Floris, 2006).

Biological control agents have been evaluated in vineyard studies and commercial products are available in some countries, for example Aq-10 biofungicide (containing the mycoparasitic fungus *Ampelomyces quisqualis*, Ecogen, Inc., USA) and Serenade® (containing the bacterium *Bacillus subtilis*, AgraQuest, Inc., USA) for control of powdery mildew and Botry-Zen® (containing the fungus *Ulocladium oudmanseii*, Botry-Zen Ltd, New Zealand), Trichodex® (containing the fungus *Trichoderma harzianum*, Makhteshim-Agan, Israel) and Sentinel® (containing *Trichoderma* sp., Agrimm Technologies Ltd, New Zealand) for control of botrytis bunch rot. Biological control of botrytis bunch rot was reviewed recently by Elad and Stewart (2004) and Elmer and Reglinski (2006). Compost extract (Elad and Shtienberg, 1994) and aerated compost extract (Palmer *et al.*, 2006, 2007), which contain large but poorly defined populations of microorganisms, have met with some success at the research level, although standardisation of extracts from a diverse range of starting materials has proved challenging (Scheuerell and Mahaffee, 2006).

Although alternative materials and products have controlled powdery mildew and botrytis bunch rot in some conditions, they may fail to provide adequate control when disease pressure is high and where environmental conditions are ideally suited to disease development or are unsuitable for biological agents (e.g. Reynolds *et al.*, 1996; Elad and Stewart, 2004; Crisp *et al.*, 2006a,c; Elmer and Reglinski, 2006). Integration of biopesticides and chemical fungicides with disease forecasting systems has allowed commercially acceptable control of disease with reduced

chemical inputs (Shtienberg, 2004). Furthermore, the inclusion of one or two sulphur sprays at the critical period around flowering and fruit-set in a program comprising regular application of canola oil plus potassium bicarbonate provided commercially acceptable control of powdery mildew (Bramley *et al.*, 2008) in conditions where full season programs of alternative 'soft chemicals' did not. Therefore, growers who wish to reduce inputs of conventional fungicides might consider applying alternative materials at phenological stages other than the critical time around flowering and fruit-set. However, Shtienberg (2004) pointed out that growers may be reluctant to risk reducing applications of conventional fungicides unless there are strong economic advantages, such as consumer demand or the need to comply with regulations, or compelling philosophical or medical reasons. Crisp *et al.* (2006a) suggested that growers keen to use alternative materials should adopt cultural practices known to minimise disease risk and experiment with a small area of their vineyard first to assess the efficacy of alternative strategies in their environment, as well as ensuring that their program is acceptable to the winemaker.

13.6 Future prospects

Spectroscopic methods are commonly used in wineries to assess grape and wine quality attributes and, once established, allow fast, cost-effective analysis. However, as with any correlative method to predict properties from spectra of unfractionated samples, chemometric calibrations to quantify powdery mildew and botrytis bunch rot in grapes require reference methods to gather calibration input data. Also, seasonal and varietal variations in sample matrix properties must be taken into account, so a reference assay is important for ongoing validation of calibration. This could be achieved using quantitative PCR; research that is in progress in Adelaide (Scott *et al.*, 2007). Spectroscopy has potential for use in the field or at a grape receival point, with little or no sample preparation, and the ultimate application is using spectroscopy for 'chemical imaging' to visualise fungal contamination *in situ* (Dambergs and Stummer, 2007).

Electronic nose technology offers potential for measuring volatile or odorant compounds associated with contaminated grapes. This involves sampling the headspace, transferring the volatile compounds to a sensor which converts them to electrical signals related to the concentration of particular components, and processing the data (Peris and Escuder-Gilabert, 2009). This approach has been used to study spoilage of red wine by *Brettanomyces* yeast (Cynkar *et al.*, 2007). Although measurement of 1-octen-3-one, which contributes to the mushroom aroma of grapes with powdery mildew and botrytis bunch rot (Darriet *et al.*, 2002; La Guerche *et al.*, 2006), is unlikely to allow discrimination between these two diseases, identification of compounds unique to a particular fungus or disease may provide the required specificity.

Biosensors, in which recognition of a target molecule or compound by a biological (or biochemical) receptor combined with a physical transducer results in a detectable signal (Wang, 2000), can be used to detect and quantify various

compounds. The application of biosensor technology in monitoring environmental pollution (Rodriguez-Mozaz *et al.*, 2006) and analysing food- and waterborne pathogens and associated toxins (Rasooly and Herold, 2006) has been reviewed recently. The use of a laccase biosensor to estimate polyphenol content of wine (Gamella *et al.*, 2006) suggests the possibility of using biosensors capable of reacting with fungal components or secretions, or marker compounds in juice or wine made from diseased grapes, to detect and quantify contamination. Biosensors used in microarray format to detect particular DNA sequences (Wang, 2000; Lievens and Thomma, 2005) have potential for high-throughput detection of DNA sequences unique to *B. cinerea*, *E. necator* and other microorganisms that contaminate grapes, juice and wine.

In terms of sustainable management of fungal diseases, elucidation of mode of action of 'soft chemical' and biological agents may facilitate more consistent control. Integration of these alternative materials with cultural practices, such as thinning of foliage in the bunch zone (Gubler *et al.*, 1987), may enhance outcomes, particularly in organic and biodynamic viticulture. However, further research is required to address concerns about possible negative effects of alternative materials on wine quality.

Sequencing of the genomes of *B. cinerea* (Broad Institute, 2009) and *E. necator* (Cornell University, 2009) may yield new targets for breeding disease-resistant grapevines and complements research on plant genes such as *Run* 1, which confers resistance to powdery mildew in *Muscadinia* grapes (Barker *et al.*, 2005). Again, research on effects on grape and wine quality is integral to such breeding programs.

13.7 Conclusions

Fungi have the potential to spoil grapes and wine when conditions are conducive for disease. Tolerance thresholds for fungal contamination vary according to winery specifications, supply of and demand for grapes, and wine style. Adoption of rapid, objective and quantitative methods for assessing contamination will assist in making decisions about pricing and processing of grapes. Tests are available commercially for estimating contamination by, and activity of, *B. cinerea*, based on immunological and enzyme assays, respectively. There is not yet an equivalent for quantifying contamination by powdery mildew. Methods based on detection of pathogen DNA and spectroscopy are being developed and, in addition to technology based on biosensors, offer potential for high-throughput detection and quantification of fungal contamination of grapes and wine in the future. Research on alternatives to conventional fungicides has identified candidate materials, and further research on efficacy and effects on grape and wine quality is required.

13.8 Sources of further information and advice

General information about grapevine disease may be found in: *Grape Pest*

Management (Flaherty *et al.*, 1992); *Diseases and Pests – Grape Production Series 1* (Nicholas *et al.*, 1994, reprinted 2007); *Compendium of Grape Diseases* (Pearson and Goheen, 1988); *Viticulture. Volume 2, Practices* (Coombe and Dry, 1992). New editions of the latter two are in preparation. *The Biology of Botrytis* (Coley-Smith *et al.*, 1980); *Botrytis: Biology, Pathology and Control* (Elad *et al.*, 2004); *The Powdery Mildews – a Comprehensive Treatise* (Bélanger *et al.*, 2002) contain information about the biology of *Botrytis* species and powdery mildew fungi, and more specific information about the effects of *Botrytis cinerea* on grape and wine quality can be found in *Handbook of Enology Volume 1, The Microbiology of Wine and Vinifications* (Ribéreau-Gayon *et al.*, 2006a) and *Wine Microbiology and Biotechnology* (Fleet, 1992). Immunological and nucleic acid-based approaches for diagnosis of plant disease were reviewed by Schaad *et al.* (2003), Ward *et al.* (2004) and Lievens and Thomma (2005), and molecular pathology of *B. cinerea* was reviewed by Williamson *et al.* (2007). Recent research on *B. cinerea*, *E. necator* and the diseases they cause can be accessed via proceedings of the International Workshop on Grapevine Downy and Powdery Mildew, and the International Botrytis Symposium.

The website cropwatchonline.com.au provides general information on disease recognition and management, and the following organisations and/or websites provide country-specific information and research publications: Grape and Wine Research and Development Corporation, Australia (gwrdc.com.au); Viticulture and Oenology Research Programs in the USA (http://winegrapes.tamu.edu/research/researchnational.html); Organisation International de la Vigne et du Vin (http://www.oiv.int/uk/accueil/index.php).

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14

Controlling ochratoxin A in the vineyard and winery

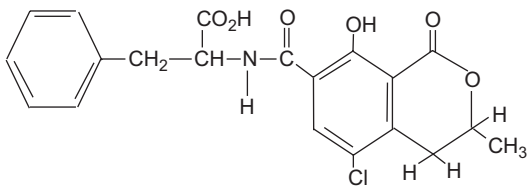
P. Battilani and A. Silva, Università Cattolica del Sacro Cuore, Italy

Abstract: The chapter summarises the current availability of data on the toxicology of ochratoxin A (OTA) and public concern over human exposure to it, taking into account that the International Program on Chemical Safety/World Health Organisation has recently indicated a need for further study and evaluation with respect to human health potential. The fungi responsible for the presence of OTA in grapes and wine are defined, as are favourable meteorological conditions and cropping systems. A short description of efforts devoted to understanding the problem (i.e. research projects) is included. Grape processing has a significant impact on the fate of OTA during the main stages of winemaking (crushing, maceration, alcoholic fermentation, malo-lactic fermentation, bottling, ageing), and critical steps of the process have been identified. Post-harvest strategies for the control of OTA in wines are focused on developments in the field of biological decontamination of OTA through the use of yeasts and lactic acid bacteria. Worldwide surveys have determined the main risk factors. The approach of risk assessment is described and data on wine consumption and the related risk of OTA ingestion are reported with some comments on the effective related risk. The approach of a decision support system (DSS) is described, including the development of a predictive model, good agricultural practices and good manufacturing practices to minimise OTA in grapes and wine.

Key words: ochratoxin A, grapes, *Aspergillus carbonarius*, winemaking, risk assessment.

14.1 Ochratoxin A (OTA) and its effect on health

The first report of ochratoxin A (OTA) in wine in 1996 (Zimmerli and Dick) elicited a prompt reaction from scientists who devoted much effort to clarifying the



Acidity:
pKa carboxyl group = 4.4
pKa phenolic hydroxyl group = 7.3

Molecular formula: $C_{20}H_{18}ClNO_6$
Molecular weight: 403.82 g/mol

Fig. 14.1 Molecular structure of ochratoxin A (Van der Merwe *et al.*, 1965).

extent of contamination, its origin and possible management. Quality and safety are important for a beverage such as wine. A guarantee of quality during the whole process is significant for consumer acceptability, but safety assurance is obligatory for the protection of human health.

14.1.1 Chemical characters of OTA

The ochratoxins are a group of mycotoxins that contain a dihydro-isocoumarin moiety linked to L-beta-phenylalanine by an amide bond. OTA, (R)-N-[(5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1H-2-benzopyran-7-yl)carbonyl]-L-phenylalanine (CAS No. 303-47-9) (Fig. 14.1), and its ethyl ester (ochratoxin C) are the most toxic compounds. The isocoumarin moiety is known as OTα, and is commonly reported to be less toxic than OTA.

OTA is a natural weak fluorophore and it is quite a stable compound, heat resistant and susceptible to extreme exposure to UV light; strong acid conditions cause hydrolysis of the amide bond, and strong bases open the lactone ring, reformed by acidification (Pohland *et al.*, 1992). OTA can be converted into OTα and L-β-phenylalanine by heating under reflux for 48 h in 6 M hydrochloric acid (van der Merwe *et al.*, 1965) or by hydrolysis with carboxypeptidase A (Pitout, 1969).

14.1.2 Toxicity of OTA

OTA is a secondary metabolite of moulds that contaminate food and feed and has been shown to be nephrotoxic and hepatotoxic, causing kidney and liver cancer in mice and rats. The nephrotoxicity in monogastric animals is well documented and results in significant economic losses in the swine and poultry industries.

OTA has been found to be a potent renal toxin in all the animal species tested (EFSA, 2004). The extent of renal injury is dose-dependent, but also associated with the duration of exposure, as OTA accumulates in renal tissue. National Toxicology Program studies in the USA showed that OTA can induce renal tumours in rodents at high dosages.

OTA has been linked to Balkan Endemic Nephropathy and the development of tumours in the urinary tract in humans. The International Agency for Research on Cancer classified OTA as a possible carcinogen to humans (group 2B) (IARC, 1993). Some early epidemiological data suggested that OTA might be involved in

the pathogenesis of distinct renal diseases and otherwise rare tumours of the kidneys in certain regions of the Balkan Peninsula. However, these epidemiological data are incomplete and do not justify the classification of OTA as a human renal carcinogen; a correlation between carcinogenicity and exposure to OTA is not confirmed in humans (EFSA 2006; FAO/WHO, 2007).

The half-life of OTA in humans is about 35 days; blood concentration is considered to represent a convenient biomarker of exposure and has been used in epidemiological studies. Recently, Mally *et al.* (2007) noted that human blood levels of OTA, in areas with relatively high dietary exposure, are at least two orders of magnitude below the mean concentration of OTA in the blood of rats that is known to cause nephrotoxicity and kidney tumours with long-term treatment.

Recent scientific evidence indicates that the site-specific renal toxicity as well as the DNA damage and genotoxic effects of OTA, measured in various *in vivo* and *in vitro* studies, are most likely attributable to cellular oxidative damage. Furthermore, advanced chemical analytical procedures failed to demonstrate the existence of specific OTA–DNA adducts. Based on these results, the Scientific Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA) used a threshold-based approach in its risk assessment of OTA. A tolerable weekly intake (TWI) of 120 ng/kg of body weight (bw) for humans (EFSA, 2006) was fixed on the basis of the lowest observed adverse effect level of 8 μg/kg bw per day for early markers of renal toxicity in pigs, the most sensitive animal species. Recent analyses of the dietary exposure of adult European consumers to OTA revealed that the weekly exposure ranges from 15–60 ng OTA/kg bw, including high consumers of foods containing OTA. This rate of exposure is below the TWI value of 120 ng/kg bw as derived by the EFSA Panel (EFSA, 2006).

Results on OTA toxicity cannot be considered conclusive and further investigations into the mechanism of OTA toxicity and into interaction among food and beverages components and mycotoxins are necessary to refine risk analysis and the definition of legal limits for maximum content in grapes and their derivatives, as is the case in other food products.

14.1.3 Interaction of OTA with other compounds

Some studies have been conducted to understand the possible interaction between OTA and compounds naturally occurring in grapes. The effect of pretreatment with antioxidant compounds, such as catechins, epigallocatechin gallate and epicatechin gallate, on OTA toxicity were investigated in a pig cell line, used as a model for kidney toxicity *in vitro* (Costa *et al.*, 2007). The pretreatment with catechins prevented OTA-induced cell death and reduced OTA-induced reactive oxygen species (ROS) production and DNA fragmentation. *In vivo* studies on the protective effect of substances with antioxidant and free radical scavenging activities have provided further evidence of the possible role of oxidative stress in OTA-induced toxicity and have suggested that the antioxidant flavonoids of red wine may carry out a protective role against OTA nephrotoxicity (Bertelli *et al.*, 2005).

A significant reduction in hepatic and renal damage caused by OTA was reported in mice fed with grape juices contaminated with OTA (Jeswal, 1998). Toxic concentration of OTA in red wine could be, to some extent, counterbalanced by the positive effect of resveratrol and its derivatives, whose amount has been shown to be related to OTA contamination in grapes (Bavaresco *et al.*, 2003; Perrone *et al.*, 2007). The potential protective effect of red wine has been studied in rats, and the results showed that red wine limited oxidative damage measured as renal lipohydroperoxides, reduced and oxidised glutathione and renal superoxide dismutase activity.

In contrast, Ranaldi *et al.* (2007) pointed out possible synergy between OTA and some red wine components, such as polyphenols, in the induction of apoptotic cell death. OTA toxicity in the presence of de-alcoholised red wine on the barrier function of the intestinal mucosa was evaluated, using the human Caco-2/TC7 cell line, an established *in vitro* model of the absorptive intestine. OTA alone did not induce significant apoptosis, while some components of red wines acted in a synergistic manner on cells apoptosis producing dramatic and permanent effects on barrier function of the intestinal cells.

14.2 Black Aspergilli and ochratoxin A production in the vineyard

There are few fungi involved in OTA presence in grapes and wine belonging to *Aspergillus* section *Nigri*. OTA is produced in the vineyard and meteorological conditions and cropping systems can influence the development of these fungi and also impact OTA production. Efforts devoted to understanding the problem are summarised in the following sections.

14.2.1 Fungi responsible for OTA presence in grapes and wine

Aspergillus section *Nigri*, the so-called black Aspergilli, includes all fungi responsible for OTA presence in grapes (Battilani and Pietri, 2002; Da Rocha Rosa *et al.*, 2002; Sage *et al.*, 2002). The taxonomy of *Aspergillus* section *Nigri* is very complex; it is based on the shape of conidial heads, in particular the distinction is between uni- and biseriates conidial heads (Raper and Fennel, 1965). Among the uniseriates, *A. aculeatus* and *A. japonicus* have been isolated from grapes. Their identification at species level, based on morphology, is not easy or relevant in practice because they have been occasionally reported, but never confirmed as, OTA producers. A new species was recently described, *A. uvarum* sp. strains, similar to *A. aculeatus* but atypical, distinguishable by amplified fragment length polymorphism (AFLP) analysis and by metabolite profiles (Perrone *et al.*, 2008); however, this species is unable to produce OTA.

Among the biseriates, *A. carbonarius* is quite easily distinguishable because of the size of its conidia, which are bigger than those produced by all the other biseriates species; a high percentage of its strains (Teren *et al.*, 1996; Heenan *et al.*,

1998), or all strains according to other authors (Cabanes *et al.*, 2002; Sage *et al.*, 2002), are OTA producers. A new species was recently described, *A. ibericus* (Serra *et al.*, 2006b), which includes strains isolated in Spain and Portugal very similar to *A. carbonarius* but unable to produce OTA.

Several other species are included among biseriates, but there is no agreement regarding their identification based on morphology. The molecular approach clearly distinguishes two groups, the *niger* and the *tubingensis* types, rather than two species (Accensi *et al.*, 1999; Perrone *et al.*, 2006a). The *niger* type includes a low percentage of OTA producers, around 5–10% (Bau *et al.*, 2006), and it is only recently that positive isolates have been confirmed in the *A. tubingensis* type (Perrone *et al.*, 2006b). All these species are commonly considered to be *A. niger* aggregate.

Several field surveys, starting from 1999, confirmed *A. carbonarius* as the main cause of OTA presence in grapes in Europe (Battilani *et al.* 2006a; Bejaoui *et al.*, 2006; Belli *et al.*, 2006; Serra *et al.*, 2006a; Tjamos *et al.*, 2006; Varga and Kozaciewicz, 2006), Israel (Guzev *et al.*, 2006), Lebanon (El-Khoury *et al.*, 2006), Tunisia (Lasram *et al.*, 2007) and Australia (Pitt, 2000; Leong *et al.*, 2006a).

Their significance is due to the high percentage of *A. carbonarius* strains that can produce toxin at relatively high levels; 70–100% of the *A. carbonarius* strains are reported to produce OTA when grown *in vitro* versus 2–20% of the strains from the *A. niger* species aggregate (Battilani *et al.*, 2003a; Bejaoui *et al.*, 2006; Bellí *et al.*, 2006; Serra *et al.*, 2006a).

14.2.2 Black Aspergilli in the vineyard

Black Aspergilli usually overwinter in soil (Leong *et al.*, 2006a), but they are present on berries from fruit-set and, their incidence increases as the fruit grows and matures, with their numbers peaking at ripening (Battilani *et al.*, 2006b). Among black Aspergilli isolated from berries, members of the *A. niger* species aggregate are the principal group in all growth stages, including ripening, in different countries and years, with respect to isolates of either uniseriate *Aspergillus* spp. or *A. carbonarius* (Leong, 2007). *Aspergillus carbonarius* counts increase between *véraison* and pre-harvest, with the highest incidence at fruit maturity. Black Aspergilli has problems penetrating healthy berries at the early growth stages, while later they are favoured by occasional openings and tender skin, and the fungal incidence depends on the conduciveness of the ripening period and the risk of berry splitting (Leong *et al.*, 2006b).

The incidence of berries infected by black Aspergilli at harvesting is significantly related to latitude and longitude, with positive west → east and north → south gradients reported in Europe. The pattern of berries infection is similar in different years, but the infection incidence is highest in the hottest and driest year. Thus, the meteorological conditions contribute significantly to the explanation of the spatial distribution of black Aspergilli (Battilani *et al.*, 2006b).

OTA is produced while the grapes are in the vineyards and is not normally detected before early *véraison* (Battilani *et al.*, 2004a). This is apparently in

contrast with research results that show a positive correlation of OTA with titratable acidity and negative correlations with reducing sugar concentration (Serra *et al.*, 2006a), which suggest higher conductivity in early stages of berry maturity. However, at this stage, it is very rare to find those wounds on berries that are crucial for black Aspergilli penetration. Field trials confirmed that the last 20 days of ripening are the most important for OTA synthesis (Battilani *et al.*, 2006a). Must obtained from apparently healthy bunches can contain OTA, but berries with black mould are more contaminated; this is probably related to the finding that 60–70% of OTA are accumulated in the conidia in different isolates of *A. carbonarius* (Atoui *et al.*, 2007).

14.2.3 Ecology of black Aspergilli

Fungi development is strongly related to environmental conditions, mainly air temperature and relative humidity, but a relevant role is played by available water (a_w) of the medium, related to the water the fungi can use, not linked with chemical compounds in the berries. Fungi in *Aspergillus* section *Nigri* produce abundant conidia, especially with temperatures higher than 30 °C (Parra and Magan, 2004) and they germinate rapidly, especially with a_w of 0.90–0.99 and a temperature of 25–35 °C. Black Aspergilli can grow between 10 and 37 °C; the optimal temperature for *A. carbonarius* is between 20 and 30 °C and between 30 and 35 °C for members of the *A. niger* species aggregate. Optimal OTA production occurs at 15–20 °C and decreases significantly at 30–37 °C (Mitchell *et al.*, 2004; Bellí *et al.*, 2005).

Available water is also very important for black Aspergilli. The optimal a_w for growth is 0.98, which is similar to the a_w in berries during ripening. Growth rate varies by species, with members of the *A. niger* species aggregate fastest and *A. carbonarius* strains the slowest and more sensitive to a_w; growth of black Aspergilli has never been observed at an a_w of 0.85, and only a few isolates can grow at 0.89 a_w (Mitchell *et al.*, 2003, 2004; Bellí *et al.*, 2004a,b, 2005). The optimal conditions for growth of *A. carbonarius* were between 0.92 and 0.99 a_w, although the highest toxin production occurred when a_w was between 0.95 and 0.99 (Bellí *et al.*, 2004*a*, 2005; Mitchell *et al.*, 2004).

14.2.4 The role of cropping system and pest and disease management

Meteorological conditions and region of origin are crucial for OTA production in grapes (Serra *et al.*, 2006c; Battilani, 2008), although proximity to the sea and the cropping system are also important. Nevertheless, high variability in OTA contamination was observed in clusters grown on the same vine in the same vineyard (Battilani *et al.*, 2006c). The first report investigating marked differences between years, grape varieties and trellising systems compared two grape varieties, Malvasia nera and Negroamaro, grown in south Italy in 1999 and 2000. Contamination levels between 0 and 13 ppb were reported (Battilani *et al.*, 2003b), with Negroamaro managed on 'alberello' trellising and harvested in 1999 showing the highest contamination.

The cropping system clearly has an effect, but the role of each variable is difficult to quantify. Grape varieties show wide susceptibility to black Aspergilli infection and OTA contamination either *in vitro* (Battilani *et al.*, 2004*a*) or in field trials. In the field, the trellising system may influence the incidence of black Aspergilli and the amount of the OTA contamination. In particular, clusters that are closer to the soil appear more contaminated, even if an effect of proximity to the soil on the amount of OTA present has not yet been proven. The type of soil can contribute substantially to the level of contamination, with clay soil being the most conducive.

Black Aspergilli are considered saprophytes, responsible for secondary rots, and wounds of both mechanical and biological origin are important entry sites (Bellí *et al.*, 2007b). *Lobesia botrana* (*Lepidoptera: Tortricidae*) is the major grape berry moth in vineyards of Southern Europe, where it usually completes three to four generations a year, depending on the weather conditions in late summer. First-generation larvae damage flowers, while the succeeding larval generations feed on berries at different stages of maturity. OTA content in berries and pest damage are related, probably due both to the increase of entry points for fungi and the role of larvae as spore vectors (Cozzi *et al.*, 2006).

Powdery mildew is the most conducive pathogen for black Aspergilli. Berries infected with powdery mildew are often misshapen, have rusty spots on the surface or split open during ripening when inoculum of black Aspergilli is readily available (Minguez *et al.*, 2004; Hocking *et al.*, 2007). The relationship between *Botrytis cinerea* and *A. carbonarius* was studied *in vitro* and competition between the two species was observed; they stopped growing when they met on the media. Interestingly, *B. cinerea* was able to degrade OTA when present in the matrix (Valero *et al.*, 2008a). The relevance of this interaction has not yet been studied in the vineyard. Data on the interaction of pests and diseases with black Aspergilli are limited, but their good management in vineyards certainly results in a decrease in OTA content in berries at harvest. This end result can be confirmed comparing neighbouring vineyards, managed with different crop protection approaches (Hocking *et al.*, 2007).

Some trials involving the direct control of black Aspergilli were managed using several active ingredients. *In vitro* trials reported the effectiveness of different compounds in reducing both fungal growth and OTA content in bunches, but only the cyprodinil/fludioxonil combination was confirmed as effective in all field trials managed in France, Spain, Greece and Italy (Tjamos *et al.*, 2004; Kappes *et al.*, 2006; Bellí *et al.*, 2007a; Valero *et al.*, 2007). This combination of active ingredients was originally developed for the control of grey mould, caused by *B. cinerea*, with the same application schedule (Kappes *et al.*, 2006). The most effective single treatment was 21 days before harvest (stage D), but a second treatment at early *véraison* (stage C) was recommended under high risk conditions. Special attention must be paid in using carbendazim because it was shown as an elicitor of OTA production, at least in *in vitro* trials (Medina *et al.*, 2007).

Various biological agents have been considered for black Aspergilli control, including yeasts that occur naturally on grapes. Isolates of *Cryptococcus laurentii*

and *Aureobasidium pullulans* were signalled as the most promising (Bleve *et al.*, 2006), but several strains of *Au. pullulans* were confirmed as effective both *in vitro* (de Felice *et al.*, 2007) and in vineyard (Dimakopoulou *et al.*, 2008).

The cropping system of grapes is relevant also for resveratrol synthesis, the compound supposed as active in the mitigation of toxic effects of OTA. At least two factors, grape variety and soil type, have been shown to be relevant. According to Vezzulli *et al.* (2007), stilbene-synthase gene expression was induced by *A. carbonarius*, and stilbenes were produced in the two grape varieties studied, but the amount was significantly higher in Castor, which displayed few disease symptoms compared to Barbera. Moreover, Bavaresco *et al.* (2008) found a greater production of *trans*-resveratrol and ε-viniferin in berries, infected by *A. carbonarius*, collected from vines grown in calcareous soil compared to those from neutral soil.

14.3 Fate of ochratoxin A in the winery

Grape processing has a significant impact on the fate of OTA during the main stages of winemaking (crushing, maceration, alcoholic fermentation, malo-lactic fermentation, bottle-ageing), and critical steps in the process have been identified. Important aspects of current studies in post-harvest strategies for the control of OTA in wines are presented, focusing on biological decontamination of OTA through the use of yeasts and lactic acid bacteria.

14.3.1 Unit operations in winemaking

The diversity and quality of wine result from grape cultivar, location and the soil type of vineyards, climate and the winemaking process. The basic process in winemaking is recurrent and modifications are applied for special wine types (Fig. 14.2). Grape clusters are harvested and transferred to the winery where they are crushed and destemmed to obtain must.

In white winemaking the must may be obtained by pressing the whole cluster because this procedure allows the minimum time between berry breakage and juice separation and can provide juice with low flavonoid content and sometimes with low levels of suspended solids. The must is treated with sulphur dioxide in order to prevent oxidative reactions and microbiological spoilage and it is clarified by static or dynamic settling. The contact between must and pomace is a fundamental operation for colour and flavour extraction and it is kept during maceration in red winemaking.

Alcoholic fermentation, which is the basic step of winemaking, is carried out by indigenous or selected yeasts to convert the sugars in ethanol and takes from 10–20 days. After the operations of solid–liquid separation (drawing-off, racking) most red wines undergo malo-lactic fermentation using indigenous or selected lactic acid bacteria, while only a relatively small portion of the world's white wines is deacidified by the lactic acid bacteria. The malo-lactic fermentation is included

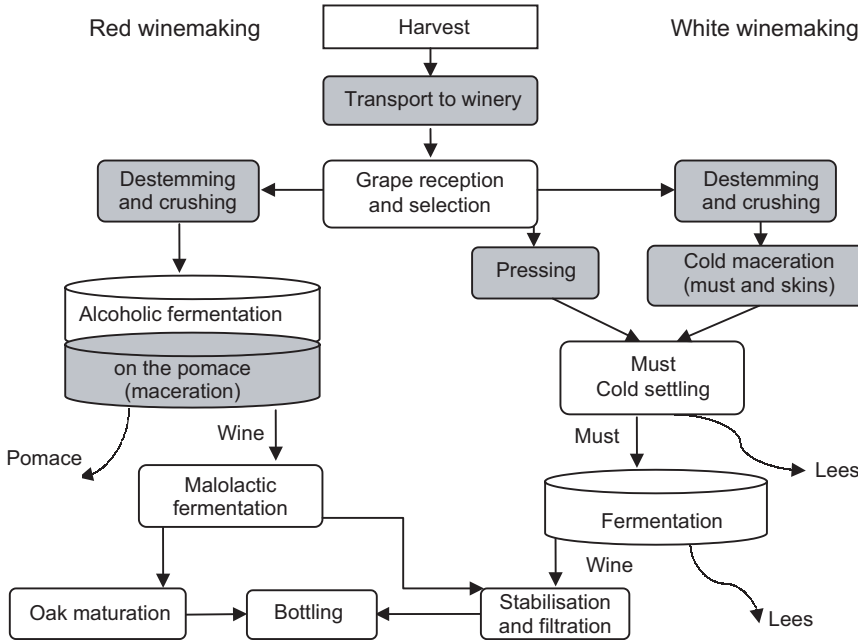


Fig. 14.2 Operations in red and white winemaking that might impact ochratoxin A concentrations.

in the red winemaking depending on the climate of the vineyards, the acidity of the wine and the type of wine. Some white wines are stored on the yeast lees for several months after the alcoholic fermentation. This 'sur lies' treatment is employed to provide the release from the lees of compounds which have a positive influence on wine flavours.

Some red wines are aged in barrels and the wood ageing process lasts months or years. Most wines require stabilisation and filtration before bottling, and the use of fining agents is a common winery practice. It involves the addition of adsorbants to reduce the level of certain compounds in wine (i.e. proteins, tannins).

During the whole process, grape clusters, must and wine are susceptible to various safety and quality hazards. Controls necessary for the critical quality characteristics of the final product are established with quality assurance processes while, for safety, the hazard analysis of critical control point must be used (Christaki and Tzia, 2002; Alldrick, 2003).

14.3.2 Fate of OTA during winemaking

The fate of OTA during vinification and the role of unit operations have been studied by several authors. Grazioli *et al.* (2005, 2006) focused on red winemaking, carrying out full-scale fermentations in different wineries in the south of Italy with two naturally OTA-contaminated grape varieties (Negroamaro and Primitivo) and

in different vintages. The results showed that no OTA is produced during winemaking and the balance of OTA in wine during winemaking is negative. The OTA present in grapes is released to the juice during crushing; maceration increases OTA content, while alcoholic and malo-lactic fermentations and solid–liquid separation reduce OTA in wine. As far as identifying winemaking OTA critical control points, the process can minimise the hazard, but it cannot assure the absence of the toxin. In order to manage the hazards of OTA in winemaking and to verify that OTA concentration in wine is lower than the legal limit of 2 μg/L (European Commission, 2006), OTA analyses in must and in wine at the end of alcoholic fermentation would be enough, since several winemaking practices (e.g. racking, clarification and filtration) reduce OTA concentration.

A small-scale fermentation trial was conducted by Leong *et al.* (2004). Red (Shiraz) and white (Chardonnay and Semillon) grapes were inoculated with *A. carbonarius* spore suspensions before harvest; 80% OTA reduction was observed during vinification of both red and white wine. The same authors reported (Leong *et al.*, 2006c) that 24% of OTA originally present in crushed red grapes passed into must and 72% OTA reduction was recorded in wine after first racking. OTA removal was reported to result from binding either to grape solids and proteins (Fernandes *et al.*, 2003) or to yeast and bacteria cells after fermentations.

Lasram *et al.* (2008) conducted two assays of red and rosé microvinification, with artificially and naturally contaminated grapes. The results from the different assays showed that the maceration of pomace has an effect on the increase of OTA content in red wine whereas the alcoholic fermentation has a reducing effect. However, the spontaneous malo-lactic fermentation showed no effect on the OTA content in wine. Storage of red wine in tanks followed by draining caused a decrease of OTA of about 55%. Clarification with a gelatine fining agent contributed to the removal of up to 58% of OTA from red wine. The positive effect of fining agents was confirmed by Castellari *et al.* (2001).

Solfrizzo *et al.* (2007) investigated with microvinification and at industrial level the distribution of OTA between solid and liquid fractions during vinification, in wine and winery by-products. Microvinification showed that only 4% of the OTA present in grapes is released in wine, whereas most of OTA is retained in pressed grape pomace and 1% is retained in the lees. The results obtained with the microvinification were confirmed at an industrial scale. Similarly, Leong *et al.* (2006c) reported that 9% of OTA present in grapes is released into wine, but intensive pressing of the pomace increased OTA concentration in wine, and the level of OTA in this product is approximately four times higher compared to the red wine traditionally obtained (Gambuti *et al.*, 2005).

All of these studies indicate that winemaking results in large reductions of OTA during processing, although the percentage reduction varied considerably between the studies. A tentative summary of all results available was reported by Battilani (2008). Starting from an assumed contamination of 5 μg/kg of OTA in grapes, the contamination decreases in all the steps in white winemaking and the final content could be between 0 and 3 μg/kg depending on compounds used for clarification and yeast responsible of alcoholic fermentation. In red winemaking, after an

increase of OTA content, it can remain around the initial level or a bit higher after alcoholic fermentation. Following operations, when applied, can reduce OTA content in wine to values around 3 or 1 μg/kg when lactic acid bacteria and clarification compound applied have low or high efficacy in decontamination.

14.3.3 Removal of OTA during winemaking

The reduction of OTA during winemaking, based mainly on the adsorption mechanism, has provided additional impetus to investigate a strategy to eliminate/reduce OTA and to protect consumer health. In general, adsorption involves the accumulation of molecules from a solvent onto the exterior and interior surfaces of an adsorbent. The surface phenomenon is a manifestation of complex interactions between the adsorbent, the adsorbate and the solvent (Kurtbay *et al.*, 2008; Var *et al.* 2008). OTA is a weak acid; it is partially dissociated at the pH of wine (3.0–3.8) and carries a negative charge that may interact with a positively charged surface. OTA may also react by means of carboxylic group and phenol moiety that could be adsorbed through hydrogen bonding and/or charge transfer complexes and interactions of two π-electron orbitals (hydrophobic adsorbent, as i.e. carbon; Huwig *et al.*, 2001).

Many methods to control the OTA concentration during winemaking have been proposed and the removal of the mycotoxin by adsorption with fining agents has been most frequently studied (Dumeau and Trioné, 2000; Castellari *et al.*, 2001; Silva *et al.*, 2003, 2007). These studies showed that most of chemical adjuvants have little effect on the removal of OTA at the dosages currently employed in wine production, and that active charcoal is the most effective. Castellari *et al.* (2001) found that potassium caseinate and activated charcoal showed high adsorption capacity for OTA. Var *et al.* (2008) reported that activated charcoal is an efficient agent that could be used to remove OTA both in buffered solutions and in white wine, while bentonite displays a low affinity for the mycotoxin.

Previous studies, performed on red wines, pointed out that charcoal-based products are highly effective to reduce OTA contamination (Silva *et al.*, 2003). The effectiveness of charcoals was related to the type of product and, for each commercial preparation, amount employed and treatment time significantly affected the level of OTA removal. Dose and contact time are very important variables when a practical application is considered. Since carbon is a very porous non-soluble powder with a relatively unspecific adsorption capacity, it can produce substantial depletion of wine aroma and phenolic profile (Gambuti *et al.*, 2005). Consequently, the contact time and the amount of charcoal to be used are preferred to be as low as possible (Silva *et al.*, 2007). Treatments with oak wood fragments have an effect on OTA reduction depending upon the quantity of wood chips and powder used (Savino *et al.*, 2007).

The use of specific materials that adsorb OTA and the possibility of using microbiological-binding agents to remove the mycotoxin have been tested. Chitosan, chitin, chitin-glucan, and chitin glucan hydrolysate of fungal origin were tested for the removal of OTA in red, white and sweet wines enriched in OTA with

doses of 0.1, 0.5 and 2 μg/L. After two days, OTA was reduced by 56.7–83.4% in red wine, by 53.4–64.5% in white wine and by 26.1–43.5% in sweet wine. These compounds could be useful for the removal of OTA, thereby improving wine safety (Bornet and Teissedre, 2007).

Different yeast by-products, yeast walls and hulls and inactivated yeast, were tested by Silva *et al.* (2007), and the maximum OTA reduction was generally obtained after eight days of contact at 20 °C; a reduction of 40–50% OTA was determined by the addition of 100 g/hL of yeast walls or 40 g/hL of yeast hulls. Removal of OTA from must and wine using oenological yeast strains has also been reported. Bejaoui *et al.* (2004) investigated the efficiency in removing OTA from laboratory medium, synthetic grape juice medium and natural grape juice by viable and dead oenological *Saccharomyces* strains compared with a commercial yeast wall additive. A significant decrease of OTA levels was observed in synthetic and natural grape juices with the addition of many of the growing strains reaching a maximum of 45%, but no degradation products were detected. OTA removal was enhanced with dead cells of yeast, indicating that adsorption, not catabolism is the mechanism involved. The authors concluded that the OTA removal was rapid and improved by dead yeasts, more efficient than commercial yeast walls. Caridi (2006) and Caridi *et al.* (2006) examined the performance of 20 strains of *S. cerevisiae* in removing naturally present OTA during vinification. Considerable strain-to-strain variation was observed and residual OTA ranged from 10–60%. The authors concluded that the yeast cell walls were responsible for much of the reduction in OTA.

A remarkable difference among wine yeasts and between active dry yeast and yeast lees has been reported in the OTA sequestering activity (Garcia-Moruno *et al.*, 2005; Cecchini *et al.*, 2006). When yeast lees obtained at the end of alcoholic fermentation were used, the optimal results were obtained with those from white wine (Garcia-Moruno *et al.*, 2005). The difference found in the treatments with white lees and red lees might be due to competition between polyphenols and OTA for the same binding site on the surface of the yeast cells (Caridi, 2007). This aspect is important because most recent technologies are oriented to applying the well-known technique of 'vinification sur les lies', used successfully with white wines, in red wines (Vivas *et al.*, 2003; Pérez-Serradilla and Luque de Castro, 2008).

The use of bacterial strains for OTA reduction in winemaking requires further evaluation because the literature available is limited and the results are contradictory. The decontaminating effect of lactic acid bacteria was not observed by Fernandes *et al.* (2007) while it was documented by Grazioli *et al.* (2006) and Fumi *et al.* (2008). The OTA removal studies were carried out in laboratory and full-scale trials by using naturally contaminated wines having different OTA levels and *Lactobacillus plantarum* and *Oenococcus oeni* selected strains. The results were species and strains dependent and a biodegradation took part in wine. This hypothesis was supported by OTα appearance during malo-lactic fermentation and during the bacteria starvation (Fumi *et al.*, 2008).

14.4 Ochratoxin A in wines internationally

Several surveys have been conducted all over the world taking into account national or international grapes derived products. They are summarised to point out main risk factors related to the area of production.

14.4.1 Analytical methods

The availability of reliable analytical methods for OTA determination in must, wine and relevant by-products is important for the risk management of OTA contamination in the wine food chain. Methods for analysing OTA in a large variety of commodities are critically reviewed by Monaci and Palmisano (2004). Liquid chromatography with fluorescence detection (LCD–FLD) is the most widely used analytical technique. High-performance liquid chromatography (HPLC) methods employ a reversed-phase column and an acid mobile phase, so the carboxyl group of OTA is in the undissociated form. Recent advances have included the coupling of HPLC to a mass spectrometer using electrospray ionisation (Timperio *et al.*, 2006). Separation by normal-phase TLC and detection by fluorescence are regarded as a technique for OTA estimation, because of its low cost and adaptability (Monaci and Palmisano, 2004). Different analytical methodologies are reported in a worldwide inter-laboratory study on three different wine matrices, red fortified wine, white wine and white liqueur wine, by Ratola *et al.* (2006).

The validated analytical methods used HPLC with fluorimetric detection coupled with immunoaffinity column (IAC) clean-up (Visconti *et al.*, 2001). This method has been approved as a reference method in 2003 by the European Committee for Standardisation (2003) and this is the official method adopted by the Association of Official Analytical Chemists (AOAC, 2002). Basic steps of OTA analysis include sampling, extraction, clean-up and concentration, separation, detection and quantification and confirmation of positive findings. The clean-up can be carried out by liquid–liquid partitioning using aqueous Na-bicarbonate or by solid-phase extraction (Zimmerli and Dick, 1995; Varga and Kozakiewicz, 2006).

Recently, a number of methods for OTA purification with solid-state extraction have been proposed using derivatised silica with C8, C18 and cyanopropyl stationary phases (Monaci and Palmisano, 2004). Interferences are removed by a washing step and then the analyte is eluted with an organic solvent. This procedure is non-selective and the clean-up levels achieved may be insufficient; therefore, IAC is used after solid-state clean-up. For grapes, solid sample, an extraction step with an organic solvent such as acidified chloroform, is necessary and purification through an immunoaffinity column is suggested (Serra *et al.*, 2004).

Besides chromatographic techniques, immunochemical methods, especially various enzyme-linked immunosorbent assays (ELISA), have been developed for rapid screening of OTA in grapes and wines. In an inter-laboratory survey, ELISA could successfully be used to determine OTA content of wines, if IAC extraction

and clean-up precede ELISA detection (Varga and Kozakiewicz, 2006). Recently, immunoassays techniques were tested by Flajs *et al.* (2009); the results of ELISA and HPLC analysis of OTA in naturally contaminated red wines correlated well ($r = 0.821$), and the correlation was better at higher OTA concentrations. In contrast to HPLC, ELISA could not detect very low OTA concentrations.

14.4.2 Occurrence of OTA in wine

OTA has been related to wine contamination since 1996 (Zimmerli and Dick, 1996). After 1996, several surveys were conducted to control the presence of OTA in wine and grape products (Battilani *et al.*, 2004a). Recently, overviews of OTA contamination were reported by Blesa *et al.* (2006), by Mateo *et al.* (2007) and by Visconti *et al.* (2008). In Spain, the highest OTA concentration was 15.25 μg/L in dessert wine. Except for this wine type, the highest concentrations were < 4.5 μg/L. The EU limit of 2.0 μg/L is not applicable to liquor or dessert wines with > 15% alcohol content. In Italy, the OTA presence in wine has been extensively surveyed. OTA incidence is higher in red wines (78.4%), followed by rosé and dessert and white wines. The highest concentration of OTA (7.63 μg/L) was found in a red wine. In Germany, a concentration of 7 μg/L was found in Italian red wine exported to Germany (Majerus and Otteneder, 1996; Majerus *et al.*, 2000). In France, OTA was found in 29 wines (0.01–0.27 μg/L), but a value of 0.78 μg/L was found in a French red wine exported to Germany (Ospital *et al.*, 1998). In Portugal, a survey of 340 domestic wines showed that OTA was detectable in 20.3% of the samples, and the highest level was 2.1 μg/L (Ratola *et al.*, 2004). In Greece, OTA was detectable in more than 66% of samples and both red and sweet wine showed the highest concentrations (Markaki *et al.*, 2001; Soufleros *et al.*, 2003; Stefanaki *et al.*, 2003). More than 50% and 100% of samples analysed, respectively, in Cyprus and Turkey contained detectable levels of OTA (Ioannou-Kakouri *et al.*, 2004; Anli *et al.*, 2005).

Grape products originating from south Europe and north Africa are more affected than those from the temperate regions of central Europe (Zimmerli and Dick, 1996; Otteneder and Majerus, 2000; Pietri *et al.*, 2001; Valero *et al.*, 2008b), following a trend of decreased prevalence and concentration in wines from southern regions compared with northern regions and in red compared to white wine. In the Mediterranean basin the proportion of wine in which OTA is detected is very high (> 50%) in some countries, but only a few wines contained concentrations exceeding the legal limit fixed by the EC.

The range of OTA content in wine produced in Europe varied between 0.01 and 3.4 μg/L. The highest values were reported in some samples of dessert wines and in wines made from dehydrated grapes. Several surveys have reported in this type of wine occurrence ranging from 57% to 100% and high OTA concentration, up to 7.0 μg/L (Valero *et al.*, 2008b). Occurrence of OTA in wines from USA, Canada, South America, Australia and New Zealand is reported as lower. In Australia and South Africa, red and white wines did not show significant differences in OTA

Table 14.1 Occurrence of ochratoxin A in red wines

Countries	Range (μg/L)	Number of samples	Positive (%)	Reference
Croatia	0.012–0.047	7	100	Domijan and Peraica, 2005
Italy	< 0.01–7.63	38	97%	Visconti *et al*., 1999
	< 0.01–3.18	96	82%	Pietri *et al*., 2001
	< 0.06–2.9	81	69%	Bononi and Tateo, 2003
	< 0.03–0.76	78	55%	Finoli *et al*., 2004
	0.23–0.91	5	100%	Shepard *et al.*, 2003
	0.003–0.19	8	75%	Burdaspal and Legarda, 1999
	0.01–2.00	23	61%	Lo Curto *et al.*, 2003
	< 0.01–0.10	44	18%	Larcher and Nicolini, 2001
	< 0.01–7.50	773	69%	Brera *et al*., 2008
	0.02–0.14	100	85%	Rolle *et al.*, 2008
Spain	< 0.06–0.32	28	46%	Lopez de Cerain *et al.*, 2002
	< 0.003–0.60	72	92%	Burdaspal and Legarda, 1999
	< 0.003–0.022	14	64%	Zimmerli and Dick, 1996
	< 0.010–0.190	6	50%	Majerus *et al.*, 2000
	< 0.01–0.50	6	50%	Markaki *et al.*, 2001
	0.05–0.53	61	29%	Blesa *et al.*, 2004
	< 0.05–4.24	130	18%	Bellí *et al.*, 2004c
France	0.002–3.4	12	100%	Markaki *et al.*, 2001
	< 0.01–0.27	21	57%	Ospital *et al.*, 1998
	0.004–0.452	8	100%	Burdaspal and Legarda, 1999
Greece	0.002–2.35	8	100%	Markaki *et al.*, 2001
	< 0.05–2.69	104	68%	Stefanaki *et al.*, 2003
	< 0.02–2.51	14	64%	Soufleros *et al.*, 2003
Portugal	0.011–0.02	2	100%	Burdaspal and Legarda, 1999
	0.002–0.5	6	100%	Markaki *et al.*, 2001
Turkey	< 0.01–0.81	51	86%	Var and Kabak, 2007
	0.05–1.92	35	100%	Anli *et al.*, 2005
Morocco	0.04–3.24	20	100%	Filali *et al.*, 2001
	0.551–0.554	3	100%	Markaki *et al.*, 2001
South Africa	0.07–0.39	9	100%	Shepard *et al.*, 2003
South America	< 0.03–1.33	45	24%	Shundo *et al.*, 2006
South America	0.03–0.07	22	32%	Rosa *et al.*, 2004
Australia	0.05–0.62	344	14%	Hocking *et al.*, 2003
Canada	< 0.008–0.39	36	14%	Ng *et al.*, 2004
Several countries[a]	< 0.05–0.75	26		Chiodini *et al.*, 2006
Several countries[b]	0.05–0.20	580	17%	Soleas *et al.*, 2001
Several countries[c]	< 0.003–0.39	79	70%	Zimmerly and Dick, 1996
Mediterranean wines[d]	< 0.008–2.32	37	68%	Ng *et al.*, 2004
Worldwide origin	< 0.01–3.31	305	54%	Ottender and Majerus, 2000

[a]France, Germany, Italy, Spain, South Africa.
[b]Argentina, Australia, Canada, Chile, Central Europe, France, Germany, Italy, New Zealand, Portugal, South Africa, Spain, USA.
[c]Algeria, Argentina, Austria, Croatia, California, France, Italy, Macedonia, South Africa, Spain, Switzerland, Tunisia.
[d]Algeria, Cyprus, France, Greece, Italy, Spain, Turkey.

Table 14.2 Occurrence of ochratoxin A in white wines

Countries	Range (μg/L)	Number of samples	Positive (%)	Reference
Croatia	< 0.022	7	57%	Domijan and Peraica, 2005
Italy	< 0.01–0.97	9	44%	Visconti *et al.*, 1999
	0.01–0.08	3	100%	Shepard *et al.*, 2003
	< 0.003–0.006	6	33%	Burdaspal and Legarda, 1999
	< 0.01–0.02	27	7%	Larcher and Nicolini, 2001
	< 0.01–1.95	290	44%	Brera *et al.*, 2008
	< 0.03–0.47	34	68%	Finoli *et al.*, 2004
Spain	< 0.07–0.2	12	58%	Lopez de Cerain *et al.*, 2002
	< 0.003–0.267	43	81%	Burdaspal and Legarda, 1999
	0.05–0.76	24	17%	Blesa *et al.,* 2004
	< 0.05–1.13	60	7%	Bellí *et al.*, 2004c
France	< 0.003–0.085	6	67%	Burdaspal and Legarda, 1999
	< 0.01–0.02	4	25%	Ospital *et al.*, 1998
Portugal	0.003–0.020	4	50%	Burdaspal and Legarda, 1999
	< 0.02	64	0%	Festas *et al.*, 2000
Greece	< 0.05–1.72	118	53%	Stefanaki *et al.*, 2003
	< 0.02–0.87	13	54%	Soufleros *et al.*, 2003
Turkey	0.02–0.34	8	100%	Anli *et al.*, 2005
Morocco	0.028–0.18	7	100%	Filali *et al.*, 2001
USA	0.010–0.019	2	100%	Burdaspal and Legarda, 1999
South America	< 0.02–0.03	15	13%	Rosa *et al.*, 2004
Australia	0.05–0.50	257	16%	Hocking *et al.*, 2003
Canada	< 0.004–0.16	43	23%	Ng *et al.*, 2004
Several countries[a]	< 0.05–0.22	16		Chiodini *et al.*, 2006
Several countries[b]	0.05–0.10	362	4%	Soleas *et al.*, 2001
Several countries[c]	< 0.003–0.178	24	100%	Zimmerli and Dick, 1996
Mediterranean wines[d]	< 0.004–3.72	41	51%	Ng *et al.*, 2004
Worldwide origin	<0.01–1.36	60	25%	Ottender and Majerus, 2000

[a]France, Germany, Italy, Spain, South-Africa.
[b]Argentina, Australia, Canada, Chile, Central Europe, France, Germany, Italy, New Zealand, Portugal, South Africa, Spain, USA.
[c]France, Italy, Switzerland.
[d]Algeria, Cyprus, France, Greece, Italy, Spain, Turkey.

contamination. US wines had no quantifiable or low OTA levels (Soleas *et al.*, 2001; Ng *et al.*, 2004). A survey of 601 samples of Australian wine was undertaken by Varelis *et al.*, 2003; OTA was detected in 90 (15%) wines, but at low levels, with 85% of positive samples containing < 0.2 μg/L and only one > 0.5 μg/L. In South African wines, OTA was detectable with a mean of 0.16 μg/L and 0.24 μg/L in white and red wines, respectively (Shephard *et al.*, 2003). Data reported are summarised in Tables 14.1, 14.2 and 14.3.

Table 14.3 Occurrence of ochratoxin A in special wines

Countries	Wine type	Range (μg/L)	Number of samples	Positive (%)	Reference
Italy	Marsala	0.29	1	100%	Visconti *et al.,* 1999
	Marsala	0.315–1.594	2	100%	Burdaspal and Legarda, 1999
	Marsala	0.044–0.337	2	100%	Zimmerli and Dick, 1996
	Special wines	0.003–0.040	4	75%	Burdaspal and Legarda, 1999
	Vermouth	< 0.003	2	100%	Zimmerli and Dick, 1996
	Dessert wines	< 0.01–1.90	28	64%	Brera *et al.,* 2008
	Special dessert wine	< 0.03–3.66	22	95%	Finoli *et al.,* 2004
	Special dessert wine	< 0.001–3.86	15	60%	Pietri *et al.*, 2001
Spain	Fondillon	0.05–0.38	6	50%	Blesa *et al.,* 2004
	Moscatel and Malaga	0.003–2.54	14	93%	Burdaspal and Legarda, 1999
	Moscatel	0.05–0.40	7	43%	Blesa *et al.,* 2004
	Jerez and Montilla-Moriles	0.003–0.254	27	85%	Burdaspal and Legarda, 1999
	Sparkling	0.003–0.037	12	83%	Burdaspal and Legarda, 1999
	Sherry	0.029–0.054	2	100%	Zimmerly and Dick, 1996
	Special wines	< 0.05–15.25	20	45%	Bellí *et al.,* 2004c
France	Special wines	0.003–0.024	4	50%	Burdaspal and Legarda, 1999
Greece	Dessert wines	< 0.05–2.82	18	83%	Stefanaki *et al.,* 2003
	Retsina	< 0.05–1.75	8	75%	Stefanaki *et al.,* 2003
	Sweet	< 0.02–3.20	7	86%	Soufleros *et al.,* 2003
Portugal	Special wines	0.003–0.029	4	75%	Burdaspal and Legarda, 1999
	Port	< 0.003–0.017	6	100%	Zimmerli and Dick, 1996
Germany	Special wines	0.003–0.016	7	57%	Burdaspal and Legard,a 1999
European countries	Special wines	< 0.02–27.79	121	49%	Valero *et al.,* 2008b

14.5 Risk assessment: contribution of wine in human exposure to ochratoxin A

This section describes the approach of risk assessment and reports data on wine consumption and related risk of OTA ingestion with some comments on the effective related risk. OTA present in the human body is related to the ingestion of small quantities present in several food commodities, such as coffee, cereals, dried fruits, grapes, beer and wines (Mantle, 2002). Wines, particularly red wines, were considered to be the second source of OTA intake for humans with 15% after cereals (Anonymous, 1998; Pietri *et al.*, 2001). Otteneder and Majerus (2000) reported that wine consumption contributes only 2% to the OTA intake on the basis of the results of national studies performed by the German Ministry of Health. In some European countries, the consumption of wine gives a value about 0.30 ng/kg bw for Spain (Lopez de Cerain *et al.*, 2002), 0.70 for Switzerland (Zimmerli and Dick, 1996), 3.70 for Greece (Stefanaki *et al.*, 2003), 2.00 for France (Markaki *et al.*, 2001) and 0.2 ng/kg bw per day in Sweden (Thuvander *et al.* 2001). Recently Brera *et al.* (2008) evaluated the exposure of Italian population to OTA ingestion from drinking wine. The results indicated a daily intake for wine consumers from 0.59–1.24 ng/kg bw/day and from 0.33 to 0.90 ng/kg bw/day for the total population, in the worst case accounting for 9.8% of tolerable daily intake (TDI). The contribution of wine to daily intake could be considered neglible in comparison with the tolerable daily intake (TDI) of 17 ng/kg bw defined by the Scientific Committee on Food of the European Commission and 16 ng/kg bw suggested by the WHO Committee of Experts on Food Additives (FAO/WHO, 2007).

Table 14.4 shows the estimated exposure to OTA due to wine consumption in different countries; it was obtained by combining the OTA occurrence with data sets of wine consumption. Based on available data, a range of OTA content in wine was reported for all countries that published contamination data (Table 14.4). The number of samples considered differed significantly between countries; in particular, few samples were analysed in Croatia (14 wines) and more than 1500 wines were considered in Italy. Nevertheless, a supposed mean contamination was defined for each country. This variable differs considerably and ranges from 38.5 mL/week (Brazil) to 1065 mL/week (France). The distribution of samples is not normal, but more similar to a Poisson distribution, and 1/5 of the maximum contamination level was then chosen as the supposed mean. Each author reported the number of positive samples and the total samples analysed; the samples were hypothesised as representative of the country and the percentage of positive samples was consequently derived. Annual wine consumption in different countries was reported by the Organisation Internationale de la Vigne et du Vin (OIV) (www.oiv.int) and consequent OTA ingestion per week was computed; a constant wine consumption during the year was considered. Weekly intake of OTA/kg bw was then computed for women and men. Based upon a body weight of 55 kg for women and 75 kg for men, the OTA ingestion ranges from 3.12 ng/week to 433.3 ng/week. Data show that the estimated weekly intake is higher for women, but it represents 6% of TWI suggested by EFSA, and it is also 7% of the tolerable

Table 14.4 Assessed weekly intake of ochratoxin A due to wine drinking in different countries where surveys were conducted

Reported contamination		Supposed mean (μg/L)	Positive samples (%)	Wine consumption (mL/week)	OTA ingestion (ng/week)	Weekly intake (per kg bw)[b]	
Countries	OTA (μg/L)[a]					Women (55 kg)	Men (75 kg)
Australia	< 0.2–< 1.0	0.2	15	430	12.90	0.23	0.17
Brazil	0.01–1.33	0.3	31	38.5	3.58	0.07	0.05
Croatia	0.01–0.05	0.01	80	784	6.27	0.11	0.08
France	< 0.01–0.78	0.2	27	1065	57.51	1.05	0.77
Germany	< 0.01–1.36	0.3	23	462	31.88	0.58	0.43
Greece	0.001–3.4	0.7	100	619	433.30	7.88	5.78
Italy	< 0.01–3.31	0.7	50	894	312.90	5.69	4.17
Morocco	0.028–3.24	0.6	100	19.6	11.76	0.21	0.16
Spain	< 0.003–3.19	0.6	50	611	183.30	3.33	2.44
Switzerland	< 0.003–0.39	0.08	82	756	49.59	0.90	0.66
Turkey	< 0.006–2.23	0.45	90	7.7	3.12	0.06	0.04

[a]Outliers were not included in the range of OTA reported.
[b]Tolerable weekly intake reported by EFSA (2006) is 120 ng/kg bw.

week ingestion suggested by WHO committee of food additives (FAO/WHO, 2007). Even if data confirmed a major exposure in Mediterranean areas (Greece, Italy, Spain), the exposure values indicate that wine contribution does not represent a serious risk factor for consumers. So the situation can be considered 'safe'.

14.6 A decision support system to minimise ochratoxin A in wine

The approach of a decision support system (DSS) is described below, including the development of a predictive model, and good agricultural practices and good manufacturing practices to minimise OTA in grapes and wine are detailed.

14.6.1 Development of decision support systems for the safe management of crops

A decision support system (DSS) is planned to support operators in decision making, taking into account all available knowledge. A DSS is a tool that can help both farmers and technicians working in vineyards and cellars to manage grape growth and winemaking, in the grape – *A. carbonarius* system, to minimise OTA contamination in wine.

The core of a DSS is a simulation model able to predict the behaviour of the pathogen *A. carbonarius*, intended as the effect on the host plant, as a function of the relevant factors, mainly ecological parameters and the cropping system. Information on geographic area and vineyard, as well as real time meteorological data, are input for a simulation model. Data output consist of a prediction of the pathogen effect on yield, in this case especially in terms of quality and safety, obtainable with the optimisation of the cropping system. The DSS can also consider the post-harvest period; the pathogen is no more active, but the unit operations play a role that can be determined and considered.

System analysis is an approach demonstrated to be useful in different disciplines where a system is studied by distinguishing its major components and characterising their changes and the interconnecting elements (Leffelaar, 1993).The system structure in plant pathology includes pathogen, host, environment, human actions and their relationships (de Wit, 1994). A simple way to represent a complicated system, like a pathosystem, is to draw a relational diagram (Leffelaar, 1993) that shows the status of the system at a certain moment and its dynamics over time.

DSS implementation follows the step-by-step approach (Rossi *et al.*, 1997), beginning with problem definition, managed with the support of growers, technicians, advisors, consumers, researchers, or any other actor in the agro-food production. In the second step, a predictive model is developed. A model is a simplified representation of a system, which is a limited part of reality and contains interrelated elements. The relational diagram (Leffelaar, 1993), drawn using information from step 1, helps to organise the available knowledge, pointing out

relevant elements, their relationships and feedback and lack of information. Quantitative relationships, defined by mathematical functions contribute to predict fungal development. Predictive models must be validated, and data collected in the past, in proper experimental trials and on farm, representative of a wide range of situations, are compared to model predictions. The last step is the refinement of the model, taking care of final user feedback and weak points underlined during validation (Teng *et al.*, 1980).

14.6.2 Decision support systems for OTA in grapes and wine

Knowledge available on *A. carbonarius*, its development on grapes and fate of OTA during winemaking is substantial, even if not exhaustive, and the base for a DSS development is ready. All available information can contribute to drawing the relational diagram of the *A. carbonarius*–grape pathosystem (Fig. 14.3).

The inoculum is always observed on berries and, consequently, it is not a limiting factor and its quantification is considered not relevant. Spore germination has been studied on grape juice agar, grape flesh and grape skin, and quantitative relationships can be developed to correctly predict germination (Mitchell, 2006). Specific trials are available on spore survival in different conditions, and these data could contribute significantly to a good prediction of spore behaviour (Leong *et al.*, 2006b). Black Aspergilli were able to grow at all considered temperatures, but each step of 5 °C was relevant for fungal growth. At 5 °C fungi grew very slowly, at 10 °C, growth rate was not different, then it increased until 30 °C and decreased at higher temperatures. The maximum growth rate occurred at 30 °C, for *A. carbonarius* isolates. Growth rate decreased progressively going from 30 to 5 °C,

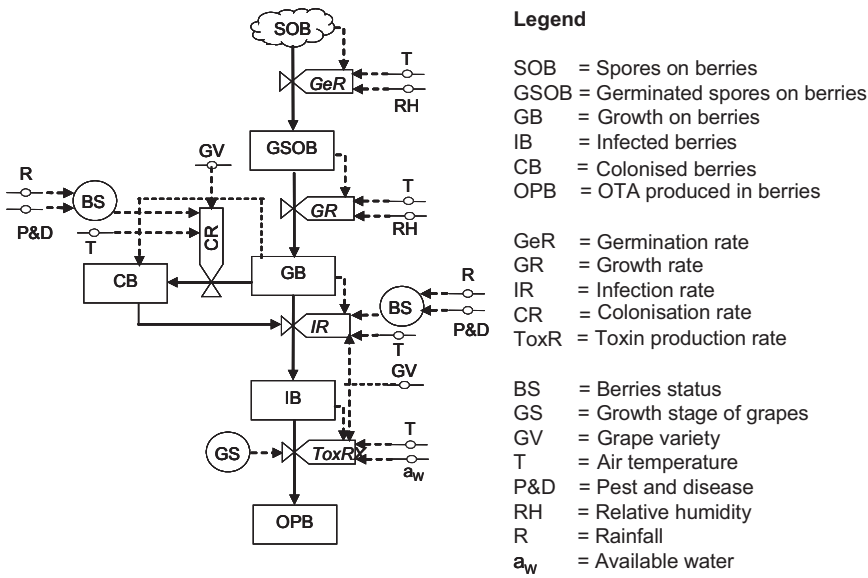


Fig. 14.3 Relational diagram of *A. carbonarius*–grape pathosystem.

but for *A. carbonarius* they were not different at temperatures between 15 and 25 °C. Growth rate of black Aspergilli in relation to temperature was well described by the Analytis equation, with R^2 =0.95 for *A. carbonarius* (Battilani *et al.*, 2003b). It depends on temperature and it will be improved, if necessary, with further data to be collected in different humidity conditions.

The mycelium of *A. carbonarius* can stay on skin and colonise berries (CB; see Fig. 14.3) or grow inside and infect berries (IB; see Fig. 14.3). Colonisation and infection rates are influenced by the same variables. Temperature is always a relevant factor because of its influence on growth rate, but berries status (BS) and grape varieties (GV) are definitely relevant. Talking about BS, the main aspect concerns presence/absence of wounds on skin. Openings favour fungus penetration and development inside the berry, and it was confirmed in many trials that infection is very rapid (Battilani *et al.*, 2003c; Bavaresco *et al.*, 2003). Many factors can determine skin damages, but rain (R), especially during ripening, and pest and disease (P&D) presence play a main role. A correlation was found between OTA content in berries and *L. botrana* damages, as cited before (Cozzi *et al.*, 2006).

Among pathogens, powdery mildew seems the most conducive for black Aspergilli. Infected berries are often misshapen or have rusty spots on the surface, and severely affected fruit often split open, mainly during ripening when the inoculum of black Aspergilli is more relevant. No quantitative data are available on this issue. Infection of healthy berries was observed *in vitro*, but time and inoculum concentration requested suggest that this way of infection should probably be infrequent in the field.

Grape variety is a further relevant factor that can favour berries infection and its relevance was shown both *in vitro* (Battilani *et al.*, 2004b) and in field trials (Battilani *et al.*, 2003b). This factor needs to be further studied, because the reasons that make a variety more or less susceptible were not understood. It is not easy to quantify colonisation or infection of berries, but a yes/no response seem sufficient to run the model, at least for the first validation. OTA seems more concentrated in the outer part of the berry, in the skin and very close layers (Pietri *et al.*, unpublished data) and only infected berries were considered to quantify OTA production (OPB; see Fig. 14.3).

A. carbonarius is able to produce relevant amounts of toxin in the range 15–25 °C. The optimum temperature for OTA production varied among isolates; 15 °C was much better for some strains, while comparable results were obtained in the range 15–25 °C for other isolates (Battilani *et al.*, 2003b; Mitchell *et al.*, 2003, 2004; Bellí *et al.*, 2004a,b, 2005).

A specific trial was managed with 60 strains of *A. carbonarius* isolated from grapes in Italy to test on synthetic grape medium, representative of grape composition at early *véraison*, the effect of strain on fungal growth and OTA production. Two temperatures were considered: 15 and 25 °C. The cluster analysis resulted in five clusters: two of them, cluster 1 (15% of considered strains) and cluster 5 (18% of considered strains), consider the situation at the temperature extremes, taking into account only those isolates able to produce a high amount of toxin at just one

temperature, 25 °C and at 15 °C, respectively. All the other clusters consider intermediate situations. The geographic origin of strains was not relevant (Giorni *et al.*, 2006) and the role of strains was not well defined, but they can be separated in two groups, one including producers of high amount of OTA and the second with low OTA producers. This must be considered in OTA prediction. No equations were found able to describe the rate of OTA production by *A. carbonarius*, but several factors such as temperature, a_w, grape growth stage (Battilani *et al.*, 2003c) and grape variety (Battilani *et al.*, 2004b) can contribute to determine the risk level. A prototype model could be elaborated based on this relational diagram and mathematical functions, and it is likely that it will be available in the near future.

14.6.3 Mitigation of OTA in grapes production and processing

Research managed during the last 10 years gave many answers to questions related to OTA presence in grapes and wine and, according to the results available, the following good practices can be suggested:

- good pest and disease control, preferably with fungicides also active against black Aspergilli
- rational fertiliser supply
- rational irrigation, when appropriate
- appropriate canopy management to control plants vigour
- timely harvest at ripening, no delay especially with damaged and mouldy berries
- minimise the time interval between harvest and crushing; refrigerate the grapes when crushing will not take place within a short time
- discard bunches with visible mould, especially black moulds
- add sulphur dioxide to grapes
- control OTA level in must after crushing
- in case of high OTA risk, reduce the maceration time, use yeasts, lactic acid bacteria, and chemical adjuvants proven to be effective against OTA
- an additional control after the alcoholic or malo-lactic fermentation can be added if the OTA level in must is above the legal limit
- further use of by-products from winemaking should be checked for OTA contamination before their usage as food or feed ingredients.

Good manufacturing practices are currently under discussion by Codex Alimentarius for the adoption of a procedures in order to minimise OTA level in wines on the basis of the code of sound vitivinicultural practices (resolution Vitioeno 01/2005) recommended by the OIV (www.oiv.int).

14.7 Future trends

There is still a lack of knowledge on this subject, and many future needs require summarisation. Many research projects have contributed to the state of the art

regarding OTA in grapes and wine, and mycotoxin content can be controlled both in vineyard and winery. Nevertheless, there is lack of knowledge in some areas. The role of grape varieties and trellising system are not yet known and efforts could be devoted to this, even if it is not easy to study these aspects in vineyard. Pests and diseases are confirmed as playing a relevant role, but quantitative data are poor. The effects of oenological operations have been confirmed and decontamination tools have been proposed, but quantitative data are very variable between studies; therefore trials managed with a previously agreed-upon approach would possibly lead to more consistent results.

A project, whose acronym is MYCORED, was recently granted by EC in the 7th framework program and hopefully it will contribute to filling the gaps, together with other projects ongoing in all interested countries all over the world.

14.8 References

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15

Advances in grape processing equipment

M. Christmann and M. Freund, Geisenheim Research Center, Germany

Abstract: The aim of grape processing, in simplest terms, is to extract the juice from the berries. It influences the must composition and, moreover, has a decisive influence on the subsequent wine quality. This chapter describes the importance of the sub-steps of harvesting techniques, grape transport, grape reception and pressing techniques. Here, the focus is on the interaction of the quality of the raw material and grape processing techniques.

Key words: gentle grape processing, harvesting techniques, grape transport, grape receiving, pressing techniques.

15.1 Grape processing

15.1.1 Grape processing steps

Grape processing, which ranges from the harvesting technique to transport and reception to the pressing process, exerts a decisive influence on the constituents and thus on the quality of the subsequent wine (Fig. 15.1). In the course of grape processing, the grape is more or less damaged throughout the various technical sub-processes during which the berry skin is torn and the juice flows from the cells. At the same time, this activates a number of biochemical processes that influence juice composition. The non-homogeneous solid/liquid phases are largely separated by pressing, during which an increase of problematic constituents can be detected. These problematic constituents in the grape are primarily found in the stems, the seeds and the berry skins. Damaging these not only creates more solids with increasing mechanical stress but also leads to increased extraction of undesirable constituents.

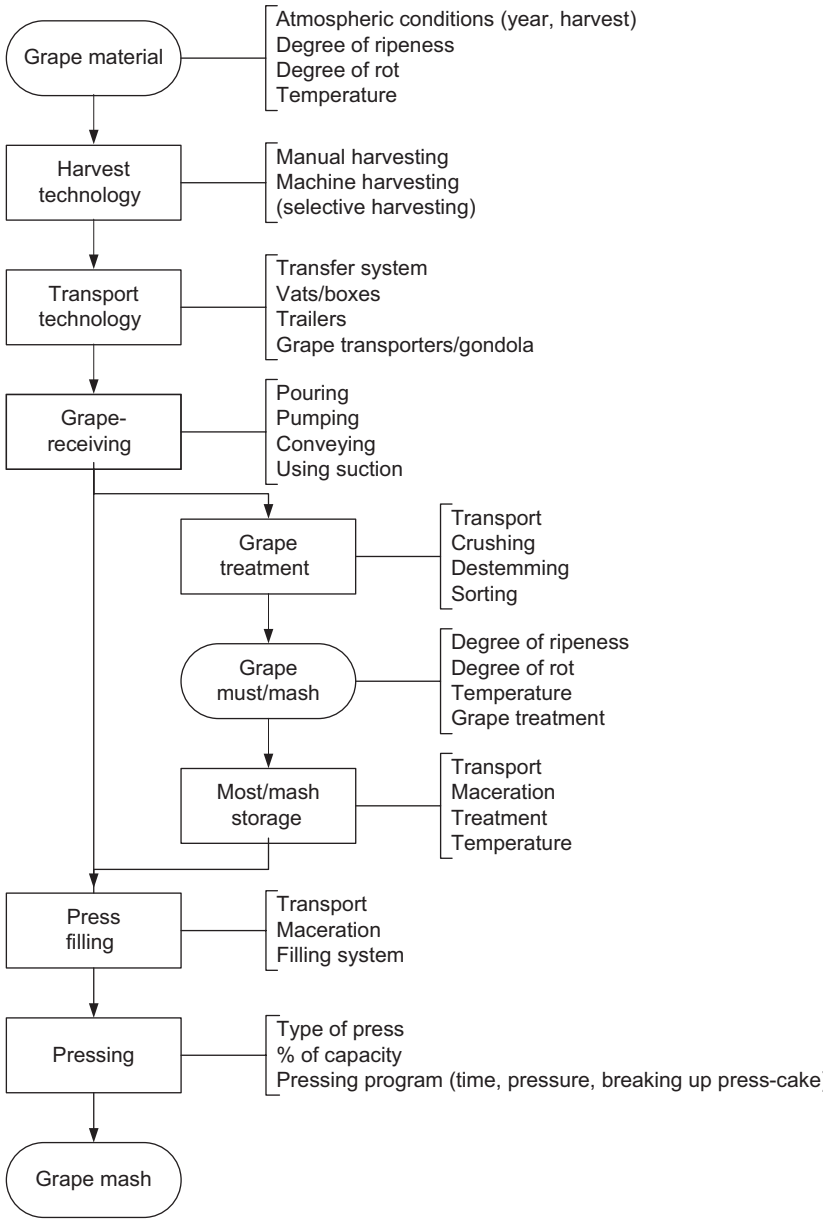


Fig. 15.1 Grape processing steps and influencing variables.

15.1.2 The influence of the time of harvesting and the composition on the technology

The effect of mechanical stress on the grape material during the processing depends largely on the firmness of the berry tissue and its resistance to the applied

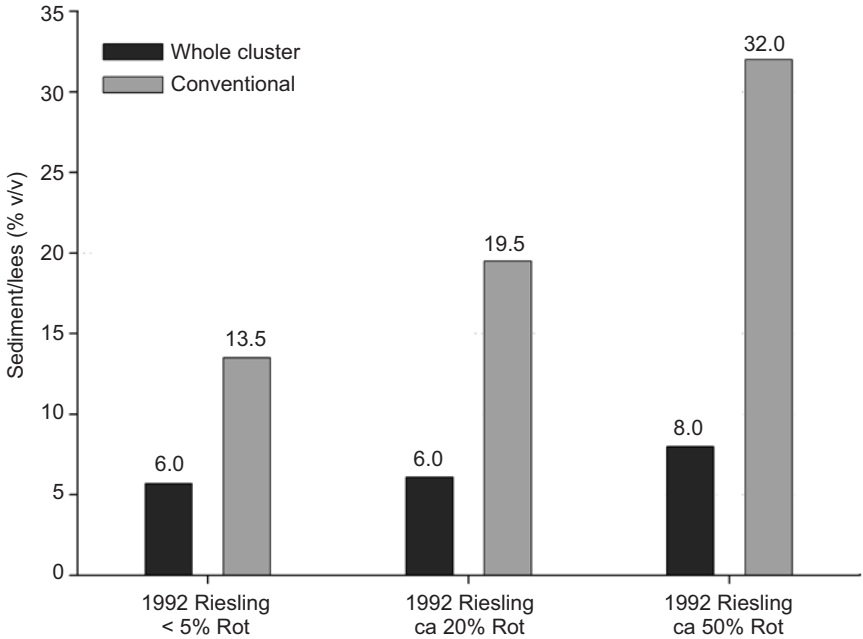


Fig. 15.2 Sediment relative to grape processing and berry firmness (Seckler, 1997).

forces. This resistance is determined by the grape variety but also by the degree of ripeness and the degree of rot of the harvested grapes. Both variables decrease the firmness of the berry skin and thus the resistance and increase the sensitivity to mechanical stress. Ultimately, the result is that, with increasing ripeness and rot, the harvested grapes become more sensitive to mechanical stress by processing (Fig. 15.2) (Seckler 1997).

15.1.3 Basic principles of careful grape processing

Harvesting technique

The condition of the grapes has an influence on the harvesting technique. Thus, Fig. 15.3a shows that machine harvesting with an opportunity for further processing – in this example, transport in standard vats – can be equal to manual harvesting in terms of quality if the harvested grapes are healthy and undamaged. It is only in relation to the technique used for further processing – in the case study, a grape transporter with screw and eccentric screw pump is used – that an adverse effect that becomes noticeable in the form of more solids. If the same technique is used for unsound grapes, the differences become more apparent. Damage, and thus more solids, are produced since the grapes cannot be selected during the harvest and the grape material is under mechanical stress during the shaking and transport process in the machine. While this may still be acceptable in the case of the

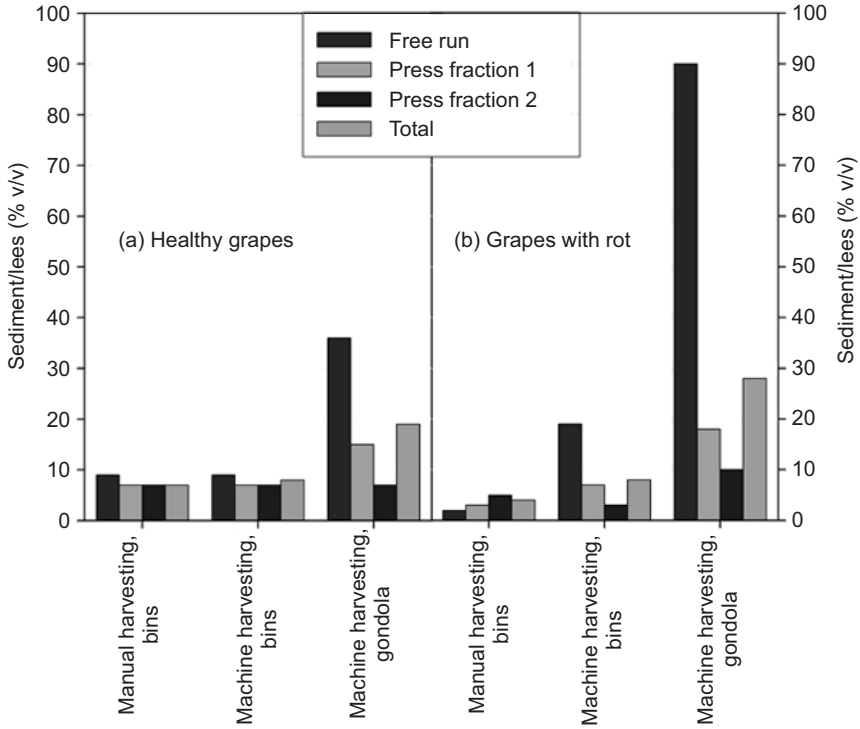


Fig. 15.3 Sediment relative to harvesting technique and berry firmness (Seckler, 1997).

example in Fig. 15.3b when using an adapted conveyor technique, machine harvesting with grape transporter, screw and eccentric screw pump influences the quality to an extent that cannot be tolerated.

Transport technique and grape reception

It becomes apparent from the results shown in above (harvesting technique) that the transport technique, and especially the unloading system, play prominent roles. In practice, in addition to vats, cases and various forms of transport vehicles, grape transporters equipped with an auger conveyor or a vibrating conveyor belt have gained acceptance for reasons of labour efficiency (see Section 15.3).

Apart from the processing capacity, however, the mechanical stress of the harvested grapes also has to be considered. This is illustrated using the example of the grape transporter with a screw and a pump. As shown in Fig. 15.4a, the production of substantial quantities of sediments can be prevented by changing the pump rotational speed while keeping the diameter constant. In contrast, changing the pump diameter (Fig. 15.4b) does not have any serious impact. In order to ensure a quality-oriented mode of operation with these systems, the criteria described in Section 15.3.1 need to be observed.

Figure 15.4c shows clearly that, despite optimizing the pump parameters

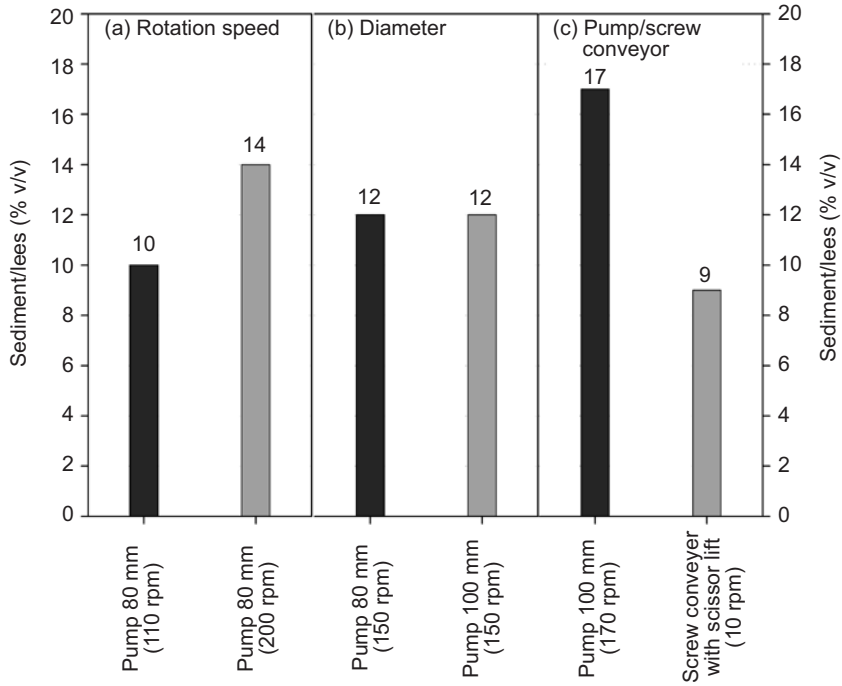


Fig. 15.4 Sediment relative to grape reception: (a) 1998 Müller-Thurgau/rotation speed; (b) 1997 Riesling/diameter; (c) 1998 Riesling/gondola with and without pump (Seckler *et al.*, 2001).

unloading exclusively by means of a screw results in higher quality. This naturally requires appropriate conditions to transport the grapes to further processing without additional pumping. Options are a natural slope, scissor lift systems and conveyor belts (see Section 15.3) (Seckler *et al.*, 2001)

Maceration time

Unloading the grapes is followed by a maceration time, depending on the basic conditions and the objective. From a quality point of view, this maceration serves to obtain constituents located in the berry skin such as aromas, minerals and phenolic substances that partly influence the taste; on the other hand, from a labour efficiency point of view, maceration leads to an increase of juice output, among other things, while the pressing time is shortened, allowing for increased pressing capacity or better utilization of the pressing capacity due to the various options for mash storage and pre-draining.

The grapes are torn to facilitate the release of juice. Depending on the region, ripeness, grape variety and other processing measures (storage, standing time, conveyance, etc.) the grapes are not only crushed but also destemmed. As a consequence of the enzymatic cell disruption taking place during standing time, all constituents primarily located in the berry skin increasingly pass over into the juice

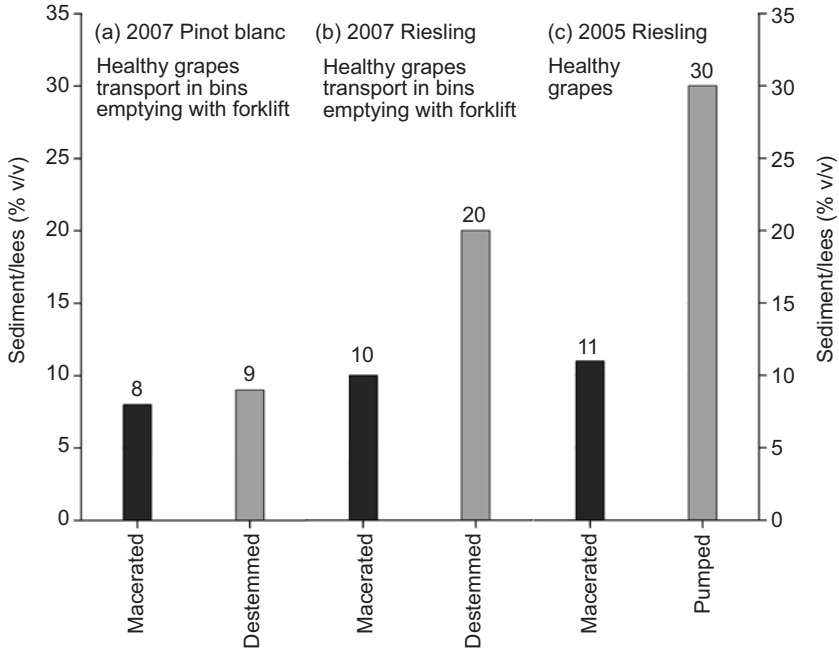


Fig. 15.5 Sediment relative to grape treatment: (a) macerated/destemmed; (b) macerated/destemmed; (c) macerated/destemmed (Seckler *et al.*, 2008).

and influence its characteristics accordingly. The manner of doing this depends to a large extent on the composition of the berry constituents and thus, among other things, on the grape variety, the yield, the degrees of ripeness and rot and, not least, on the processing steps before and after maceration time. For this reason, the preceding as well as the subsequent processing steps need to be considered in addition to the characteristics of the harvested grapes in order to determine the necessity for and the duration of the maceration time. This is illustrated in Fig. 15.5. In the case of Pinot blanc (Fig. 15.5a), which is suited to being destemmed (Freund and Seckler, 2007), only slight differences in solid reaction can be observed between crushing and destemming. However, in the case of Riesling, a destemmer imposes excessive mechanical stress on the grape material (Fig. 15.5b). Considering the influence of the pumping process (Fig. 15.5c), which in extreme cases precedes and/or follows destemming, a maceration time conducive to the wine quality cannot be assumed. If further factors are involved, such as overripeness, rot, machine harvesting, or respective transport techniques that initiate the enzymatic disruption of grapes already in the vineyard and thus, to a greater extent, predispose them to mechanical stress, the adverse effect is even more increased. Figure 15.6 shows further that the destemming process not only creates more solids but also produces less juice yields in both Pinot blanc (Fig. 15.6a) and Riesling (Fig. 15.6b), which results from the fact that the stems are missing, leading to increased compaction and clumping and to a reduced loosening effect.

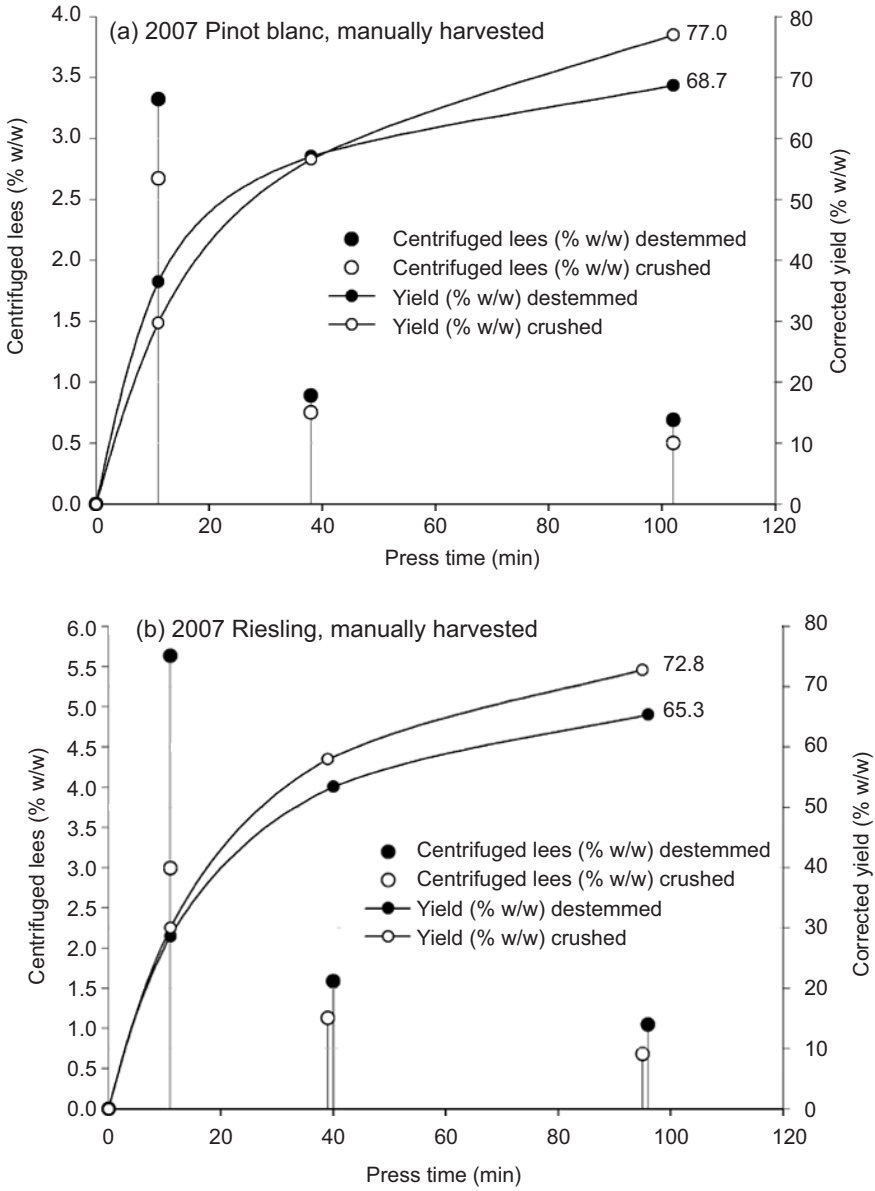


Fig. 15.6 Chronological sequence on corrected yield and centrifuged lees relative to grape treatment (Seckler *et al.*, 2008).

Table 15.1 Maximum volume flow in must pipes

Must pipe		Volume flow	
∅	Cross-sectional area (A)	v = 0.7 m/s	v = 1.0 m/s
60 mm	0.0028 m²	7 m³/h	10 m³/h
80 mm	0.0050 m²	13 m³/h	18 m³/h
90 mm	0.0064 m²	16 m³/h	23 m³/h
100 mm	0.0079 m²	20 m³/h	28 m³/h
125 mm	0.0123 m²	31 m³/h	44 m³/h

$$\dot{V} = A \times v \left[\text{m}^2 \times \frac{\text{m}}{\text{s}} \times \frac{3600\ \text{s}}{\text{h}} \right] = \left[\frac{\text{m}^3}{\text{h}} \right].$$

$\dot{V}$ = volume flow [m³/h].
A = cross-sectional area [m²].
v = flow velocity [m/s].
Source: Seckler *et al.* (2001).

Press loading and the pressing process

The storage of mash is followed by filling the press, which can be carried out by way of the cover or by means of an axially arranged valve – the so-called axial filling. In the case of axial filling, draining during filling is additionally offered, mainly for reasons of labour efficiency. Since the press basket can rotate during filling, much larger amounts of grape mash can be placed in it. Table 15.1 illustrates that the price for this additional amount of press capacity is increased solids content and the resulting adverse effects.

The same goes for depressurized pre-draining which can also be selected in many pressing programmes in the case of filling by way of the cover, in order to increase the press capacity. In the case of axial filling, as described above, further effects depending on the harvested grapes are added by the necessary use of a pump. This pumping process results in increased cell disruption, and thus in a larger amount of free-run juice during pressing. Despite the increased solids content that can be observed, a higher corrected yield for the pumped fractions can be expected in the case of healthy grape material (Seckler *et al.*, 2008). If, however, pre-damaged grape material or grape mash is transported into the press, the berry tissue is increasingly damaged and the solids already created during processing are fragmented. This leads to colloidal solids having an adverse effect on the pressability.

Filling the press is followed by the actual pressing process which, with regard to its effect on quality, may only be judged in connection with the basic conditions already described – grape variety, year of vintage, degrees of ripeness and rot, production steps before pressing. Thus, the preparatory steps such as destemming, pumping and maceration time have a greater impact on the pressing result than the parameters given by the pressing programme such as the pressure pattern, the configuration of cycles and pressure levels, the moment of loosening and loosening repetitions as well as the selection of maximum pressure.

15.2 Mechanical harvesting

15.2.1 General

The machine harvester was developed to make one of the most time-consuming processes in winemaking more efficient. About 160–300 hours are needed to pick one hectare (2.4 acres) of grapes by hand, while using a machine harvester takes only 0.6–1.2 hours. Economically, the advantages are quite obvious. Machine harvesting costs 400–550 Euros per hectare while hand picking costs from between 1600 and 2000 Euros per hectare.

During the 30-year history of developing machine harvesters, the functions have been expanded and optimized, so the current models are multifunctional high-tech machines. The market is serviced by four main brands: (ERO, Braud/New Holland, Pellenc and Gregoire). These brands make both self-propelled and pulled harvesters, with different specifications and quality levels. Even though all machines harvest using the same principles, the brands have different ways of implementing these techniques (Schwarz and Keicher, 2009).

15.2.2 Functionality

The pulled and self-propelled machines work using the same mechanical dynamic swing–shake method. This method involves the separation of the grapes and berries from the stems/vines through the effect of the action on the trellising system. The adherence of the fruit, which can vary substantially according to variety and ripeness, is overwhelmed by the swinging power of the shaking head, which causes the grapes or berries to separate from the vine – harvesting. The swinging of the vine is transferred through the shaker sticks or bows which can be run with different frequencies. To be able to react to different circumstances, the frequency of the bows can be adjusted, as well as the amplitude. Two systems of shakers are used by the different companies. Braud and Pellenc have bow-formed shakers that are mounted and adjustable on both sides. ERO and Gregoire use drop-formed sticks that transfer the swinging and shaking from one end of the stick to the vineyard row. Compared to older systems of directly beating the grapes from the vine, these systems are much gentler on the vineyard and the harvested fruit. The fruit, once shaken off the vines with the swing–shake method, lands on the bucket conveyer and gets transferred to the holding bins. Different manufacturers have specific ways of separating out leaves, stems and other MOG (materials other than grapes). The holding bins are built-in parts on all machine harvesters and can be transferred to other containers sideways or backwards (Schwarz and Keicher, 2009).

15.2.3 Technology

Even though the principles of the harvesting are the same, the different manufacturers have different technical solutions, accessories and additional equipment. The goal is to optimize the harvest while at the same time relieving the strain on the

operator. Certain quality assurances come through optimization of the equipment while some are directly dependent on the operator. Decisions on the settings for the harvester are more important for quality assurance than the direct operation of the harvester. A successful harvest depends upon experienced operators and their optimal utilization of the equipment (Schwarz and Keicher, 2009).

15.2.4 Technology specific to each manufacturer

Braud/New Holland
The harvesting head of the VL-model allows for complete control over every single rod, which supports easy adaptation to different trellising systems. The gap between rods can be controlled in the cockpit as well, in order to fit varying conditions. The harvesting of grapes and/or berries is completed with the patent-protected basket-collection system. The basket-collection system minimizes losses of juice and berries and seals the harvester off from the ground. VL models are run with electronic speed-control to assure a constant harvesting speed. They can be ordered with emptying capacity to the rear or side of the harvester. To maximize the use of this machine, it is also possible to buy different accessories (i.e. hedging head, pruning head, defoliating head, etc.) that make the machine deployable year-round (Schwarz and Keicher, 2009).

Pellenc
The frequency, amplitude and spacing of the shakers can be set with controls contained in the cockpit. Up to six different harvesting programs can be saved and loaded with the push of a button. Pellenc machines are equipped with an 'Active head' system, which automatically performs horizontal corrections of up to 15 cm. With the help of a sensor, the posts are identified and the shake frequency is automatically slowed to protect their integrity. Out of the factory, all machines are ready for year-round implementation with a variety of specialized accessories like the Braud, but Pellenc is the only company that offers all accessories and extensions manufactured by their own company (Schwarz and Keicher, 2009).

ERO
Using ultrasonic and mechanical sensors the harvester has built-in automatic steering (over grape rows), which allows for easy operation. The shaking mechanism automatically turns off when going over posts in order to spare posts and shaking rods. Besides removing leaves and other MOG using heavy-duty suction blowers, the ERO has a rotating rake mounted in the harvesting tunnel that discards MOG to the side. A 'steep vineyard' package with permanent four-wheel brake system and a differential lock and safety device for steep vineyards with up to a 40% slope can also be ordered. The predominantly small vineyard parcels in Germany can be easily harvested with an option to raise the maximum street legal speed to 40 km per hour, which is especially useful for contract harvesting (Schwarz and Keicher, 2009).

Gregoire
The French manufacturer offers both pure harvesters and multifunctional machines with harvesting capabilities. Food-grade plastic thatches seal the harvesting head with conveyor belts running the fruit up to the containers on each side. To ensure consistent results, the speed can be regulated and/or programmed. Included in the comprehensive controls are adjustable shaker intervals and, like the ERO, the operator's cabin is mounted in the centre, which facilitates operation and quality control (Schwarz and Keicher, 2009).

Summary
Within the large variety of specific solutions, several functions available on grape harvesters can be identified which will most likely become this industry standard. Automatic speed control, steering, height adjustment of the harvesting head and easier programmability of these settings are being continually developed (Schwarz and Keicher, 2009).

15.2.5 Optimization of grape processing with mechanical harvesters

To raise the level of efficiency of grape harvesting and processing, as well as to improve the quality of both, harvesters can be accessorized with additional equipment. All manufacturers offer a built-in destemmer, though type and manufacturer differ. Pellenc focuses on its own destemming and cleaning system that it has developed, which reduces MOG to a minimum. The Braud/New Holland offers a built-in belt-destemmer option that is gentler on the grapes. ERO uses a more traditional cylinder/basket destemmer, which adds more flexibility; it is not built-in but can be mounted as an accessory.

Another current step in mechanization is the addition of a decanter to make it possible to complete the grape processing in the grape harvester itself. This would make it possible to store an intermediate product directly out of the vineyard. ERO and Westfalia are developing this system and are planning an upcoming commercial launch (Schwarz and Keicher, 2009).

15.2.6 Cable-drawn steep-slope grape harvesters

Mechanization of steep slope vineyard grape harvesting would bring efficiency and possibly prevent a massive decline in steep vineyards in Germany and elsewhere in Europe. To develop the technologies necessary for such a complicated project, the cooperation of several companies was vital. Durmatec created a prototype that is currently in its second year of testing (Fig.15.7). While the principle behind harvesting is the same as for any other grape harvester, the difficulty of creating a safe and efficient way of coming up and down steep slopes made a massive overhaul of the system unavoidable. The drive system on this special harvester is regulated between a hydrostatic traction drive on the harvester and specially developed winches attached to the supporting trailer. Both winches on the trailer keep the grape harvester safe in case of problems and help climb

Fig. 15.7 Durmatec cable-drawn steep-slope grape harvester.

slopes of up to 60%. The system is being optimized to make operation of the machine easier, but it will soon be available to fill a gap in the niche market of steep-sloped grape harvesters (Schwarz and Keicher, 2009).

15.2.7 Future outlook

The difficulties of being able to produce affordable machines while still improving the quality and keeping up with the ever-rising requirements for record-keeping and quality control are leading to a necessity for automatic documentation of all completed tasks. The mechanization of the harvest should ease the workload on management as well as saving money for the company. The automatic documentation should not create more work, and it should be legal standard documentation (i.e. cannot be manipulated). With the help of GPS (global positioning system) systems currently used in some larger agricultural companies, it will be possible to retrace the wine to the grape/vineyard. Also, optimized routes can be calculated using automatic documentation of harvesting, transportation and time used for setting up. Even data from the specific yields of vineyards or vineyard portions can be collected and used to choose and separate different quality levels (Schwarz and Keicher, 2009).

15.2.8 Documentation

Using a combination of the field record system and satellite navigation technology, several documentation systems have been realized (Fig.15.8 and 15.9.). To

Fig. 15.8 ERO grape harvester.

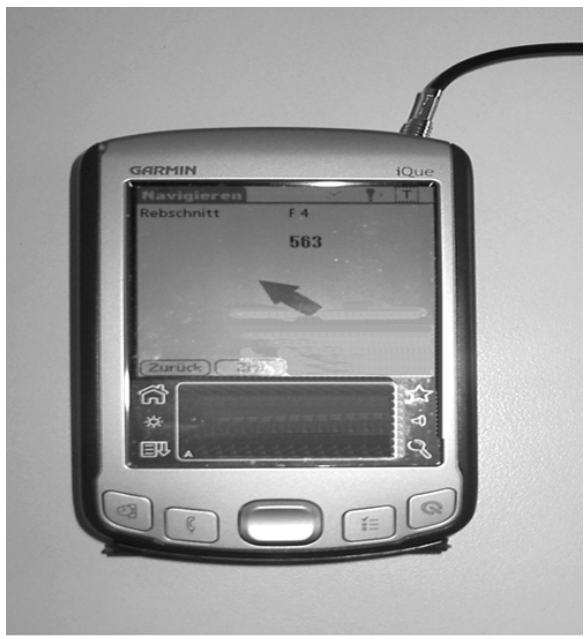


Fig. 15.9 Garmin satellite receiver.

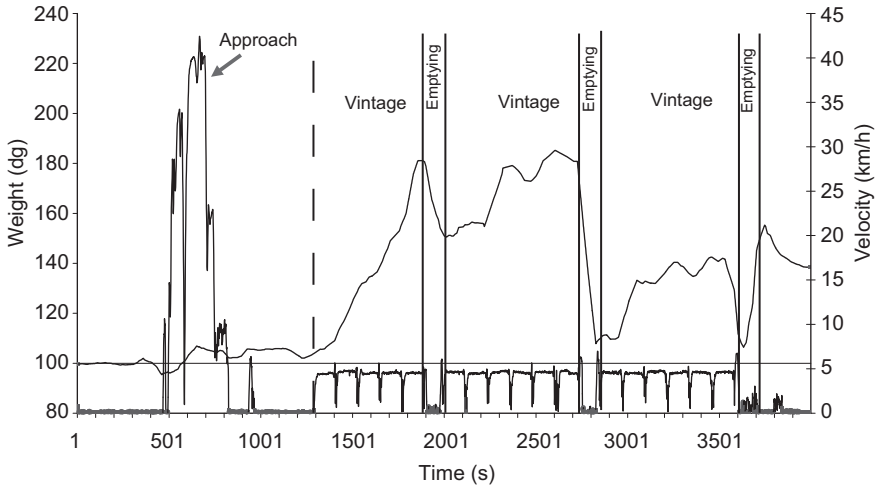


Fig. 15.10 Distribution of work-, transport-, and idle-time of a grape harvester. Averages: 65% work, 19% transport and 16% idle time (Schwarz and Keicher, 2008).

demonstrate this, an ERO Grapeliner SF 200 was equipped with a GPS receiver and the respective software while another ERO Grapeliner was equipped only with an airplane-style black box system. The first system interacts with the machine operator, i.e. it must be fed assignments, and after the workday it must be readout. The second system only sends position, time, speed and the status of the harvester to a server via GPRS (general packet radio service).

The first results of the data analysis show substantial differences regarding operating figures such as machine utilization, transportation and changeover time. Figure 15.10 shows that at Company No. 2, 65% of the total operating hours were used for harvesting, while Company No.1 only required 34%. This shows that logistics and planning are a large factor in the efficiency of the company. Furthermore, the data collected can be used to further raise the efficiency of the system and create more accurate billing statements. The data can also be used to create a more differentiated pricing, for example, transport and cleaning may be less expensive than harvesting or vice-versa (Schwarz and Keicher, 2009).

15.2.9 Yield monitoring

The black box system also has four analogue and four digital inputs that can be utilized in any combination. By this means, other important information can be collected, such as emptying of the tank or status of the destemmer or by adding special accessories, the yield can be measured as it is picked (in principle).

Yield monitoring can be realized in several ways (see Chapter 12). Theoretically, a system similar to how combine harvesters in the grain industry measure the yield, with a volume or mass flow rate measuring device or a permanent gravimetric scale

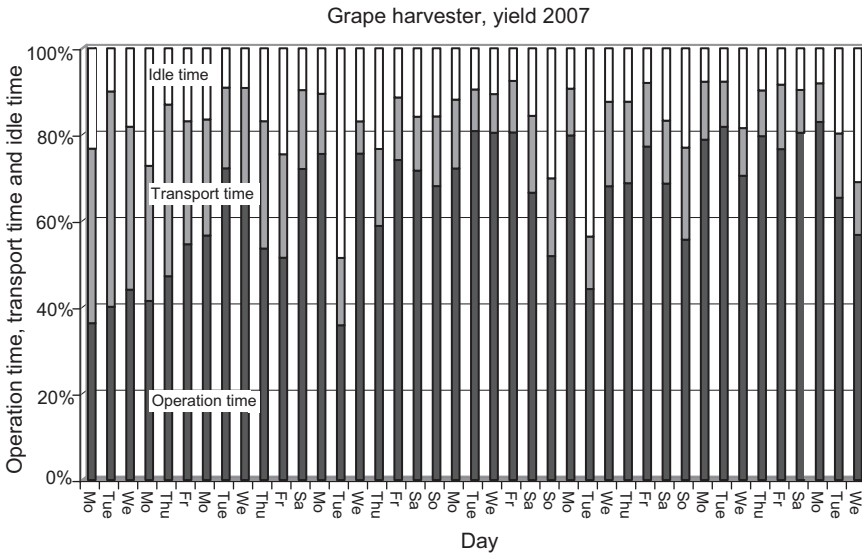


Fig. 15.11 Using weighing equipment data to give operation, transport and idle time (Schwarz and Keicher, 2009).

of the tank, can be conceived. Figure 15.11 shows the problems with the gravimetric scale; the zero value is not always the same, which could be caused by incorrect placement or shifting, and the values do not grow steadily, which could be caused by sloping of the vineyard or leaning and shifting of weight. However, after being tweaked and perfected this system offers a good way to monitor yield (Schwarz and Keicher, 2009).

15.3 Grape transportation systems

15.3.1 From the vineyard to the winery

It is clear from the section on harvesting technique that the transport technique plays a prominent role in grape and wine quality. Apart from the traditional variants such as vats, large- and small-capacity cases, trailers (lined with tarpaulins) and must tanks, the grape transporter especially has gained particular acceptance. It is usually composed of a single-axle or two-axle chassis on which a stainless steel trough is built that is open to the top and is tapered downwards (between 2000 and 7500 litres depending on the version). Due to the diversity of internal grape transport within the winery, different types of construction have emerged.

When using grape transporters with lifting equipment (Fig. 15.12), the containers can be lifted by means of hydraulic lifting elements to the filling height of the destemming machine or of the press. This allows for emptying without conveyor belt or must pump. The machine is driven entirely by tractor hydraulics. Turning

Fig. 15.12 Grape transporter with lifting equipment and vibration discharging.

the screw on and off and opening and closing the cover flap is done by remote control, a procedure which is advantageous in view of occupational safety and allows operators to coordinate the procedures during and after emptying.

For discharging the grapes from the grape transporters, there are materials-handling techniques using screws, vibrations or conveyor belts, which allow for emptying the grape transporters without mechanical damage to the harvested grapes. Since, depending on the harvesting technique and the harvested material, a certain amount of must is always released during transport, it is reasonable to have predraining equipment integrated in the bottom. This is made up of a tank at

Fig. 15.13 Discharging the grapes from the grape transporters: (a) only with screws; (b) with screws and pump (Zickler, 2009).

Table 15.2 Lees deposit after sedimentation and filling quantity for a pneumatic press (press cage capacity 1800 l) depending on the method of filling (Riesling, degree of rot 25%)

Method of operation	Filling quantity		Lees deposit	
	[kg]	[%]	[% v/v]	[%]
Filling through filler neck – from above –	2184	100	13.1	100
Filling through central opening – rotation every 2 min –	2885	132	17.8	136
Filling through central opening – continuous rotation –	3054	140	28.0	214

Source: Seckler *et al.* (2001).

the bottom of the trough that is separated by a perforated plate. Thus, the juice that has accumulated can be drained before emptying the transporter.

Grape transporters without lifting equipment require additional conveying systems for further transport in-house. This can be a natural or constructional slope, conveyor belts, conveyor screws or pumps, respectively (see Section 15.3.2). For screw discharge, there are grape transporters equipped with a flange-mounted eccentric screw pump (Fig. 15.13). After coupling a hose, these transporters allow pumping directly onto the destemming machine, into the must storage container or onto the press. Apart from all its advantages from a labour efficiency point of view, there is the disadvantage of mechanical stress so that the solids and phenol contents are increased by pumping, to an extent which depends on the grape material (see Section 15.1.3).

To counteract these disadvantages, the following should be kept in mind (Seckler *et al.*, 2001):

- The conveyor screw and the eccentric screw pump should be optimally matched, i.e. the screw and the pump have the same capacity.
- The larger the screw diameter, the more carefully the product is handled.
- Larger grape transporters should be equipped with a loosening shaft to prevent undercutting of the harvested grapes (tunnelling).
- Pump rotor diameter should be 80–100 mm.
- An additional crushing device should not be used because of the additional production of solids.
- The conveying speed should be limited to a maximum of 0.7 m/s for non-destemmed mash or whole grapes and to a maximum of 1.0 m/s for destemmed mash.
- Subsequent piping should match the pump diameter (Table 15.2).

15.3.2 Grape transport within the winery

The transport from the vineyard to the winery is followed by grape reception and processing. The grape reception and thus the internal grape transport are co-determined by the following parameters:

- upstream transportation system (see Section 15.3.1)
- reception capacity per unit of time
- total reception volume per season
- proportion of red and white wine
- proportion of manual and machine harvesting
- varietal diversity
- system parameters influencing the quality
- occurrence of variants of quality categories and development of wine from individual vineyard sites
- intensity of cleaning and service operations on the respective system
- cost of acquisition, operating costs and follow-up costs of the respective system
- subsequent grape processing measures for quality improvement.

Grape reception

There are a number of options for unloading grapes from large- and small-capacity cases: by means of a fork or a shovel, with a suction plant, a crane hoist or a tipping device and, not least, with a fork lifter equipped with a hydraulic slewing ring. Grape transporters with a screw and a pump can fill the destemming machine or the press directly via a hose coupled to it. Grape transporters without a pump require additional conveying systems for unloading, such as a natural or constructional slope, conveyor belts, conveyor screws or pumps, respectively, in connection with a reception hopper or a reception bin. Suction plants can also be used. The same applies to the cart lined with tarpaulins and similar tiltable vehicles for grape transport. Often large wineries and winegrowers' cooperatives also use tipping devices integrated into the reception station. Thus, vehicles without their own tipping device can also be unloaded quickly.

Grape transport

Apart from the transport and the grape delivery, the internal grape or mash transport is primarily influenced by the further processing steps of grape treatment and mash storage. Here, the focus should again be on avoiding mechanical stress. Thus, grape and mash transportation should be reduced to the bare minimum needed. Table 15.3 illustrates a number of alternatives for reception with regard to their effects on the development of solids.

The ideal mash transportation taking advantage of constructional differences in height most often cannot be put into practice for constructional reasons. Therefore, pumps are normally used that increase the solid content of the must. For this reason, Troost (1988) recommends conveying speeds as low as possible and a large hose diameter. Seckler *et al.* (2001) suggests a conveying speed of a maximum of 0.7 m/s for non-destemmed mash or whole grapes and a maximum of 1.0 m/s for destemmed mash (Table 15.2). Moreover, the conduit should have a smooth inner wall and should have as few bends, curves or narrow sections as possible to minimize friction. These authors mention the hose pump as a gentle must pump. In practice, however, eccentric screw pumps and piston pumps are normally used. Maul (1987) discovered in extensive tests that with every pumping

Table 15.3 Influence on lees ratio by different ways of grape reception

Procedure	Lees ratio [% w/w]
Method 1 Standard vat from vineyard – press room – hoist – crushing – hoist – to press	2.3
Method 2 Standard vat from vineyard – press room – (hoist) – crushing – hoist – pumped into press	2.9
Method 3 Direct transport from vineyard – press room – filling of press by rotary vane pump (turbo) with crushing device	4.4
Method 4 Direct transport from vineyard – press room – filling of press by worm type pump with crushing device	3.5

Source: Maul (1987).

or transport operation the sediment content is increased by 0.5–1% v/v depending on the system.

The must pump is selected taking into account various aspects. On the one hand it should be suitable for cleaning and for maintenance but, on the other hand, it should have appropriate dimensions. When the rotor diameter is increased, the required rotational speed decreases leading to a substantial reduction in mechanical stress.

When planning and setting up winery and pressing equipment, it is necessary to select pumping and piping systems with appropriate dimensions. Tapers in the systems, i.e. transitions from larger to smaller diameters, should be avoided since this leads to increased mechanical stress due to an increase in flow velocity. Shear forces that are created due to friction at the mash conduit wall, especially at bends, further increase the solid content (Troost, 1988).

In the past, the grape delivery and the subsequent processing were mostly considered with a focus on large processing capacities. Various aspects of quality-oriented grape processing such as increased use of large-capacity bins or boxes, grape transporters without pumps and grape sorting have lately led to a change in thinking so that further conveyor techniques for internal transport of whole grapes or mash are being used. Among others, these include:

- constructional slope
- roller conveyors for transporting cases and vats
- hoists for cases and vats
- forklifts for cases and vats
- movable, liftable hoppers and troughs
- belt conveying
- oscillating conveying
- screw conveying
- must pumps (e.g. eccentric screw pumps, hose pumps, piston pumps)
- combinations of the various techniques.

One option is to transport the grapes and mash in larger containers, e.g. in large-capacity bins or stainless steel troughs by means of hoisting devices, industrial

trucks or roller conveyors that can allow for further sub-processes such as destemming or sorting when combined with tipping, hoisting and conveying devices.

While the options mentioned usually have an intermittent mode of operation, a continuous mode of operation can be provided by means of continuous handling equipment. This includes belt, oscillating and screw conveyors and, ideally, gravity conveyors.

The main component of a belt conveyor is an endless rotating belt supported by carrier rollers, slide ways or an air film. It is driven by at least one driving drum via a frictional connection. Conveyor belts are suitable for horizontal, slightly rising or tilting conveyance (Table 15.4). The destemming machine or the press can be filled slantwise directly via a predraining trough integrated into a filling hopper and via a food-grade rubber band equipped with lugs.

In the case of screw conveyors, the conveying element is a full or interrupted helicoid being rotated around its axis and moving the grapes or mash forward in a trough or pipe. Due to gravity and the friction between the transported material and the wall of the trough, this type of conveyance prevents the transported material from being rotated. Grapes can be loaded and discharged at any point of the conveyor screw by means of doors in the bottom of the trough, e.g. directly above the presses. Usually full screws are used for grape transport. To prevent blockage in the trough, it should only be filled half full. The screw diameter is between 100 and 1250 mm at a rotational speed between 40 and 180 r.p.m. depending on the screw diameter and the sensitivity of the transported material (Martin *et al.* 2008). Due to the large frictional force and the high rotational speeds required, conveying to a height is not recommended. Table 15.5 sums up the advantages and disadvantages of screw conveyors.

In the case of oscillating conveyors – also called vibratory conveyors – steady-state oscillations are generated in a rigid conveyor channel by means of a drive system – often an unbalanced motor. During the forward movement of the channel, these oscillations transmit the inertia forces to the grapes or the mash which are thus moved forward during the backward movement of the channel. Due to this mode of operation, conveying uphill or downhill can only be performed up to a tilt of about 15°. Although the channels are simply constructed, in the case of higher power consumption, these systems are subject to heavier wear due to the heavy demands on the drive system. Thus, the use of these oscillating conveyors is usually limited to grape acceptance and grape sorting where the dispersal of the grape material is utilized.

In the case of gravity conveyors, the grapes or the mash slide on an inclined conveyor line during which the frictional resistance is overcome by means of gravity. This includes straight, curved or helically arranged slides which should have a tilt angle of 30–60° depending on the condition of the grape material (Martin *et al.*, 2008). To accelerate the transported material to ca 0.5–1.5 m/s, the front part of the slide should be designed steeper. Down pipes are also used for mash storage tanks and for press filling if the conveying depth is not too large. Due to their simple, low-maintenance and inexpensive construction, they are mainly

Table 15.4 Advantages and disadvantages of belt conveyors for transporting grapes and must (adapted from Martin *et al.*, 2008)

Advantages
• High velocity and output quantity at low power demand
• Careful grape/must transport
• Low acquisition cost and maintenance, low wear
• Easy installation of belt weighing devices to determine the output (continuous and total)
• Suitable for long distances even at heavy loads (multiple motor drums with steel-reinforced belt strap
Disadvantages
• Inclined conveyance is limited (max. 18°–20° slope)
• Only straight conveyance
• Some belt types have low tolerance of hot and very frictional substances
• Labour-intensive cleaning

Table 15.5 Advantages and disadvantages of screw conveyors for transporting grapes and must (adapted from Martin *et al.*, 2008)

Advantages
• Dust-tight transport
• Low occurrence of malfunctions
• Grape input and output along the whole processing line
Disadvantages
• High power demand caused by continuous friction and mixing processes
• Inclined conveyance is limited
• Suitable for short distances (up to 40 m) only
• Mechanical wear of screw and hopper
• Not suitable for sensitive materials

used for existing constructional slopes or slopes made by hoisting devices and as connecting links between powered continuous conveyors.

Depending on the task and the operating conditions, it may be reasonable to combine the transport systems described. If multiple systems are integrated in the procedure, their capacity needs to be matched to each other and to the other machines since congestions and idling usually lead to increased solid content and total phenol concentration. Intensely pre-drained mash is also relatively difficult to convey. In this case, grinding the mash results in abrupt increases in the solids content and total phenol concentration.

15.4 Grape treatment

15.4.1 Crushing and destemming

To achieve the basic aim of grape processing, i.e. to extract juice from the berries, a separation of the solid and liquid phases (pomace/juice) is required, which is usually obtained by means of mechanically generated pressure. To quicken the

release of juice and to increase the juice output, the grapes are crushed. In conjunction with a maceration time, the juice flow can thus be improved and both desired and unwanted extraction processes can be accelerated. Traditionally, this is achieved by means of crushing mills designed to tear the berries but not to grind them.

In red winemaking and the corresponding fermentation on skins it is customary to remove the stems. For this purpose, destemming machines are used with an optional grape mill downstream. In white winemaking, however, depending on the winegrowing region, the grape variety and other processing measures such as mash storage, maceration time, mash transportation, etc., the berries are also separated from the stems.

The main objective of destemming is to remove herbaceous, unripe, green stems to prevent diffusion of undesired phenolic substances and absorption of further unwanted water-soluble substances. Consequently, it is carried out for the following reasons:

- removal of herbaceous, green stems to reduce extraction of bitter phenolic substances in the form of catechins, epicatechins, gallic acid, and other sensorially negative substances during the fermentation on skins or the maceration time (e.g. 3-isobutyl-2-methoxypyrazine in the case of grape varieties such as Sauvignon blanc and Cabernet Sauvignon)
- cleaning (stems, leaves, wood) of grapes harvested mechanically
- allowing for manual or mechanical grape sorting
- preventing blockage when conveying mash by means of a pump or piping for transport, cooling or heating
- increasing the pressing capacity while potentially impairing the pressability (the stems act as a drainage system)
- improving the distribution of treatment agents such as sulphite, dry ice, or liquid carbon dioxide.

Considering the context described in the section on harvesting technique above, from a quality point of view, the use of a grape mill is preferable to a destemming machine for processing white grapes or red grapes used for rosé wine making. This holds especially true for grape varieties with very delicate stems (see Table 15.6). For destemming, the degrees of ripeness and rot and, particularly, the suitability of the grape variety play an important role. Thus, the various grape varieties not only require different separating forces to separate berries and stems, but their stems are also delicate to different extents. Despite the need for destemming, the fact remains that according to the aspects described in Section 15.1.3, attention should be paid to mechanical stress. Thus, it can be deduced that the harvested grapes should preferably enter the destemming machine without major damage by means of consistent and continuous feeding. This applies particularly to grapes harvested mechanically and to overripe or rotten grapes. Thus, filling should be carried out as carefully as possible via belt, oscillating or screw conveyors. If the berries are crushed, the destemming machine should be followed downstream by a crushing mill. The mash should then consistently be transported further without shear forces

Table 15.6 Classification of grape varieties regarding required energy and grade of quality for destemming process (data base from 2002 and 2003, location Geisenheim Rheingau/Germany)

		Required energy input[a]		
		Low	Average	High
Destemming quality[b]	Good	Sauvignon blanc Silvaner Saphira Müller-Thurgau Rondo	Pinot gris Pinot noir Pinot Meunier St Laurent	Chardonnay Lemberger Carbernet Franc
	Average	Gewürztraminer		
	Bad		Pinot blanc	Riesling Regent

[a]Required energy = low < 1.8 N
average 1.8–2.2 N
high > 2.2 N.
[b]Destemming quality = good < 0.5% w/w
average = 0.5–1.0 N % w/w
bad > 1.0 % w/w grapes in must.
Source: Freund and Seckler (2007).

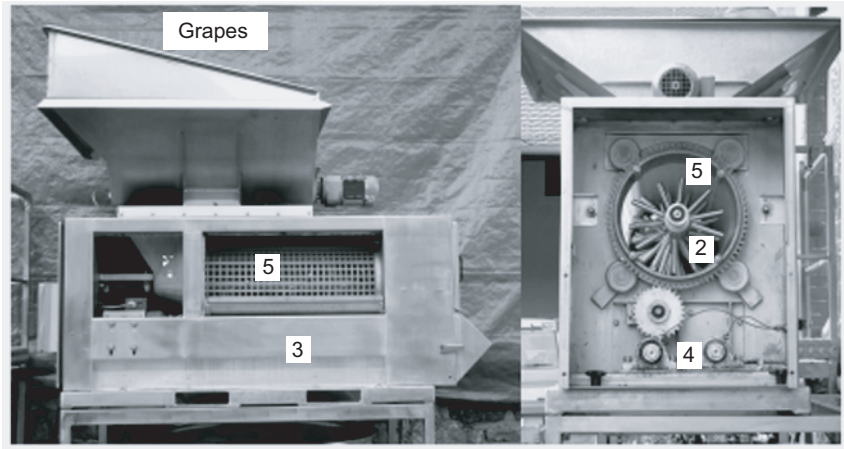
(see Section 15.3). This applies particularly to mash macerated by means of maceration time or even by means of fermentation on skins.

Three different construction types are currently available for destemming – the basket destemmer, the link-belt destemmer and the high-frequency destemmer. The aim of quality-oriented destemming is a degree of pollution of the mash of < 1% w/w (Vinsoneau and Vergnes, 2000; Weik, 2003; Reichert, 2004).

Basket destemmers

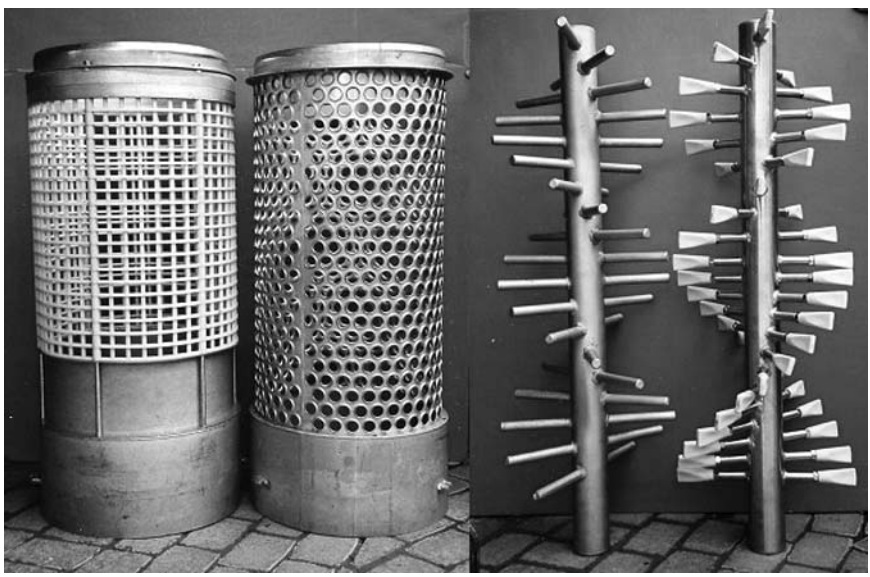
The basket destemmer is made up of a pin shaft movable on a travel path and rotating in a revolving cleaning drum. The harvested grapes are transported into the inside of the cleaning drum via a hopper and by means of a screw. The rotating pin shaft picks up the grapes and knocks the berries off the stems. Due to gravity and guided by baffles, the berries fall through the holes of the cleaning drum into a system-specific conveying system (screw, pump, conveyor belt, container). Depending on the construction, a grape crusher is attached directly below the basket drum (Fig. 15.14). Apart from the condition of the grapes, including the grape variety, the degrees of ripeness and rot and mechanical pre-damages induced by upstream processing steps and consistent filling by a continuous transport system matching the capacity, the quality of the destemming process is determined by machine parameters influencing the application of energy to the mash. Among others, these include:

- rotational speed ratio of shaft/basket
- rotational speed of shaft and basket
- velocity of circulation
- construction of shaft and basket (Fig. 15.15a,b)
- direction of rotation of shaft and basket.



1 Auger/screw conveyor
2 Arms/paddles
3 Crusher rollers
4 Drive for crusher rollers
5 Perforate cylinder

Fig. 15.14 Construction of basket destemmer.



(a) (b)

Fig. 15.15 Different construction of basket (a) and shaft (b) (Seckler *et al.*, 2006).

Due to the diversity of influencing factors, an ideal setting of the machines can only be achieved taking the grape material into account. The basic principle is that with increasing rotational speed of the pin the amount of berries in the steep output is reduced. Due to the higher application of energy, the berries are separated more effectively from the stems. However, the stems are also more badly damaged and broken into small pieces that get through the holes of the drum. The rotational speed ratio between shaft and basket should be between 1:8 and 1:10 and the rotational speed of the shaft should be 350–500 r.p.m. depending on the manufacturer.

To reduce the shear forces inherent in the design of a basket destemmer, there are versions available whose pin shaft can be oscillated by means of floating mounting on a rubber buffer and an additional unbalanced motor. The vibration drive induces a longitudinal movement of the pin shaft of 2–4 mm at a frequency of 2300 oscillations/min and a reduction of the circumferential velocity of the shaft by 50–60%. Thus, a gentle destemming shall be allowed despite low rotational speed (Armbruster, 2007).

Link-belt destemmers

The grapes are fed to the link-belt destemmer via the filling hopper, and are then pre-drained and transported on a link belt to the four destemming rollers. The frequency-controlled drive of the link belt and of the two pairs of pin rollers allows for setting different speeds so that the harvested grapes are picked up and destemmed by the front or the rear pair of pin rollers depending on the degree of ripeness. The berries separated from the stems can be collected for each pair of pin rollers in separate collection drawers and are thus sorted. When the destemming rollers are raised, the link belt can additionally be used as a whole grape conveyor belt for filling grape presses or fermentation tanks. (Fig. 15.16a,b). This type of destemmer is used by the Braud/New Holland company for their harvesting machines (see above).

(a) (b)

Fig. 15.16 Link-belt destemmers 'EUROSELECT': (a) at work; (b) separate berry collecting from each pair of pin rollers (Reichert, 2004).

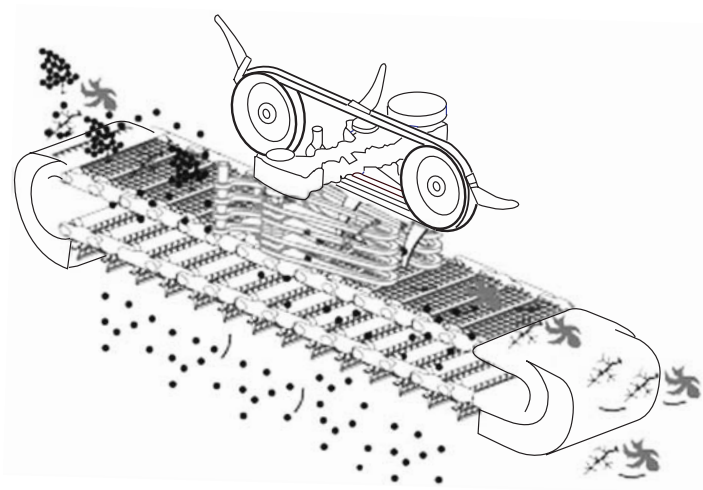


Fig. 15.17 High-frequency destemmers 'Selectiv' Process Winery' (Pellenc, 2008).

High-frequency destemmers

The operating principle of the linear high-frequency destemmer of the Pellenc Company is the mechanical dynamic swing–shake method (Fig. 15.17) and is borrowed from the mode of operation of the harvesting machines (see Section 15.2). The combination with a sorting table ensures a very gentle harvest of grapes with a low degree of damage to the berries and a very low degree of foreign materials in the mash.

The grapes are transported via a conveyor belt to the link belt of the destemmer where berries that have already been destemmed and small foreign materials (petioles, pieces of stems) fall through the holes directly onto the sorting table. Whole grapes or pieces of stems with berries that are too large to fall through the holes are picked up by a gripper operating at the belt and are fed through the swing shakers. There, the berries are knocked off the stems, fall through the link belt and thus also reach the sorting table. The stems are ejected via the belt.

By means of the rotating roller sorter, the berries are now separated from the remaining unwanted materials such as leaves, stems and pieces of wood. Since the gap between the rotating rollers increases towards the discharge, oblong foreign bodies (pieces of wood, petioles, etc.) can align parallel to the moving direction of the rollers and are thus transported across the increasing gaps while the round berries fall into the mash tank below the sorting table. This type of destemmer is available from Pellenc as an additional device for their harvesting machines (see above) and as a fixed installation.

15.4.2 Grape sorting

In recent years, grape sorting has gained more and more in importance. This development can be observed particularly for the production of high-quality wines and the further processing of mechanically harvested grapes. While most steps of grape processing aim to maintain the quality of the grapes, grape sorting provides an opportunity to actively increase the quality of the harvested grapes by sorting out green parts of plants, as well as unripe, damaged or partly rotten berries. In the past, this was mainly done manually, either by selective manual harvesting or by means of manual sorting devices such as vibrating or belt sorting tables. For some time, automatic sorting lines have been available that select the harvested grapes using different methods and according to various criteria.

Manual sorting

In principle, manual sorting can be carried out before or after destemming the grapes. The removal of leaves and plant remains as well as rotten and unripe grapes before destemming was practised, primarily in the 1990s, in part directly in the vineyard prior to mechanical grape harvesting to prevent mixing of the juice of grapes infested with rot with that of sound grapes during transport (Jacquet and Capdeville, 1990; Vinsoneau and Vergnes, 2000).

In the production of top-quality wines, sorting after destemming with the main focus on removing foreign materials originating from the grapes or from other sources has gained acceptance. These sorting tables are mostly rotating belt or oscillating conveyors (see Section 15.3), some of which are equipped with a feeding hopper with additional holes to remove the juice accumulated due to the preceding grape processing. Depending on the design of the openings (holes) of the stainless steel vibrating tables, besides permitting juice drainage, small pieces of plants (i.e. leaves, rachises, etc.) can be removed.

The table width is usually 80 cm while the following rule of thumb is used for the length:

$$\text{length} = 0.80\ \text{m} \times (\text{number of workers}/2 + 0.50\ \text{m})$$

In case of six to ten workers at the sorting table, a belt length of 4 m and a belt speed of 8–10 m/min, 8 tons of harvested grapes processed per hour are expected (Weik, 2003). In addition, the recommended minimum distance between the workers at the sorting table is 80 cm. At a belt speed of 5–8 m/min, one anticipates a processing volume of about 10 t/h. Figure 15.18 illustrates various options for arranging people at the sorting table: the best sorting results are achieved when workers are positioned in a staggered fashion opposite each other. Sorting very healthy and ripe grapes of the varieties Merlot und Cabernet Sauvignon, Vinsoneau and Vergnes (2000) achieved a reduction of unwanted plant materials by up to 30% compared to destemming only. The disadvantages of manual sorting are the high personnel requirements as well as the resulting costs and the relatively low daily output. Moreover, it is very difficult to sort out small berries and pieces of stems.

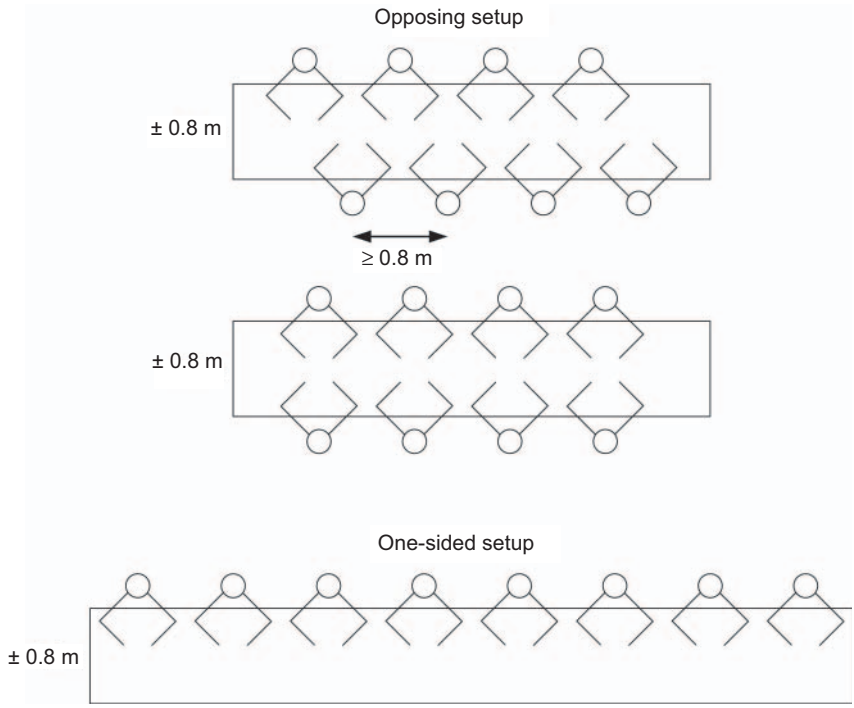


Fig. 15.18 Various options for arranging workers at the sorting table (Vivas, 2007).

Automatic sorting

Mistral sorting line

The mode of operation of these sorting lines is based on the principle of blowing undesirable materials out of the harvested grapes. For this purpose, the line is positioned downstream of a destemming machine where the berries first fall onto a traditional vibrating sorting table and are then distributed evenly due to the vibration. During this transport, juice that has accumulated as well as part of the small pieces of stems, petioles and unfertilized berries are separated by means of a perforated plate embedded in the table. Larger pieces of stems and entire rachises are separated at the end of the oscillating conveyor via a grid (Fig. 15.19, no. 1). The bar distance of the sieve grid varies between 18 and 25 mm depending on the grape variety and the size of the berries. Subsequent to the first separation step, the actual sorting is carried out by means of the air flow. The remaining berries and berry parts fall from the vibrating table past an adjustable air nozzle. By means of a thin air stream, damaged berries and parts of stems, the surface of which offers higher air resistance than the surface of whole berries, are blown off backwards and are collected separately. The whole berries are deflected very little by the air stream and fall almost vertically downwards where they are transported further. Thus, as a result, two different qualities of grape material can be separated – the

Fig. 15.19 Delta Mistral sorting line (Bucher Vaslin, 2007).

damaged berries that have been blown out with the pieces of stems and the whole berries with relatively little foreign materials.

Below the sorting area which consists mainly of the air nozzle (Fig. 15.19, no. 2) and adjustable separating plates, the berries (Fig. 15.19, no. 3) and the waste (Fig. 15.19, no. 4) are collected and can be transported further by means of system-specific transport devices such as conveyor belts, pumps or large-capacity cases. Setting the sorting line and the sorting effects is done by choice of the grids, the infinitely variable control of the air capacity of the blower and the various settings of the separating plates (Mindnich, 2006; Bucher Vaslin, 2007).

Tribaie® sorting table

Triviti's Tribaie sorting system is supposed to remove plant-based impurities from destemmed, uncrushed berries and then to make a differentiated selection based on quality. Thus, after destemming, the berries are placed onto perforated vibrating tables where they are washed with recycled must (Fig. 15.20, level 1). Rotten berries, bits of berry skin, seeds and other unwanted materials are supposed to be rinsed off. The must, which is now mixed with these undesirable materials, is drained off via a draining grid, cleaned and then led back into the cleaning cycle. At the end of the vibrating table, the grape material that has been washed is separated from the remaining unwanted materials which, due to their size, cannot fall through the holes of the vibrating table located in this section (Fig. 15.20, level 2).

From there, the berries fall onto a perforated stainless steel roller rotating

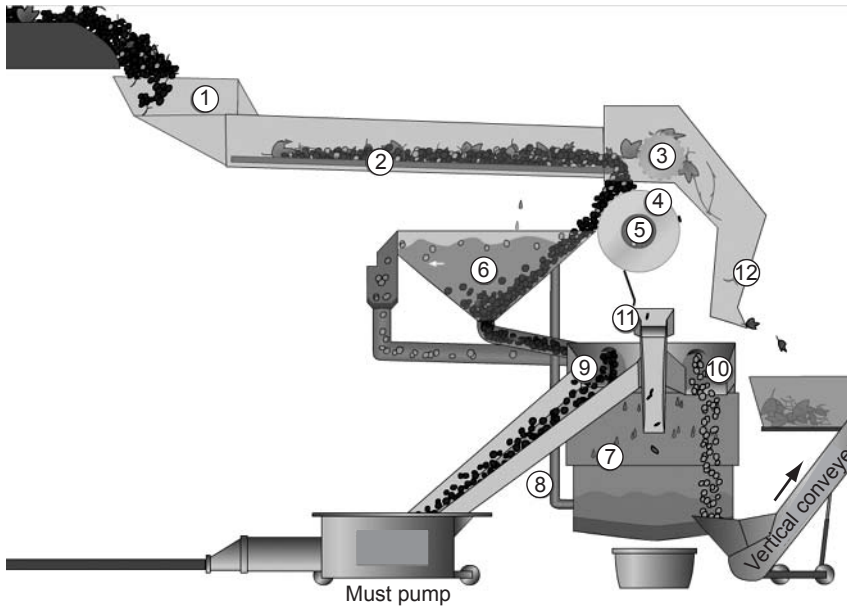


Fig. 15.20 Tribaie sorting table (Amos Distribution, 2005).

clockwise where they are sorted according to their surface properties (Fig. 15.20, level 3). Damaged, burst berries get caught at the perforated surface of the roller, are carried along, are stripped off and are then further processed separately. Whole berries slide over the surface onto a conveyor belt and, in the last step, are sorted according to their soluble solids concentration (Fig. 15.20, level 4). They get into a bath of mash or sugared water that has defined soluble solids. The healthy grapes, which have a higher soluble solids and thus a higher sugar content, sink while the lighter berries float and are washed over the edge of the basin together with the overflowing must. The submerged berries are led away at the bottom of the hopper-shaped container. Both berry fractions are conveyed by means of a vibrating conveyor belt that at the same time separates the must. The berries that have thus been sorted are further transported via a system-specific transportation system (conveyor belt, pump, etc.). The must draining off is led back into the cycle (Davaux, 2007).

Optical sorting tables

Optical sorting has already been used for decades in the fruit-processing industry, e.g. for nuts, grains, coffee beans, blueberries and peas (Brennan *et al.*, 1976). When using optical systems, the berries are filled after destemming onto a slit vibrating conveyor belt. By this means, the berries are spread out and an initial pre-draining of the berries is carried out. After spreading out, the berries reach a perforated sorting belt that accelerates the berries to about 2 m/s. At this speed, the harvest is passed alongside the optical system. Depending on the sorting criterion, this optical system is made up of camera, laser and/or LED technology allowing for

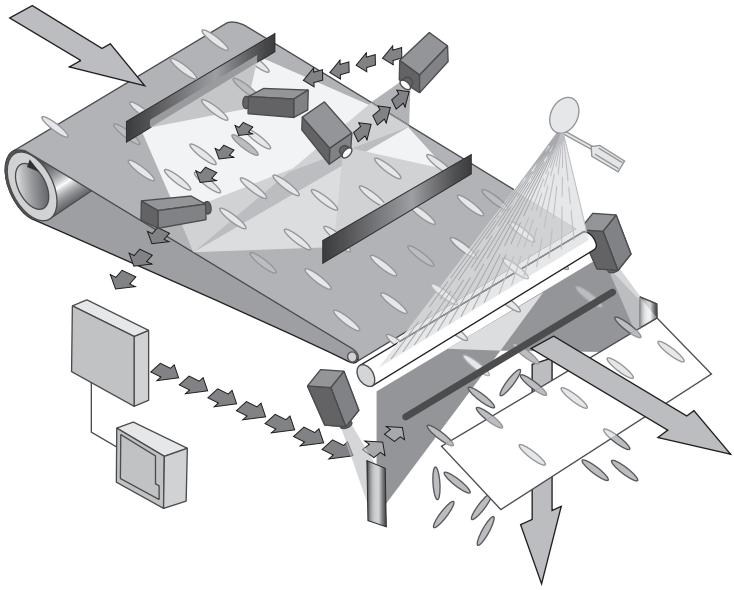


Fig. 15.21 Working principle of a camera and laser sorter on colour, structure and shape detection (Best, 2009).

systematic selection according to colour, structure and/or form. The number of optical instruments depends primarily on the resolution required (Best 2009).

The pictures thus taken by the camera are compared with the defined selection criteria previously stored in a computer. Due to inertia, at the end of the accelerator belt the individual elements fly on an inclined trajectory in the direction of the collecting system. The elements that the optical evaluation system recognized as foreign materials or generally as negative are deflected from the trajectory at an angle of 90° by means of air nozzles mounted above the trajectory and are blown into a separate collecting system. Berries that are recognized as positive are not deflected and at the end of their trajectory get into a collection container or onto another conveyor belt. The respective air nozzles are selected according to the positioning on the belt and the subsequent trajectory (Fig. 15.21).

To calibrate the machine, pictures of berries are taken in advance with the cameras which are then defined as good or bad by the operator on a touchscreen. Thus, the computer calculates rasters that can be further differentiated as needed. Due to this quick configuration and the option to save the settings adjusted to the respective requirements, adjusting to the grape material is easy. For the grape varieties Riesling, Müller-Thurgau and Kerner, Porten *et al.* (2008) achieved selection rates of 90–95%. The mode of operation of the sorting machines 'Selectiv Process Vision' by Pellenc/France, 'Delta R2 Vistalys' by Bucher Vaslin/France and 'Genius' by Best/Belgium is based on this optical system.

15.5 Presses

Depending on the processing measures that have been carried out, the grapes may reach the press whole, in a crushed or destemmed state or, with or without maceration time, via the various conveyor systems described in Section 15.3.

During the actual pressing procedure the juice is separated from the solid components of the mash by means of separation of the solid and liquid phases. At the same time, this separation process involves biochemical processes since the berry cells need to be disrupted enzymatically according to the degree of ripeness. This does not mean squeezing the juice out of the cells but implies a separation process due to pressure differences and is thus a filtration process (Hemming, 1989).

When evaluating a pressing technique with regard to gentleness of operation, parameters such as the preparatory steps, press filling (see p.554), pre-draining, break-up processes, the thickness of the press-cake, shear forces, total pressing time and the yield play an important role. High operating pressures do not improve the pressing result but rather accelerate the narrowing of the juice drainage channels. Alternating pressure and break-up processes with the pressurization level rising continuously until the end of the pressing process proves favourable; however, frequent break-up processes increase the mechanical stress on the mash. Thin press-cakes lead to shorter pressing times; however, they create more solids since there is no filtration effect. (Troost, 1988)

Even these few details show that an optimization of the pressing technique must always keep a balance between the conflicting priorities of an efficient mode of operation and product protection. Thus, considerations in terms of corporate philosophy ultimately tip the balance when choosing a pressing system.

15.5.1 Types of presses

Throughout the history of winemaking, a number of different types of presses have emerged. Basically these can be divided according to:

- the mode of operation (continuous – intermittent)
- the generation of pressure (hydraulic, mechanical, pneumatic)
- the design of the basket (horizontal, vertical)
- the juice drainage channel (closed, semi-open, open).

Different press systems are compared in Table 15.7.

When choosing a pressing system, apart from the aspects of processing capacity from a labour efficiency point of view described in Section 15.3.2 and of varietal diversity, the following criteria need to be considered:

- type of processing (whole-cluster pressing 65%, mash via filling port 140%, mash vial axial filling 200% of the drum volume)
- type of filling or arrangement of the cover doors, respectively
- variability of the filling quantity
- option of maceration time

Table 15.7 Comparison of different continuous press systems

Parameter	Decanter	Belt press	Screw press
Must condition	Destemmed	Destemmed, not destemmed	Destemmed, not destemmed
Must feed	Homogeneous (η drops when heterogenous)	Homogeneous, about 5–10 cm layer of must	Heterogeneous possible
Dejuicing suggested?	No	Yes	Yes
Operation method	Sedimentation: centrifugal force filtration by 'shear pressing'	Filtration by high pressure: 'shear pressing'	Filtration by high pressure: 'periodic pressure'
Pomace unload	Internal screw conveyor	Scrape off while belt cleaning	Periodic opening by pressure
Must fractions	1	2–3	2–3
Cleaning	CIP	Belt cleaning with water during operation	Easy cleaning because of the simple construction
Works as	Enclosed system	Open system	Open system
Must oxidation	Low when operating in special atmosphere	Unregulated	Unregulated

Source: Maüser (1995).

- operability
- level of automation
- necessity of maintenance, repair service
- cleaning
- influence of oxygen
- pomace emptying
- pressing programmes.

Due to their product protection capacity and efficient mode of operation, pneumatic horizontal presses with an intermittent mode of operation have established themselves in practice for quality-oriented modes of operation.

Because of their horizontal design, they have the following advantages, among others, over vertical pressing systems (Troost, 1988):

- a quicker flow of juice
- simpler loosening of the mash
- more economic filling and emptying from a labour efficiency point of view
- larger capacity.

Compared with the horizontal screw presses (internal or external screw), membrane presses induce less mechanical stress on the mash and are more flexible in

dealing with partial fillings, among other things. The membrane presses available today from different manufacturers are to be divided into three categories:

- closed membrane presses – tank presses
- semi-open membrane presses
- open membrane presses.

All press types mentioned have an inextensible press membrane. The press membranes are engaged either from the direction of one of the two halves of the tank shell, divided twice from the side, or in several parts centrally from the inside in the direction of the completely slotted shell. Regarding the design of the juice drainage surface, drainage bars and vertical juice drainage channels for tank presses, on the one hand, and partly up to completely slotted press drums for semi-open and open systems, on the other hand, are differentiated.

Air is pressed between the press membrane and the unperforated half of the press shell by means of compressed air. This compressed air is generated by internal compressors and, in the case of larger pressing systems > 5000 L in capacity, by external compressors. The membrane is displaced and presses the grape material in the press drum against the opposite juice drainage surface. The must flows into the must tray underneath via the slotted shell surface or via the juice drainage elements. Thus, the grape mash is drained.

Break-up processes and increases in the pressure level are initiated fully automatically by means of a fully-automatic control and pre-programmed pressing programmes (Section 15.5.2). Comparing the three designs, there are not only differences in construction but also in the potential fields of application.

Closed membrane presses (tank presses)

In closed tank presses, maceration can be carried out to improve the enzymatic disruption of the harvested material by closing the juice drainage devices using screws or valves. In the case of some press types, by means of additionally mounting pillow plates around the press basket, the mash can be cooled or heated during the maceration time or during the pressing process. In such a case, the inlet of the refrigerant, for example, goes through the axial filling.

Another advantage of tank presses is the large surface of the draining channels, which is achieved by their advantageous configuration or construction. Moreover, due to the closed tank presses, the maceration time and the pressing process proceed largely free from atmospheric oxygen. In order to be responsive to the various objectives of winemaking, such as the reaction on the susceptibility to oxidation of aroma components of different grape varieties, devices for working with inert gas (nitrogen, carbon dioxide) are offered (Fig. 15.22).

A significant disadvantage of tank presses over semi-open or open systems is the cleaning procedure. For thorough cleaning, the juice drainage bars have to be removed which can be very time-consuming depending on the press type. Consequently fully-automatic cleaning systems are usually available for presses > 5000 L capacity. These are embedded into an existing cleaning in place (CIP) cleaning cycle. Moreover, blockage of the juice drainage channels can become

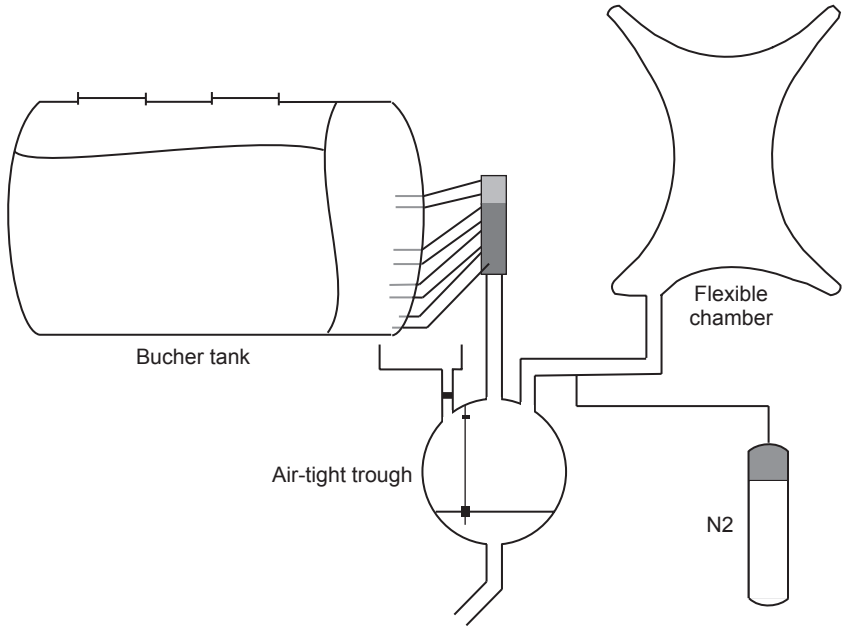


Fig. 15.22 Pressing under inert gas with gas recycling – Bucher Inertys® (Bucher Vaslin, 2009).

problematic in the case of hard-to-press grape varieties. In combination with automatic cleaning, there are blow back devices available that blow the slots clear from the drain side by means of air or inert gases.

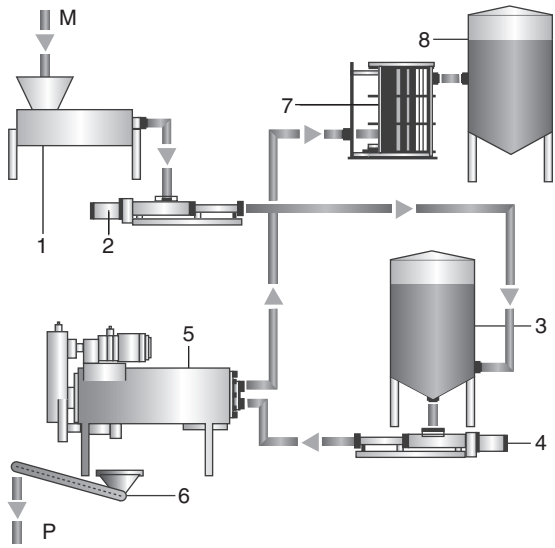
Semi-open membrane presses

Unlike in tank presses, in these systems the press shell is not closed but half of it is provided with vertical or horizontal slots. The other half is closed and acts as support for the press membrane that is pressed against the mash to be drained by means of compressed air. Due to the half-slotted press drum, a large amount of juice drains off already during filling (pre-draining). Therefore, a longer maceration time in the press is not recommended.

In terms of pressing time and surface of the juice drainage surface, there are only slight differences between the closed and the semi-open systems. Nor are there any major differences with regard to the execution of the pressing programmes and the number of break-up processes. The filling quantities measured against the capacity of the press basket do not differ. The most noteworthy differences are in the construction and the design as well as in the fact that the semi-open systems can be cleaned more easily, a process which as yet cannot be automated.

Other pressing systems

Due to their product-protecting characteristics, vertical presses attract more attention again in the field of production of base wines for sparkling wine production,



1	Stemmer	6	Extraction of solids
2	Pump	7	Plate heat exchanger
3	Mash tank	8	Must tank
4	Pump	M	Grape feed
5	Decanter	P	Solids

Fig. 15.23 Continuous juicing of fermented-mash grapes with a decanter – Westfalia VINEX® (Westfalia Separator Food Tec, 2006).

of red wines but also of premium and special wines as well as for very small wineries. Despite the high production of solids and the release of phenols, continuous systems such as band presses, screw presses and decanters (Fig. 15.23) are important, especially for large wineries, because of their impact force. This is particularly so due to the process of concentration which can be observed taking place in the wine industry.

15.5.2 Pressing programmes

To be able to respond to a varying array of grape materials, the present presses are equipped with different control programmes that allow winemakers to press their grapes according to their own objectives. Usually, these take into account the factor of time, the degree of filling, the quantity of yield and the quality that can be determined by very different constituents such as pigments, phenols, potassium and acid but also by the solids content.

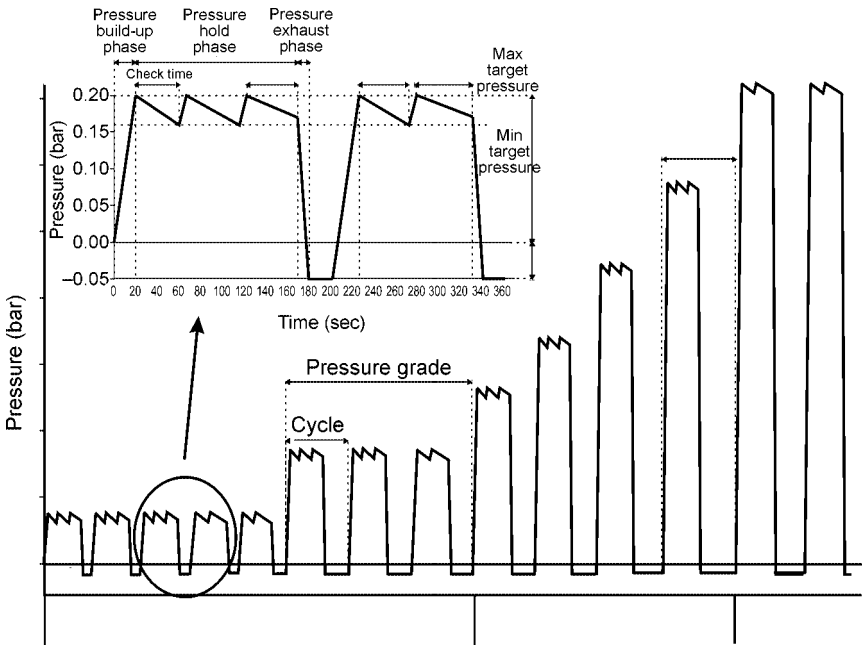


Fig. 15.24 Pressure/time diagram of a standard programme (Freund *et al.*, 2008).

These pressing programmes can be divided into two types with fundamental operation sequences – so-called model or standard programmes (Fig. 15.24) and sequential or Cremant programmes (Fig. 15.25) (Freund *et al.*, 2008).

Standard programmes

In the case of the standard programmes, the pressing programmes are described by the pressure level, its repetitions in the form of the number of cycles or the phase time, the pressure holding time and the loosening by means of the number of rotations or a unit of time and, depending on the press manufacturer, also the loosening speed and the lower set pressure. Thus, by means of these parameters, existing programmes can be changed or new programmes can be designed. In the standard programmes, the pressure level is thus considered to be the programming unit (Freund *et al.*, 2008).

Sequential programmes

Although the repetition of cycles, the number of loosening steps or amount of loosening time, and pressure holding time of the sequential programmes can be compared with the descriptions of the standard programmes, due to the staircase-shaped pressure build-up, the operation is carried out in a cycle with a maximum pressure specific to the respective cycle. After the pressure holding time, the pressure is then built up to the level of the next set pressure by means of a fixed pressure difference, until the maximum pressure of the respective cycle is obtained.

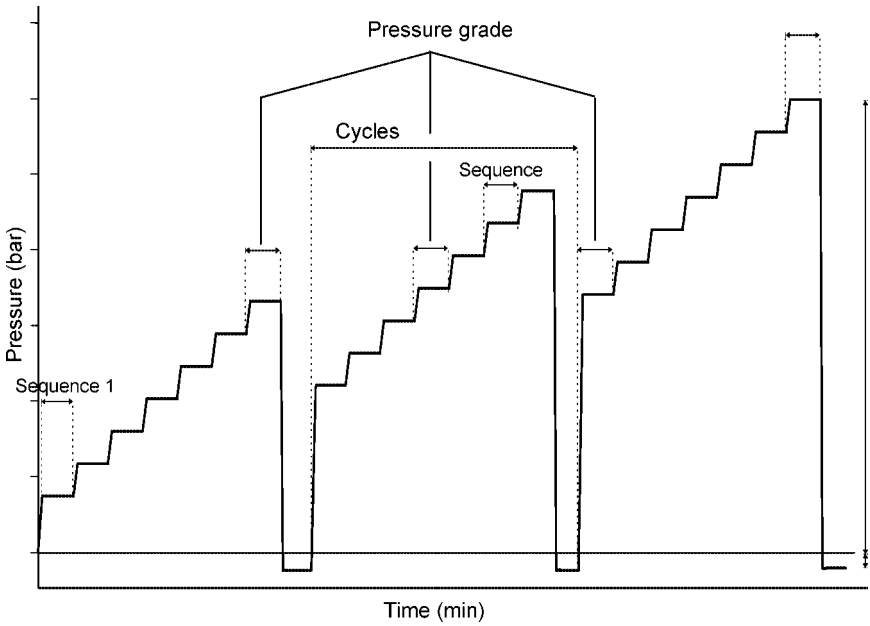


Fig. 15.25 Pressure/time diagram of a time-controlled sequential pressure programme (Freund *et al.*, 2008).

Contrary to the standard programmes, in the sequential programmes the succession of the individual steps is stored in so-called sequences. This means that every pressure level has to be stored individually in the programme with the set pressure, the pressure holding time and the loosening step (Freund *et al.*, 2008).

Self-optimizing programmes

In the case of the self-optimizing programmes, the sequential mode of operation is complemented by a weight or juice volume detection that provides data about the loss of volume per unit of time to the programme. By linking with the data on pressure build-up, holding and relief and especially considering time, the mathematical algorithms of these programmes are capable of gathering information on the pressing process and independently optimizing the pressure pattern according to the harvested grapes without requiring the programme or the operator to exactly devise pressure levels, cycles or loosening phases. By means of the integrated weight detection or a moisture measurement of the mash, the end of pressing can also be determined via programme control. Thus, these self-optimizing programmes approximate the former manual control during which the winemaker stood beside the press and stopped the increase of pressure if the juice flowed too fast, increased the pressure slightly if the juice flowed more slowly and initiated a loosening process if this was unsuccessful (Freund *et al.*, 2008).

Pressing behaviour of the grape material

Another option to classify pressing programmes is based on the characteristics of

the grape material since the control units of the presses include different pressure and time patterns that allow for responding to the character of the harvested material. To simplify matters, these programmes can be divided into programmes for easy-to-press, regular or hard-to-press grape material. Additionally, the further objective of the winegrower, i.e. that pressing should be quick and gentle, is also taken into account. Further, some manufacturers take the degree of filling of the press into account by making use of the fact that a thinner press-cake can be drained more easily because the juice drainage paths are shorter. The resulting advantage is that a partially filled press can be put under higher pressure more quickly and requires less loosening.

The question which then arises is how the pressability of the grape material is influenced. As already mentioned earlier, this includes all conditions that improve the biochemical and mechanical disruption of the berry cells and are thus, in addition to the grape, determined by the process technologies applied, the machines used and further basic conditions.

Thus, fermented red wine mash, in which the cell structure of the berries has already been destroyed to a large extent, comes under the category of easy-to-press material. A mash of ripe and overripe white grape material that has been enzymatically disrupted by means of a longer maceration time is also counted among this category. In contrast, the berry cells of heated red wine mash are decomposed insofar as pigments are released; however, the enzyme systems of the grapes will have been inactivated by the influence of heat resulting in macromolecular, colloidal, pectinaceous fragments that reduce the pressability and impede the draining of heated red wine mash. Ripe and overripe harvested material that has additionally been crushed is counted among regular material in terms of pressability. This also includes harvested material that has been pumped, but this depends on the further processing of the grapes and on the resulting mechanical stress. Rotten harvested material with a high proportion of pectin fragments and other colloidal macromolecules from the cell wall or potential rot such as botrytis can be classified as regular in terms of pressability, unless, depending on the machine technology employed, there is high stress and colloidal gelatine is created that can then substantially impair pressability. Apart from this pressing material and from unripe harvested material, grape varieties such as Silvaner, Pinot Meunier (rosé wine making) or Frühroter Veltliner that have a naturally high pectin content are also counted among the category of hard-to-press material. In these cases, a preceding maceration time improves the pressability – healthy grape material provided.

The different approaches of the various press manufacturers only allow for a rough division of the programmes in terms of easy-to press, regular, and hard-to-press. Fundamentally, however, the following can be said in general:

- the more difficult the grape material, the more slowly the pressure build-up
- the more difficult the grape material, the higher the maximum pressure
- the more difficult the grape material, the higher the cumulative pressure
- the more difficult the grape material, the higher the number of cycles

- the more difficult the grape material, the higher the number of break-up processes per cycle or per pressing
- the more difficult the grape material, the longer the pressure holding times
- the more difficult the grape material, the longer the pressing times depending on the pressure holding times and the number of cycles.

The protective pressing programmes are designed in such a way that berry skins and thus the constituents located in the skins are only slightly extracted and accumulated in the must. Thus, in connection with gentle grape processing, white must or wine can, for example, be obtained from black grapes. This basic idea is central to the Cremant programmes. Slow pressure build-up, few break-up processes and lower maximum pressures are used in an attempt to minimize the mechanical stress on the mash. In contrast, the more rapid programmes mainly work with faster and mostly higher pressure build-up which, apart from a larger loosening effort, usually results in more intense cell disruption and more solids (Freund *et al.*, 2008).

Supplementary programme elements

Depending on the manufacturers, the actual pressing programmes are supplemented by additional sub-programmes for press filling, pre-draining, repressing and press emptying. Thus, special filling programmes take into account whether the press is loaded via the cover or via an axially arranged valve. In the first case, for instance, an alternating swivel function, the so-called rocking, can remove the dumping cone of whole grapes without complete rotation. In the case of axial filling, draining during filling is additionally offered, mainly for reasons of labour efficiency. At the same time, these programmes control the closing process of the cover or the valve, the tank rotations (continuous, impulse, swivel) and, in case of axial filling, control the pressure in the press generated during filling. Moreover, the various control units of the presses offer the choice between depressurized predraining with or without rotation and direct starting of a main pressing programme.

The repressing features allow for responding to excessively moist pressing material. They usually repeat the phase of maximum pressure or part of it. Moreover, there are options available in which further predefined programmes can additionally follow a main programme. The emptying programmes control the direction of rotation, rotation intervals or cycles and often include the option to control the pomace conveyor system (Freund *et al.*, 2008).

15.6 Conclusions

When incorporating all steps of grape processing, what stands out is the particular importance of the quality of the harvested grapes. With increasing biochemical disruption of the berry tissue, caused either by ripeness or by rot, mechanical stress during grape processing has an adverse effect on the must composition and thus on the subsequent wine quality. For quality reasons, a maceration time with the aim of berry disruption particularly needs to be considered carefully against the back-

ground of overripe, rotten grapes, especially since the production of grape mash often leads to additional mechanical stress on the grape material. Generalizing, it can thus be said that with increasing ripeness and rot, grape processing should be adjusted by reducing mechanical stress up to the utmost – to whole-cluster pressing.

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Index