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Biochemical Changes throughout Grape Berry Development and Fruit and Wine Quality

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ABSTRACT

Wine is made up of more than one thousand compounds, the majority of which, such as vitamins and minerals, come from the grapes, while others, like ethanol and glycerol, are products of the winemaking process. Although sugars are either partially or completely transformed, sugar import and accumulation into the ripening berry is a major parameter of wine quality. Sugar status is directly related to the final alcoholic content of wine, and regulates several genes responsible for the development of its aromatic and organoleptic properties. Physiological ripeness is reached when the grapes achieve sufficiently high sugar levels without loosing too much acidity; however, aromatic and phenolic compound content must also be taken into account. Softening and water content are other essential characteristics of a ripe berry. From a winemaker point of view, optimal grape maturity is essential for wine quality, but is difficult to assess because it is under multifactorial control, involving grapevine cultivar variety and environmental parameters such as soil, temperature, exposure to sun, and hormonal regulation. Continued study of the key control points in grape ripening is crucial if we ultimately hope to improve grape and wine quality.

Keywords: berry ripening, hormonal regulation, malic acid, nitrogen, phenolics, potassium, sugar, tartaric acid, wine, *Vitis vinifera*

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INTRODUCTION

Grapes (*Vitis* spp.) are economically the most important fruit species in the world. In 2005, vineyard area and grape production was of 7,488,196 ha and 66,901,419 t, respectively, ranking second in fruit production (FAO 2007). Approximately 71% of this production is used for wine, 27% as fresh fruit, and 2% as dried fruit. More than 50 species are recognized in the grape genus *Vitis*, but almost all world wine is made from *Vitis vinifera*, native to the area south of the Caucasus Mountains and the Caspian Sea (Kunkee and Goswell 1996). Nowadays, wine is an integral component of the culture of many countries, a form of entertainment in others, and a libation of choice for advocates of its health benefits. Unlike many modern foods, wine's attraction relies not on strong consistent flavours, but upon a subtle array of shifting sensations that make its charm difficult to define (Bisson *et al*. 2002).

The most significant constituent of wine is water (75-90%). This 15% variation can be explained for by the amount of phenolics, organic acids, mineral salts and pectins which form the wine extract, and which differ from

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wine to wine. The second largest constituent of wine is ethyl alcohol, which, according to the type of wine, varies from 8% to 13% (v/v) or more. The sugar content of dry wines is generally less than 2 g/L; while in a botrytized sweet wine it can reach almost 200 g/L (Dominé *et al*. 2004). Some features of grape berry juice make of it a perfect medium for wine production, and these include: (1) a high concentration of sugars and other nutrients that provide a luxurious medium for the growth of wine yeasts; (2) a natural acidity, which is high enough to inhibit the growth of undesirable and noxious microorganisms during and after fermentation; (3) a high concentration of ethanol produced by sugar fermentation, which contributes to inhibit microbial activity in the product; and (4) a unique presence of aromas and flavours. Alcohol content, flavours and aromas are the principal factors used to define a particular wine (Kunkee and Goswell 1996).

In the 1990s, people's attention was increasingly drawn to the positive effects of moderate wine consumption. Indeed, the well known "French paradox" reports that, although the French diet is relatively rich in saturated lipids compared to that of other countries, the level of mortality due to coronary heart disease is reduced as a result of daily wine consumption (Renaud and de Lorgeril 1992). In fact, besides alcohol itself, which in moderate amounts helps blood flow in the body, wine polyphenols contain antioxidant properties, which are also beneficial to health (Bors and Michel 2002). Epidemiological studies indicate that a moderate alcohol and/or wine consumption may protect against the incidence of many diseases of modern society – cardiovascular disease (Cordova *et al.* 2004; Bradamante *et al.* 2005), dietary cancers (Jang *et al.* 1997; Gronbaek *et al.* 1998), ischemic stroke (Sinha *et al.* 2002; Wang *et al.* 2002), peripheral vascular disease (Kiechl *et al.* 1998; Szmitko and Verma 2005), diabetes (Stampfer *et al.* 1988; Rimm *et al.* 2005), hypertension (Gillman *et al.* 1995; Nevill *et al.* 1997; Thadhani *et al.* 2002), peptic ulcers (Aldoori *et al.* 1997), kidney diseases (Curhan *et al.* 1998; Rodrigo and Bosco 2006) and macular degeneration (Obisesan *et al.* 1998) – in addition to stimulating the resistance to infection (Cohen *et al.* 1993; Feng *et al.* 2002; Docherty *et al.* 2005, 2006) and retention of bone density (Burger *et al.* 1998; Hoidrup *et al.* 1999). The benefits of antioxidants are more pronounced in red wines since their levels of phenolic content are higher.

Wine quality largely depends on the vineyard and on the vine grower. Most of the wine compounds are produced by the plant itself, in leaves (sugars and acids), and in berry (acids and phenolics). Furthermore, some molecules related to aroma and taste are produced during the fruit development and ripening, being their spectrum specific to a given variety. Theses aromas, called "varietal" or primary aromas, are the grape's signature, recognizable by the consumer during degustation. Thus, the growth and the fructification of grapevines in the vineyard are of utmost importance to wine quality (Blouin and Guimberteau 2000). The french term "Terroir" is used by viticulturists to define a geographical and environmental origin where the grapes used for the vintage were grown (Laville 1990). This word includes characteristics such as soil composition (minerals, soil density), sunlight, climate (temperature, precipitations), topography and it can also take into account strains of microorganisms usually found on the berry skin, which take part in wine elaboration. The soil type depends on how the geological parent material has been altered and shaped by physical, chemical, and biological processes. In general, suitable soils for viticulture are those that are not particularly fertile or deep (Jaeger 2004). A "terroir" offering good conditions to a specific grapevine cultivar would then help the plant to produce high quality grapes, which is a good start for a good vintage.

A glance at the distribution of wine-growing regions on our planet shows at once that wines will grow only in certain conditions. The most important factor for viticulture is climate, and above all, temperature. Here there is a striking difference between the northern and southern hemispheres. The world map shows that vines clearly prefer moderate conditions. They seldom thrive where temperatures rise above 25ºC in the summer months of July and January in the northern and southern hemispheres, respectively. In a large part of western Europe, which is the zone that the majority of Europe's classic viticultural regions lie, average July temperatures vary between 15 and 25°C. Rainfall and drought also play an important part. It is almost impossible to grow wine with less than 200 mm of rain a year; on the other hand, too much rain also makes it difficult to cultivate grapes. A moderate climate, with adequate to relatively high rainfall, provides ideal conditions for producing both fragrant white wines with a good structure and acidity, and well-balanced red wines with good potential for maturing. All over the world, however, wine growers have found ways of adapting to natural conditions, and they have been particularly resourceful in borderline (Dominé 2004).

The development and maturation of grape berries have received considerable scientific scrutiny because of both the uniqueness of such processes to plant biology and the importance of these fruits as a significant component of the human diet and wine industry. For the winemaker, an outstanding attribute of *V. vinifera* is its ability to store enormous quantities of sugar in its berries. From the plant point of view, the ripe phenotype is the summation of biochemical and physiological changes that occur during fruit development and make the organ edible and desirable to seed dispersing animals (Giovannoni 2001). Control of the ripening timing, berry size and colouration, acidity and the relative assortment of volatile and non-volatile aroma and flavour compounds in table and wine grape cultivars are major concerns to viticulturists. Molecular and biochemical studies of grape berry development and ripening have resulted in significant gains in knowledge over the past years. Here the information available is updated regarding the physiological, biochemical, and molecular aspects of grape berry maturation that ultimately may interfere with fruit and wine quality. Understanding how and when various components accumulate in the berry is of critical importance to adjust grape growing practices and thus modify wine typology.

GRAPE BERRY DEVELOPMENT AND RIPENING

Grape berries contain three major types of tissue: skin, flesh, and seeds (**Fig. 1**). The time course of berry growth in *V. vinifera* has been interpreted differently by various authors. As grape berries develop, they display modifications in size, composition, colour, texture, flavour and in pathogen susceptibility. Grape berries exhibit a double sigmoid growth pattern (Coombe 1992). Growth first occurs mostly by cell division and later by cell expansion. From flowering to approximately 60 days afterward, a first rapid growth phase occurs during which the berry is formed and the seed embryos are produced. Several solutes are accumulated in the

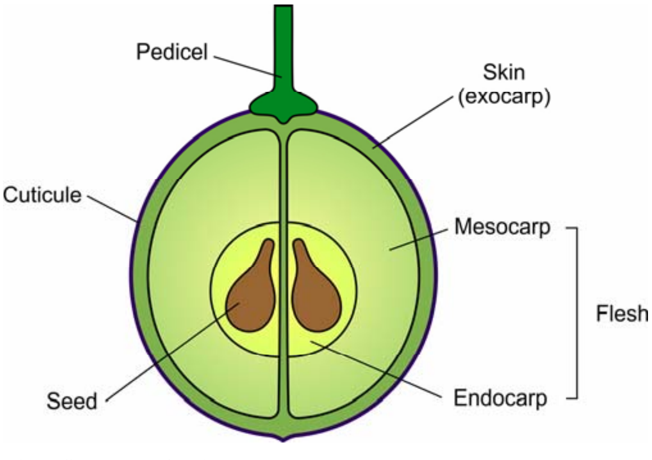


Fig. 1 Structure of a ripe grape berry.

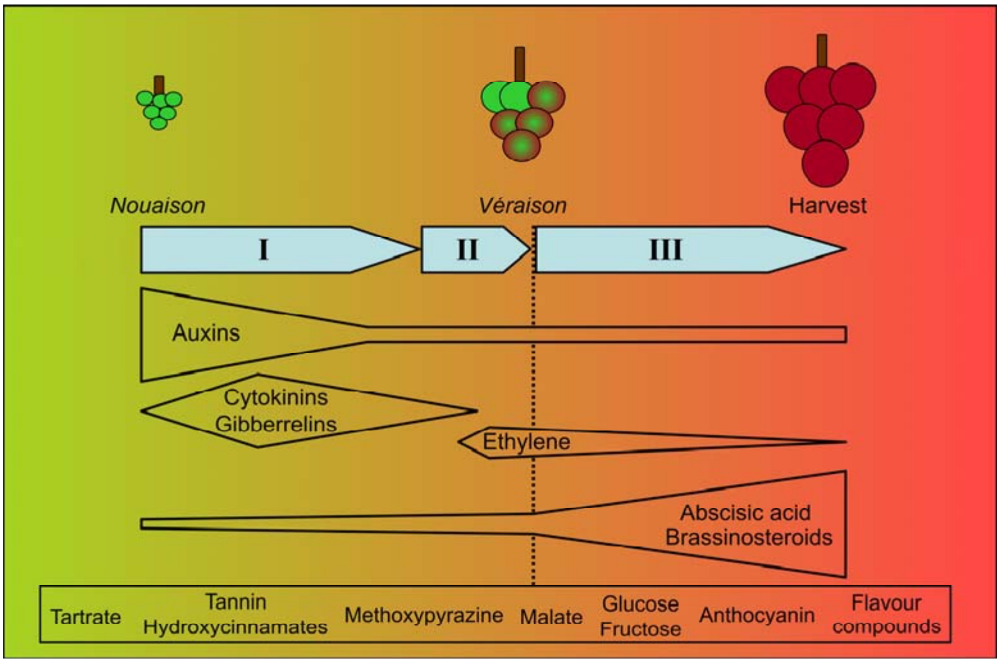


Fig. 2 Hormonal regulation of grape berry development and ripening. Hormonal content variations are indicated by arbitrary thicknesses volumes. Phase I: early fruit development; Phase II: lag phase; Phase III: berry ripening. In the bottom are indicated the main compounds that accumulate in the fruit (Coombe 2001).

berry during the first growth period, contributing in some extent to the expansion of the berry, and reach an apparent maximum around 60 d after flowering (Possner and Kliewer 1985) (**Fig. 2**). The most prevalent compounds among all the others are by far tartaric and malic acids. Tartaric acid is accumulated during the initial stages of berry development and its concentration is highest at the periphery of the developing berry. By contrast, malic acid is accumulated in the flesh cells at the end of the first growth phase. These acids confer the acidity to the wine, and are therefore critical to its quality. Hydroxycinnamic acids are also accumulated during the initial growth period. They are distributed in the flesh and skin of the berry and are important because of their involvement in browning reactions, and since they are precursors of volatile phenols (Romeyer *et al.* 1983). Tannins, including the monomeric catechins, are present in the skin and seed tissues but nearly absent in the flesh, and they also accumulate during the first growth period (Kennedy *et al.* 2000a, 2000b, 2001). Several other compounds like minerals, amino acids, micronutrients, and aroma compounds also accumulate during the first phase of berry growth and affect grape berry quality and ultimately wine quality.

Berry formation and development and the production of compounds having sensory importance are still far from being understood. Studies regarding these processes are limited as a result of the complex nature of this type of research and because the compounds produced are thought to be of less interest from a sensory standpoint. However it should be remembered that all above-discussed compounds are of critical importance to wine quality.

In most cultivars, the first growth phase is followed by a lag phase. The duration of this phase is specific to the cultivar considered and its end corresponds to the end of the herbaceous phase of the fruit. After this period of absence of growth, a second growth phase takes place, which coincides with the onset of ripening. The French word *véraison* used to describe the change in berry skin colour, which indicates the beginning of ripening, has been adopted to describe the onset of ripening. The most dramatic changes in grape berries' composition occur during this second growth phase, or ripening phase. Berries switch from a status where they are small, hard and acidic, with little sugar to a status where they are larger, softer, sweeter, less acidic, and strongly flavoured and coloured. The flavour that builds in grapes is mostly the result of the acid/sugar balance and the synthesis of flavour and aromatic compounds, or precursors taking place at this time. The development of these characteristics will largely determine the quality of the final product (Boss and Davies 2001). This ripening phase begins in August in the north hemisphere and lasts about 45 days, depending on the environmental conditions.

Overall the berry approximately doubles in size between *véraison* and harvest. Many of the solutes accumulated in the grape berry during the first period of development remain at harvest; yet due to the increase in berry volume, their concentration is significantly reduced. However, some compounds produced during the first growth period are indeed reduced (and not simply diluted) on a per-berry basis during the second period of berry growth. Among these is malic acid, which is metabolised and used as an energy source during the ripening phase, resulting in a significant decrease of its levels relative to tartaric acid, whose concentration usually remains almost constant after *véraison* (see section "Organic acids in grape berry and wine"). Tannins also decline considerably on a per-berry basis after *véraison* (see section "Phenolics in grape berry and in wine"). Aromatic compounds produced during the first growth period also decline (again, on a per-berry basis) during fruit ripening. These include several of the methoxypyrazine compounds that contribute to the vegetal characters of some wines (such as Cabernet Sauvignon and Sauvignon Blanc) (Hashizume and Samuta 1999). The decline in pyrazines is thought to be linked to sunlight levels in the cluster. Therefore, if these compounds are deemed to be undesirable (the current prevailing opinion), then canopy management can be used to reduce them. Although the first growth period contributes to the final quality of the berry, the most important event occurring during the second growth period is a massive increase in compounds, the major ones being glucose and fructose, as a result of a total biochemical shift into fruit ripening mode (**Fig. 2**).

HORMONAL CONTROL OF GRAPE BERRY RIPENING

The abundance of certain endogenous hormones over others at specific stages of fruit development and ripening indicates a possible role of these hormones during that developmental stage (**Fig. 2**). The first steps of berry development, from fecundation to *nouaison* (fruit set) are under the control of developmental hormones (auxins, cytokinins and gibberellins) that promote cell division and cell expansion. Although these hormones can be imported into the berry they are mostly produced by the seeds, or by the maternal tissues (unfertilized ovules) in case of seedless species. However, cytokonin production by seeds is not completely established for all plant species. Thus, the final size of the berry depends on the number of seeds it contains. These hormones reach a maximum concentration just before *véraison* and then decrease sharply along ripening (Coombe 1992; Blouin and Guimberteau 2000).

Hormonal control of grape berry ripening, from *véraison* to harvest, is still not very clear and may result from a combination of signals rather than being under the control of a single hormone. Indeed, whereas ethylene is the phytohormone related to climacteric fruit maturation – characterrized by a peak of ethylene production and respiration rate at the onset of ripening –, no specific hormone is clearly related to non-climacteric fruit ripening in general, and grape maturation in particular (Coombe and Hale 1973; Jiang *et al*. 2003; Giovanonni 2004). However, progresses in functional genomics favour the idea that fruit ripening displays quite general pathways whether obeying a climateric or non-climateric fruit maturation program (Giovannoni 2004; Mailhac and Chervin 2006). Three hormones may associate to regulate grape berry maturation processes: abscissic acid (ABA), ethylene, and brassinosteroids. There is a considerable accumulation of ABA after *véraison*, and it plays a role in seed maturation, acquisition of seed dormancy, resistance to water stress deficit – at later stages of ripening – but it is also specifically involved in maturation control (Coombe and Hale 1973). A delay in ABA accumulation correlated with a delay in the import of hexoses (Pirie and Mullins 1976; Davies *et al*. 1997) suggests that this hormone may promote sugar accumulation. As it will be referred to later, ABA may also positively influence the accumulation of phenolics in grape.

Recent studies have reported potential roles of ethylene signals in grape berry ripening, although the hormone is present at low but steady levels along berry development and ripening. As the impairment of ethylene perception limits the increase of berry diameter, it is an initial proof of the potential roles that ethylene signals may have in regulating the vascular fluxes associated with berry ripening (Mailhac and Chervin 2006). In addition, ethylene also seems to be implicated in the reduction of berry acidity over the ripening phase (Weaver and Montgomery 1974; Chervin *et al*. 2004). Furthermore, exogenous ethylene treatment at *véraison* was shown to enhance the accumulation of anthocyanins in grape berries (El-Kereamy *et al*. 2003). In a preliminary study conducted by Chervin *et al*. (2006) ethylene was related to sugar accumulation, leading authors to suggest that its effect in grape ripening needs to be reconsidered. Finally, there is some evidence that ethylene can have an effect on development of grape flavour and aroma (Tesnière *et al*. 2004; Mailhac and Chervin 2006). However, the effect of ethylene is modulated by the developmental stages of the fruit, i.e. fruit sensitivity to ethylene, whether there are exogenous or endogenous changes with maturation. In the grape, a higher sensitivity to ethylene has been associated with a decrease in auxin content, and fruit ripening in general is considered to involve both ethylene-dependent and -independent (i.e. developmental) processes (Coombe and Hale 1973; Lelièvre *et al*. 1997; Tesnière *et al*. 2004).

The role of brassinosteroids in grape maturation has been recently highlighted. These steroidal hormones are involved in plant growth and development, but their concentration in grape berry increase at the onset of ripening. Furthermore, an exogenous treatment of these hormones has hastened berry ripening and such a treatment could be the answer viticulturists are looking for in order to synchronize berries maturation in the same bunch (Symons *et al*. 2006).

WATER IN GRAPE BERRY AND WINE

Water is by far the most abundant constituent in must, defined as the juice of freshly pressed grapes (75-85%), and in wine (65-94%, depending on wine sugar content), acting as a solvent of volatile and fixed chemical compounds. Up to 99% of the water content in the must and in wine is absorbed by the roots from the soil. The quantity of water present on the surface of fruits and leaves when grapes are harvested and that deriving from enologic treatments can contribute up to 5% of the total water content in wine (Blouin and Cruège 2003). Vine growth and grape berry development are closely related to water availability in the soil. Berry volume per vine, which sets the limit of crop production, depends on berry number and water volume per berry. Sink size is by far the most variable factor in commercial vineyards (Delrot *et al*. 2001). Water represents 90% of the harvest weight in a crop primarily valued at a fresh weight basis, and carries most of the remaining 10% of crop weight into fruit, determining thereby fruit quality (Lang and Thorpe 1989).

Fruit volumetric growth is primarily the result of water accumulation, and hence the maintenance of fruit growth requires coordination between water and solute transport, both through the vascular tissue and at the level of individual cells. One hypothesized coordinating principle is that for fruit growth to occur there must be a favourable gradient in total water potential between the fruit and the rest of the plant. This gradient is generated both by transpiration and by an osmotic gradient (reviewed by Matthews and Shackel 2005).

Water deficit generally leads to smaller berries since it inhibits both cell division and, especially, cell expansion. It is widely acknowledged that berry size is a determining factor in wine grape quality. The implicit mechanism of this concept is that surface area/volume ratio of the approximately spherical berries decreases with the increase of berry size. Then, since anthocyanins and other phenolic compounds accumulate in the skin (Coombe *et al*. 1987), smaller berries have a relatively greater solute to solvent ratio than larger berries. However, there are effects of vine water status on fruit composition that arise independently of the resultant differences in fruit size. Indeed, the effect of vine water status on the concentration of skin tannins and anthocyanins is greater than the effect of fruit size *per se* on those same variables (Roby *et al*. 2004). The primary mechanism responsible for that is probably the differential growth responses of skin and inner mesocarp tissue to water deficit (Roby and Matthews 2003), although there may also be a direct stimulation of phenolic biosynthesis. Water stress may also affect the chemical breakdown or formation of important berry acids and flavours, and promote the conversion of carotenoid to aroma compounds. In addition, the concentration of arginine, a yeast-assimilable amino acid, is lowered. Indirectly, water stress may reduce the shading of fruit (Goodwin 2002; Keller 2005). Rojas-Lara and Morrison (1989) selectively shaded grape vines to separate the effects of shading fruit from the effects of shading foliage on berry development and on the accumulation patterns of sugar, anthocyanins, malate, tartrate and potassium in the fruit. Results showed that shading fruit and shading foliage differentially affected berry development and ripening. However, in general, shading decreases fruit colour, and the concentration of tartrate and soluble solids, and increases pH and the concentration of malate and potassium.

The timing of the water deficit is clearly important in order to determine fruit and wine composition, and irrigation can provide a means to manipulate wine sensory characteristics in the vineyard (Matthews *et al*. 1990). Although water deficit has a less pronounced effect on sugar accumulation than on berry growth, when it occurs at post-*véraison*, fruit sugar is often reduced rather than being improved. Conversely, malate decrease occurs primarily when water deficit occurs before *véraison*. Just before harvest excess water should be avoided since it can increase berry size and cause a “dilution” of solutes (sugars, acids, anthocyanins, tannins, etc.) or cracking of berries (Keller 2005; Keller *et al*. 2006).

Water influx into fruits occurs via both the xylem and phloem and most of the berry volume gain before *véraison* is due to water import from the xylem, whereas most of the post-*véraison* gain is due to water import from the phloem (Ollat *et al*. 2002; Keller *et al*. 2006). Moreover, the xylem and phloem are interconnected along the entire length and can readily exchange water and solutes (Zwieniecki *et al.* 2004). Indeed, despite the presence of intact and apparently functional xylem conduits in the berry, it seems clear that, at *véraison*, there is a substantial reduction in the proportion of water that is transported to the berry through the xylem compared to the phloem, which perhaps depends more on source and/or sink behaviour than on physical loss in xylem conductance (Bondada *et al.* 2005). At the same time, there is also a shift of phloem unloading, from the symplasmic to apoplasmic pathway that allows and is linked to the property of fruit flesh to accumulate high levels of soluble sugars (Patrick 1997; Zhang *et al.* 2006). The active removal of unloaded sugars from the mesocarp apoplast, which happens at a faster rate than water influx into mesocarp cells, explains how sugar accumulation in sink organs is associated to the shift from xylem to phloem water transport at the transient onset of grape berry ripening. Keller *et al.* (2006) have demonstrated that berry-xylem conduits retain their capacity for water and solute transport during ripening, and propose that apoplastic phloem unloading coupled with solute accumulation in the berry apoplast may be responsible for the decline in xylem water influx into ripening grape berries. In addition, the involvement of cell compartmented solutes, the rising solute concentration inside the vacuoles, make it more and more difficult for leaves to extract water from ripening berries. Xylem may serve to recycle excess phloem water back to the shoot. It is generally believed that at any given turgor, the growth rate is determined by the weakening rate of the cell wall structure (Cosgrove 2000). These two processes (turgor increase vs. wall loosening) are used to explain an increased fruit growth at *véraison* (Mathews and Shackel 2005). The shift from xylem to phloem function at *véraison* would prevent a substantial water or solute backflow from the fruit to the plant, which is increased by fruit transpiration (Delrot *et al.* 2001).

As most of the water coming into the berry after *véraison* is imported by the phloem, it has to cross biological membranes (Lang and Thorpe 1989). The hydraulic conductivity of the biological membranes is controlled mainly by the presence of specialized proteins that belong to the major intrinsic proteins (MIP) family. The aquaporin *VvPIP1A* (*Vitis vinifera* plasma membrane intrinsic protein) mediates water transport and is mainly expressed in the berries after *véraison* (Picaud *et al.* 2003). The co-expression of some aquaporins at the same time as sugars transporters, suggests a functional link between sugar and water fluxes during the processes of unloading and sugar accumulation in the vacuoles of the flesh cells (Delrot *et al.* 2001). In our lab, the aquaporin *VvSIP1* (*Vitis vinifera* small intrinsic protein, DQ086835) was cloned and its functionality is being studied by a heterologous expression in *Xenopus* oocytes. Baiges *et al.* (2001) obtained the nucleotide sequences of eight cDNAs encoding putative *Vitis* aquaporins (PIPs and TIPs) and found that most of them had higher expression in roots, eventually enhancing and regulating root water permeability.

POTASSIUM IN GRAPE BERRY AND WINE

Mineral soil composition (and density) has an essential influence on grape quality and on the organoleptic properties of wine. Grape berries are very rich in potassium which is an essential macronutrient for grapevine and grape berry growth and development. Thus, potassium is the main cation in must and wine (~ 900 mg/L; Blouin and Cruège 2003). As in other plants, potassium plays a key role in the grapevine physiology: (1) enzyme activation, (2) major control on the transmembrane potential difference of the plasma membrane, which to a large extent determines the uptake of many different cations, anions and sugars, and (3) regulation of osmotic potential, thus controlling plant water relations, turgor maintenance and growth. Although other cations may replace potassium in some of these functions, potassium plays the major role given its high concentration in plant tissues and the high permeability of plant membranes to potassium (reviewed by Mpelasoka *et al.* 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 potassium accumulates in the leaves. After *véraison*, a sharp increase in berry potassium is observed as a result of potassium redistribution from leaves to berries (Blouin and Cruège 2003). Potassium uptake by Cabernet Sauvignon berries is slow before *véraison* and strongly increases when ripening starts in the same proportion as sink strength and phloem water influx (Ollat and Gaudillere 1996).

Despite its importance to grapevine, excessive levels of potassium in berries at harvest may reduce the quality of fruit and have a negative impact on wine quality, particularly on red wines (Davies *et al.* 2006). Grape juice pH is a critical determinant of wine quality. The stoichiometry exchange of tartaric acid protons with potassium cations results in the formation of largely insoluble potassium bitartrate. This leads to a decrease in free acid and tartrate:malate ratio (Gawel *et al.* 2000), resulting in an increase of overall pH. Grape juice with a high pH often results in unstable musts and wines that are more susceptible to oxidative and microbiological spoilage. These wines have a high pH, low acidity and generally a flat taste (Somers 1977; reviewed by Mpelasoka *et al.* 2003). Also, the presence of higher levels of malate to tartrate enhances malolactic fermentation (Rühl 2000). High potassium 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.

The high pH of grape juice and wine also leads to a decrease of the colour quality and stability of red wines as a result of reduced anthocyanins ionisation at higher pH. Anthocyanins are located in the berry skin where potassium concentration is generally higher than in the pulp. Grape juice contains from 0.5 to 3 grams of potassium per liter. Grape skin contains about 9 grams of potassium per liter. Therefore, berry potassium levels are often more important to red than to white wines, because during red wine fermentation the skin is left for some period after crushing for the extraction of anthocyanins. During this period, even more potassium leaches out of the skins into the juice (reviewed by Mpelasoka *et al.* 2003).

Given the importance of berry potassium levels for wine quality and its possible deleterious consequences, understanding the dynamics and mechanisms of grape berry potassium accumulation is of extreme importance. Potassium uptake into cells occurs by multiple mechanisms. Potassium channels mediate passive low affinity potassium transport across membranes, whereas potassium carriers mediate energized high and low affinity uptake (Davies *et al.* 2006). External potassium levels are thought to determine which mechanism is used by the cell, in a way that a sufficiently high external potassium concentration will allow transport through channels; but carrier-mediated uptake becomes critical at low external potassium levels, when the gradient may not be favourable to passive transport (Maathuis and Sanders 1996; Davies *et al.* 2006). During berry development, potassium may play different roles depending on the development stage. During an initial rapid phase of berry growth, cells are divided and expanded at a high rate, during which time potassium may play an important role as osmoregulator. After *véraison*, grape berries continue to enlarge due to cell expansion driven by the increase of sugar content in the cell vacuole. Potassium may play an important secondary role in the accumulation of sugars during this phase. Recently, two cDNAs encoding putative potassium transporters from grape berries have been isolated (Davies *et al.* 2006). VvKUP1 and VvKUP2 function as potassium transporters as demonstrated by heterologous expression in an *Escherichia coli* mutant deficient in potassium uptake. The two transporters were shown to be highly expressed in the berry skin during the first phase of berry development, suggesting that, at this time, these transporters play a role either in potassium uptake into the berries or in its compartmentation into the skin cells. The transcript levels of both transporters are lower at post-*véraison* than at pre-*véraison*, but significant levels of expression were detected. It is suggested that VvKUP1 and VvKUP2 may therefore continue to contribute to potassium homeostasis throughout berry development. Other mechanisms may also be involved after *véraison* (Davies *et al*. 2006).

The levels of potassium in grape berries may be affected by numerous factors including potassium level in the soil, grape variety, and viticultural practices (Mpelasoka *et al*. 2003; Davies *et al*. 2006). A detailed knowledge of the mechanisms involved in potassium transport from the soil, xylem and phloem translocation through the vine and its accumulation in the berry, is crucial in order to develop strategies which may reduce its excessive accumulation in grape berries and thereby improve fruit and wine quality.

Other minerals, such as calcium and magnesium, together with minimal amounts of sodium and iron, are also present in grape berries. Calcium concentration is at its maximum at *véraison*, and remains stable or decreases during maturation. Calcium has a significant role in the resistance against *Botrytis cinerea* infection. In must, its content (80 mg/L) can slightly decrease because of the increase of calcium tartrate resulting from alcohol formation. Magnesium, a component of chlorophyll, is present in grapes and in must at levels similar to calcium. Grapes are poor in iron and copper, their concentrations in must and wine being approximately 10 and 1 mg/L, respectively. However, an unusual increase of these compounds in must can occur as a result of viticulture and vinification practices. Copper affects wine flavour by oxidizing thiol-containing compounds (Blouin and Cruège 2003).

SUGAR IN GRAPE BERRY AND WINE

Sugar transport and accumulation in berry

Efficient assimilation and use of nutrients by plants is of prime importance for the optimization of crop productivity. Grape berries, as typical "sink organs", rely on the use of available carbohydrate resources produced by photosynthesis to support their growth and development. The transport and allocation of sugars between the photosynthetic "source tissues" and the heterotrophic "sink tissues" is known as assimilate partitioning and is a major determinant of plant growth and productivity (Kingston-Smith 2001) (**Fig. 3**).

Our understanding of the mechanisms of carbon partitioning in grapevine has made considerable progress over the past 10 years. Sucrose produced through photosynthesis in the mesophyll of mature leaves is the main carbohydrate used for long distance transport (Swanson and Elshishiny 1958). Sucrose is loaded into the phloem by either a symplastic (via plasmodesmata) or apoplastic mechanism (reviewed by Boss and Davies 2001). According to Munch's mass flow hypothesis, sucrose, as the major osmotically active constituent in the phloem, also provides the driving force for translocating all other compounds in the phloem sap. Upon its arrival in the phloem of the berry, sucrose may be unloaded by the different pathways described above (reviewed by Boss and Davies 2001). It has been suggested that symplastic connections via plasmodesmata between the sieve tubes and the mesocarp cells remain for quite a long period during berry development. However, at the phloem/storage parenchyma interface, sufficient plasma membrane surface area is available to support exchange with the apo-

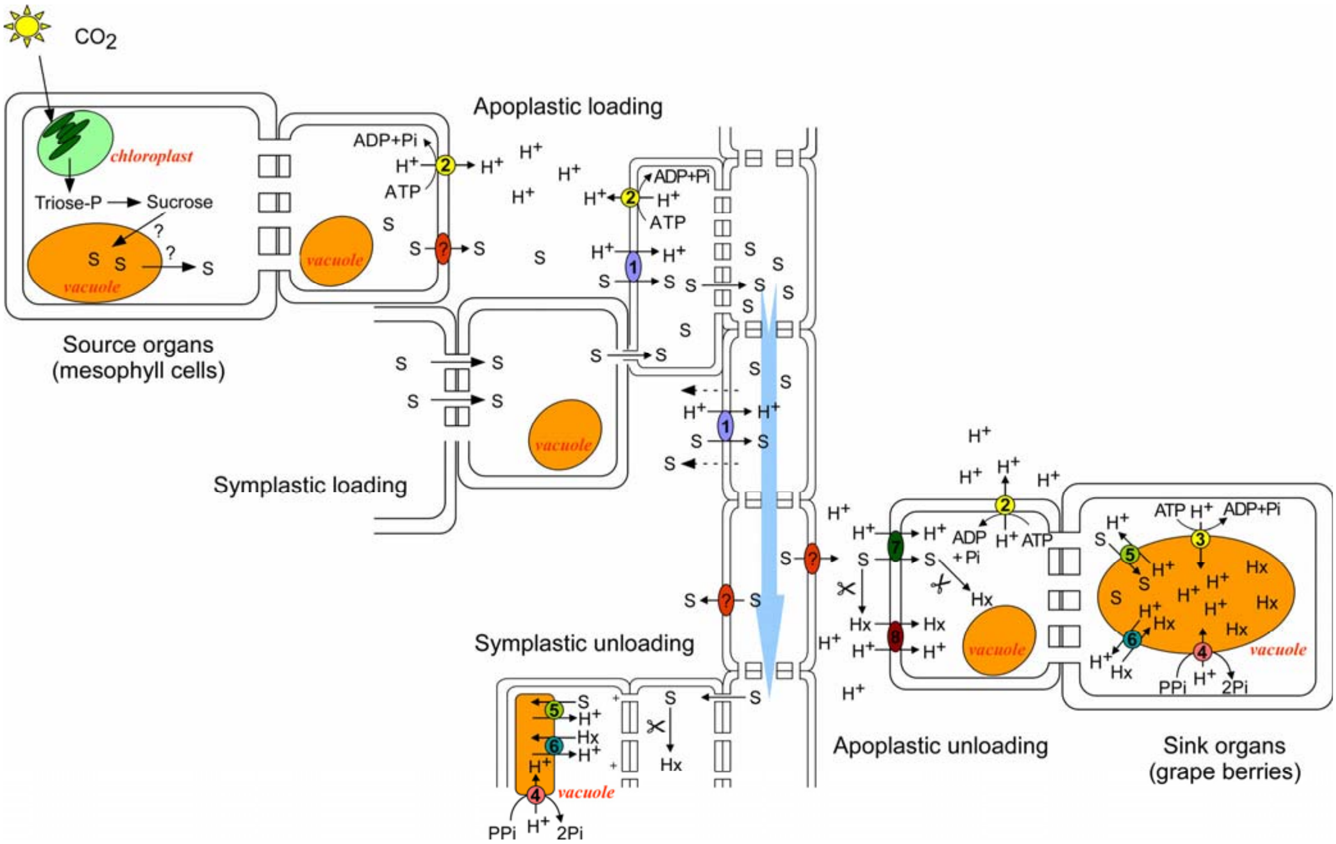


Fig. 3 Long-distance sugar transport through the phloem. From its point of synthesis in the mesophyll, sucrose may be loaded into de sieve elements/companion cell complex either through plasmodesmata or via the apoplast. Apoplastic loading requires sucrose export from the mesophyll or the vascular parenchyma and reuptake into the SE/CC complex. Hydrostatic pressure drives phloem sap movement toward sink tissue. Passive leakage can take place along the path (indicated by dashed arrows). Reuptake of leaked sucrose occurs along the phloem. Apoplastic phloem unloading implies the existence of a sucrose exporter (yet unidentified) at the sink tissue. Import of sucrose and other solutes into sink tissue may occur through plasmodesmata or sucrose transporters. In addition to plasmodesmal and transporter mediated uptake, cells in the sink take up hexoses after sucrose hydrolysis by an apoplastic invertase. The vacuolar transport system may consist of Sucrose/H^+ antiporters and Hexose/H^+ antiporters. (1) Source Sucrose/H^+ symporter; (2) Plasma membrane H^+ ATPase; (3) Tonoplast H^+ ATPase; (4) Pyrophosphatase; (5) Sucrose/H^+ antiporter; (6) Hexose/H^+ antiporter; (7) Sink Sucrose/H^+ symporter; (8) Hexose/H^+ symporter; ✂ Invertase. (Adapted from Lalonde *et al.* 1999).

plast. The high sugar concentrations found in the berry apoplast and their sensitivity to changes in phloem import rates also suggest an apoplastic unloading (Patrick 1997). Indeed, an adaptive shift of phloem unloading from symplastic to apoplastic pathway at the onset of ripening was recently clearly demonstrated for grape berries (Zhang *et al.* 2006).

The presence of an apoplastic step requires the involvement of membrane-located sugar transporter proteins mediating the exit of sucrose from the phloem, and the uptake and compartmentation of sugars across the plasma membrane and the tonoplast of flesh cells. Both disaccharide transporter (DST) and monosaccharide transporter (MST) families have been characterized in plants (reviewed by Williams *et al.* 2000). Three cDNA clones encoding homologues of sucrose transporters have been identified in *V. vinifera*, and two of them functionally characterized as proton-sucrose symporters by heterologous expression in yeast. RNA blot and microarray analysis showed that they are differentially expressed in grapevine tissues and during the grape berry development. *VvSUC11* (AF021808; also identified as *VvSUT1*, AF182445) and *VvSUC12* are expressed mainly after *véraison* when the sugar concentration increases, whereas *VvSUC27* expression is associated with early stages of berry development and with sink organs (Davies *et al.* 1999; Ageorges *et al.* 2000; Manning *et al.* 2001).

Massive accumulation of glucose and fructose in the vacuoles of mesocarp cells occurs after *véraison*. Twenty days after this period, the hexose content of the berry is close to 1 M, with a glucose/fructose ratio of 1. Because sucrose is the major translocated sugar in grapevine, the rapid accumulation of hexose characterizing berry ripening must involve the activity of invertases (Fillion *et al.* 1999). Invertases catalyse hydrolysis of sucrose provided by the phloem conducting complex into glucose and fructose. Different invertase isoforms are localized in the cell wall, cytoplasm and vacuole. Hydrolysis of sucrose by cell wall invertase may promote unloading by preventing its retrieval by the phloem, and by maintaining the sucrose concentration gradient. Both expression and activity of cell wall invertase increase around the onset of ripening and reach a high level in the late stage (Zhang *et al.* 2006). Two vacuolar invertases, *GIN1* and *GIN2*, have been cloned from grape berries. Their expression is high at early stages of berry development, but it declines greatly when hexose accumulation starts (Davies and Robinson 1996; reviewed by Boss and Davies 2001). The activity of apoplastic cell wall invertases requires the co-ordinate activity of monosaccharide transporters. In addition to a putative plastidic glucose transporter (*pGLT*, AY608701), six full length cDNAs homologous to plasma membrane hexose transporters have been cloned from grape berries and named *VvHT* (*V. vinifera* Hexose Transporter; *VvHT1*, AJ001061; *VvHT2*, AY663846; *VvHT3*, AY538259, also identified as *VvHT7*, AY854146; *VvHT4*, AY538260; *VvHT5*, AY538261; and *VvHT6*, AY861386, DQ017393) (reviewed by Agasse *et al.* 2007). So far, only *VvHT1* has been characterized functionally. The transport function of VvHT1 was directly assessed by heterologous complementation in mutated yeasts deficient in glucose transport. Uptake measurements showed that VvHT1 is a high affinity, H^+-dependent symporter with broad specificity for various monosaccharides (Vignault *et al.* 2005).

VvHT1 is expressed in sink organs like young grapevine leaves and grape berries. Microarrays and RNA blot studies have shown that *VvHT1* is strongly expressed in berries before *véraison*, that is, before hexose accumulation. A slight increase of expression has been observed by quantitative RT-PCR for the late phase of berry ripening (Fillion *et al.* 1999; Terrier *et al.* 2005; Vignault *et al.* 2005). In addition, western blot analysis using an anti-VvHT1 antibody showed that VvHT1 protein is abundant at early stages of berry development and absent from the ripening berries (Conde *et al.* 2006) (**Fig. 4**). *VvHT2* expression in the ripening berry is weak and associated with *véraison*. *VvHT6* expression during grape maturation is strong and is also associated with the *véraison* stage (reviewed by Agasse *et al.* 2007). Given the specific expression patterns of sucrose and hexose transporters (except *SUC11* and *SUC12*, which are expressed at the same time, and *VvHT2* and *VvHT6*, which are also expressed at the same time), understanding the particular functions and regulation mechanisms of each transporter is of critical importance. Recent data from *in situ* hybridization studies indicate that *VvHT1* is strongly expressed in the phloem tissues (Vignault *et al.* 2005). This high-affinity transporter may be involved in the supply of energy for the intense cell division and growth when low apoplastic sugar is available. As ripening proceeds, other sugar transporters should be involved in sugar accumulation into the mesocarp cells (Conde *et al.* 2006).

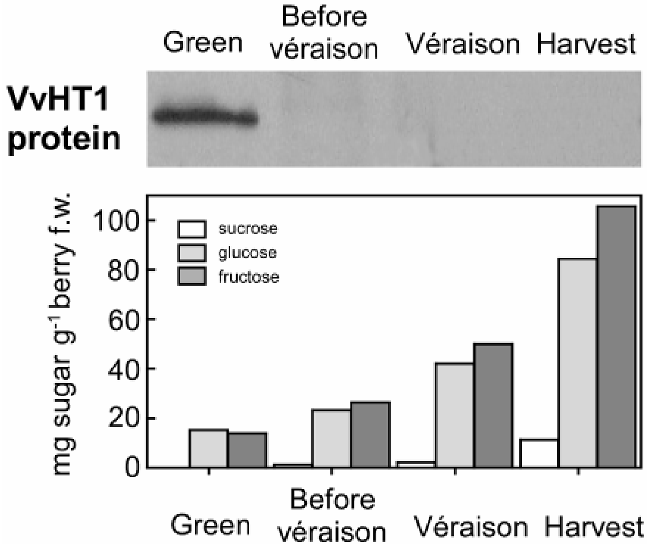


Fig. 4 Sugar content and western blot analysis of VvHT1 levels in the plasma membrane of berries flesh cells at different stages of grape berry development. (Reprinted from Conde *et al.* (2006) *Plant Physiology* **141**, 1563-1577, with kind permission from the American Society of Plant Biologists).

Sugar accumulation in the berry is regulated by complex mechanisms. The expression of DSTs and MSTs may be affected by various parameters including light, water and ion status, wounding, fungal and bacterial attacks, and hormones (Kühn *et al.* 1997). However, the mechanisms of these regulations are still poorly understood (Delrot *et al.* 2000). The vineyard fungal pathogen *Eutypa lata* can affect sugar import and cause reduction of bunch numbers (Octave *et al.* 2006). Sugars of grape in association with pathogen-resistance proteins also constitute a defence mechanism against fungi. Indeed, a simultaneous accumulation of antifungal proteins and sugars during berry ripening is correlated with the development of pathogen resistance, constituting a developmentally regulated defence mechanism against phytopathogens in the maturing fruit (Salzman *et al.* 1998). Besides their role as carbon and energy sources, sugars synthesized during the light phase can act as regulatory signals affecting gene expression. The ability to sense altered sugar concentrations is important in the context of resource allocation, allowing the plant to tailor its metabolism in source tissues to face the demands in sinks. We found recently that high glucose levels repress *VvHT1* expression involving both an HXK-dependent decrease of *VvHT1* transcript level and an HXK-independent decrease of VvHT1 protein amount in the plasma membrane (Conde *et al.* 2005, 2006). Once phloem transport has ended, any further increase in berry sugar concentration is exclusively due to water loss. The fungus *B. cinerea* can thus act positively on the sugar content in matured berries and is used by grape growers to produce sweet wines (Blouin and Cruège 2003). However, our recent results in *Pinus pinaster* cultured cells suggest that the increase in sugar content in the berry may also be related with the ability of the pathogen to enhance glucose transport (Azevedo *et al*. 2006).

Sugar content is an indicator often used to assess ripeness and to mark the harvest. Furthermore, as most of the sugar is fermented to ethanol during the vinification process, the measurement of sugar content, the so-called "must weight", allows the control of alcohol content in the wine. While indicating maturity level, "sugar ripeness" is not, by itself, the best index of optimal maturity.

Sugar fermentation

The first phase of winemaking consists in finding high quality berries and making sure that they are harvested in optimal conditions. The second phase consists in fermenting the grape juice into wine. Winemakers control the fermentation through several parameters: temperature, skin contact time, pressing technique, etc. (Eisenman 1999). During the fermentation process, glucose and fructose, the two major sugars of berries, are converted into alcohol by the action of the yeast *Saccharomyces cerevisiae*, releasing carbon dioxide and heat (**Fig. 5**). Depending on the type of wine being produced, all the sugar may be fermented into alcohol to produce a dry wine (residual sugar concentration <4 g/L), or only a portion, creating medium dry or sweet wines (sugar concentration between 12-45 g/L or >45 g/L, respectively). Whatever the origin of the grapes, different native *S. cerevisiae* strains will also be found on the surface of their skins (Cappello *et al*. 2004). As soon as the skins are damaged, these yeasts come into contact with the sweet juice. Indigenous *S. cerevisiae* strains are also found in the winery environment, several reports indicating that these strains participate in wine fermentation with different degrees of prevalence at the end of alcoholic fermentation (Ciani *et al*. 2004; le Jeune *et al*. 2006; Mercado *et al*. 2007). Some winemakers and enologists take the view that ambient yeasts are essential for the true expression of the *terroir*. However, these natural yeasts are not always present in the environment in sufficient quantities to bring about adequate fermentation. The use of chemicals in the vineyard for example can cause a drop in the yeast population. In addition, common practices of cellar cleaning and sanitation in modern winemaking and the change in the nature of winery surfaces also reduce the autochthonous yeast flora (Pretorius 2000). Increasing numbers of winemakers prefer not to take any risk, and add commercial yeasts strains, whose properties are known in detail. From a technical point of view, they must produce a significantly faster fermentation and a good output in terms of converting sugar into alcohol. The yeasts are also selected with regard to the taste and smell they generate. Generally speaking, neutral yeasts are preferable, because they do not affect the aromas of the wine. With certain grape varieties, however, strains that contribute to the development of a variety-specific aroma are chosen (Jaeger 2004). If dry wines are being made, the yeast must have a high tolerance to alcohol, so that they can survive until the fermentation is completed. Otherwise, fermentation may cease when 11.5-12% ethanol is reached, even though some sugar remains in the must. Current winemaking practices favor the use of fully maturated grapes in order to obtain high flavour intensity wines (Heux *et al.* 2006). However, these grapes also have high sugar concentrations, yielding must with elevated probable ethanol. However, excessive ethanol concentrations resulting from high-sugar musts, contrast with current public health policies and worldwide trends to decrease wine ethanol levels, and this can also affect flavour and aroma quality (Heux *et al*. 2006). In addition, high ethanol concentrations increase the risk of an incomplete fermentation. The unfermented sugars (mainly fructose) may enable growth of contaminating yeasts and bacteria, and consequently spoil the wine. Thus, when fermentation is blocked, re-inoculation with a selected yeast strain is usually required to complete sugar consumption to a residual amount. Ethanol toxicity is primarily associated with the damage of cell membranes (Leão and van Uden 1984; Alexandre *et al*. 1994) and transport systems (Cardoso and Leão 1992), and ethanol effects on yeast cell viability and on hexose transporters during vinification are responsible in many instances for hindering complete sugar consumption.

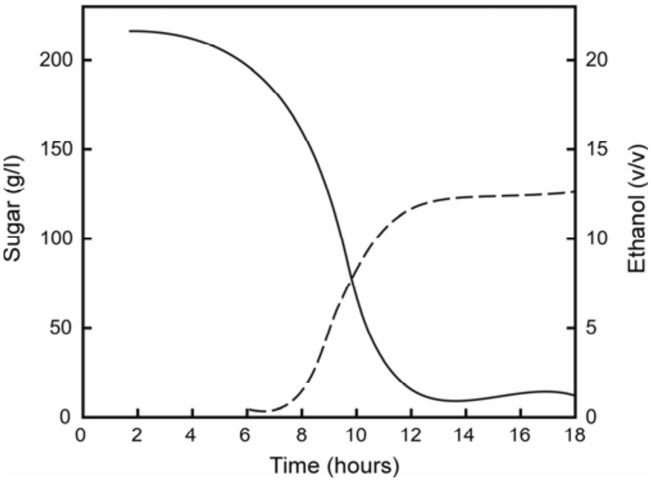


Fig. 5 Sugar consumption (full line) and ethanol production (dashed line) along an alcoholic fermentation carried out by *Sacharomyces cerevisiae* PYCC 4072 (under laboratory culture conditions).

The fermenting yeast *S. cerevisiae* has several hexose transporters (encoded by *HXT1-HXT17* and *GAL2*), six of which (Hxt1-4p and Hxt6-7p) have been characterized as the most physiologically relevant (Reifenberger *et al*. 1997). During must fermentation they perform different roles, and have different expression patterns according to their regulatory and kinetic properties: *HXT2* is expressed essentially in the lag phase; *HXT1* is then expressed from the beginning of fermentation to the stationary phase; *HXT3* is present throughout fermentation, with a maximal expression when growth stops; finally *HXT6* and *HXT7* are induced at the entry of stationary phase and remain expressed till the end of fermentation (Perez *et al*. 2005). The latter transporters are high-affinity transporters with K_m values around 1-2 mM for glucose and about 2-times higher for fructose (Reifenberger *et al*. 1997). This preference for glucose is shared by all the other hexose transporters and explains the prevalence of fructose towards the end of fermentation, and the residual amounts of this sugar that still remain in fully fermented wines. In addition to fructose, wines also contain small amounts of other sugars, such as arabinose, xylose, ribose, rhamnose, galactose, etc. The residual sugar in dry wines is enough to add mouth-feel without a perceivable sweet taste, and can balance the wine, increase viscosity, and help to mask a small defect or a slight unpleasant flavour. Preventing sugar fermentation by yeasts will produce sweet wines. One way of achieving this is the addition of pure alcohol or another high-alcohol liquor before the wine is completely fermented, exceeding the level tolerated by the yeast, as in the case of Port wines. Clear and stabilized wines can also be sweetened by sugar addition, after removal of yeast cells by filtration, or after addition of sulphite and potassium sorbate to inhibit yeast and bacteria. The normal dose level of potassium sorbate is 200 to 250 mg/L but wines treated with sorbate should also contain at least 30 mg/L of free sulfur dioxide. When potassium metabisulfite is added to wine, it produces about half its weight in SO_2. Molecular SO_2 is the biologically effective form and its concentration is strongly dependent on the wine pH (Eisenman 1999; Blouin and Cruège 2003).

Besides ethanol, small amounts of several other compounds with high impact in wine quality are produced during must fermentation. From these, glycerol is the most abundant being produced by yeasts to balance redox metabolism and also in response to environmental stress, mainly osmotic stress (Zuzuarregui *et al*. 2005). Glycerol concentration in wines usually varies from 4-9 g/L, contributing to wine sweetness and body (Remize *et al*. 2000).

ORGANIC ACIDS IN GRAPE BERRY AND WINE

Rapid changes in the acid/sugar balance occur at the onset of berry ripening. The presence of adequate levels of organic acids in the berries is a key factor to determine if the must has the potential to produce a good and stable wine. Tartaric and malic acids generally account for 69 to 92% of all organic acids in grape berries and leaves (Kliewer 1966). Minor amounts of citric, succinic, lactic and acetic acids are also present in ripened grapes.

Tartaric acid metabolism

Given the important role of tartaric acid in grape potential quality and ultimately wine quality, the elucidation and understanding of its biosynthetic pathways remains of special interest to plant scientists. Unlike most organic acids found in grapes, the metabolic origin of tartaric acid lays outside the oxidative metabolism of sugars (Loewus and Stafford 1958). Tartaric acid biosynthesis begins with L-ascorbic acid. A key step in tartaric acid formation is the cleavage of a six-carbon intermediate between either position C2/C3 or C4/C5, depending on the plant species. The former reaction yields oxalic acid (OxA) and L-threonate, which in plants of the *Geraniaceae* is then converted to tartaric acid in the leaves, whereas the latter “direct pathway” yields tartaric acid and a two-carbon compound, possibly glycoaldehyde, and is the pathway proposed to predominate in the *Vitaceae* (Loewus 1999) (**Fig. 6**). OxA and tartaric acid are formed by ascorbic acid catabolism in intact grape berries, suggesting that, in the grapevine, both pathways are functional. However, in grape berries, tartaric acid biosynthesis is limited from post-anthesis until *véraison* (Saito and Kasai 1982; Coombe and McCarthy 2000; de Bolt *et al*. 2006). Despite having established the nature of chemical intermediates in tartaric acid biosynthesis, data regarding the identification of the enzymes responsible for the proposed reactions is scarce. Moreover, attempts to identify the corresponding enzyme activities in crude cell extracts have been unsuccessful. Only very recently, combining transcript profiling of EST data and metabolite profiling of organic acids, have de Bolt *et al*. (2006) identified the gene and characterised the coded enzyme responsible for the proposed rate-limiting step in tartaric acid biosynthesis from vitamin C. L-idonate dehydrogenase (L-IdnDH) was shown to catalyse the conversion of L-idonate to 5-keto D-gluconic acid, providing evidence for a biochemical component of this pathway.

Malic acid metabolism

Contrarily to tartaric acid, malic acid levels vary greatly as berries develop and mature. As referred before, malic acid is accumulated in the fleshy cells at the end of the first growth phase, reaching its maximal value just prior to *véraison*. As in the case of tartaric acid, both leaves and immature green berries are able to form malic acid. Photosynthesis in the green berry is responsible for approximately 50% of the accumulating acids. Most of the work on malic acid synthesis indicates that β-carboxylation of phosphoenolpyruvate is the most important pathway (Meynhardt 1963, 1965; Ribéreau-Gayon 1968; Hawker 1969). Phosphoenolpyruvate carboxylase irreversibly catalyses this step. The resulting oxaloacetate is then reduced to malate by a cytosolic malate dehydrogenase (Hawker 1969; Meynhardt 1965) (**Fig. 7**).

The decrease of organic acid content that begins at the onset of ripening is associated with a sudden induction of malate oxidation. The simultaneous initiation of organic acid breakdown and sugar accumulation at the onset of ripening, which occurs 6 to 9 weeks after flowering and about midway between bloom and fruit maturity, has led to the suggestion that malic acid may be either transformed to fructose and glucose or used as carbon and energy source for respiration. Cytosolic NADP-malic enzyme is considered to play a key role in the regulation of malate breakdown catalysing the oxidative decarboxylation of malate to pyruvate and CO_2 (Hawker 1969; Possner *et al*. 1981; Ruffner *et al*. 1984). Another operating pathway is the diffusion of malic acid into the mitochondria and its subsequent degradation through the action of a mitochondrial malate dehydrogenase into oxaloacetate or, alternatively, its oxidation to pyruvate through the action of a mitochondrial NAD-malic enzyme (**Fig. 7**). It is admitted that cytosolic and mitochondrial isoforms of malate dehydrogenase would respectively participate in malate synthesis and catabolism in response to metabolic changes occurring during grape development (Taureilles-Saurel *et al*. 1995a, 1995b).

Cool regions typically produce grapes with higher concentration of malic acid and, conversely, grapes grown in warmer regions tend to have lower acidity. This negative temperature correlation with malic acid levels is due to the effect of temperature on the balance between malic acid synthesis and catabolism. The negative correlation between mitochondrial malate dehydrogenase activity and malate concentration during ripening supports the idea that mito-

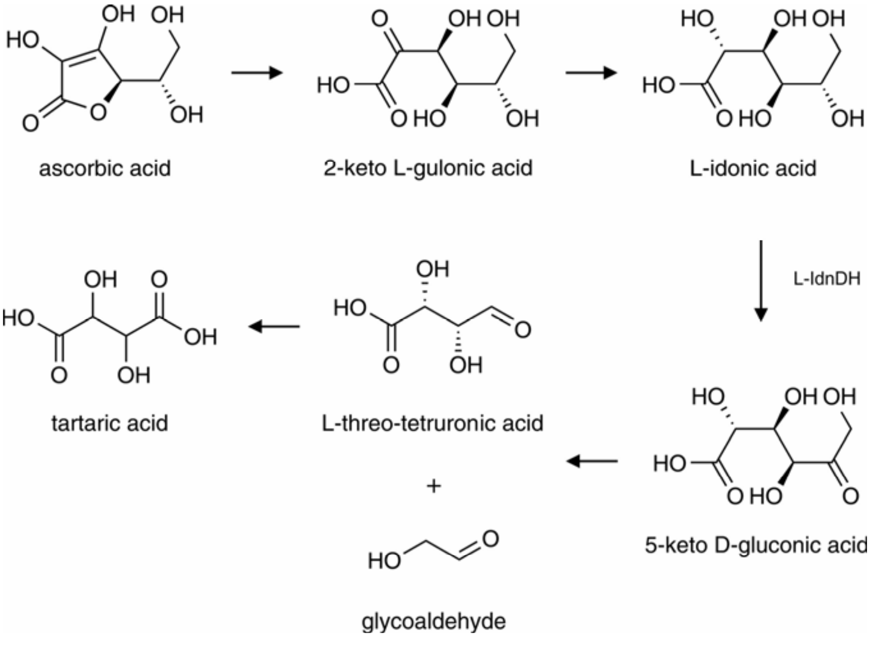


Fig. 6 Proposed pathway for tartaric acid formation from ascorbic acid in the grapevine. (Adapted from Loewus 1999). L-IdnDH, L-idonate dehydrogenase. Other enzymes are currently unknown.

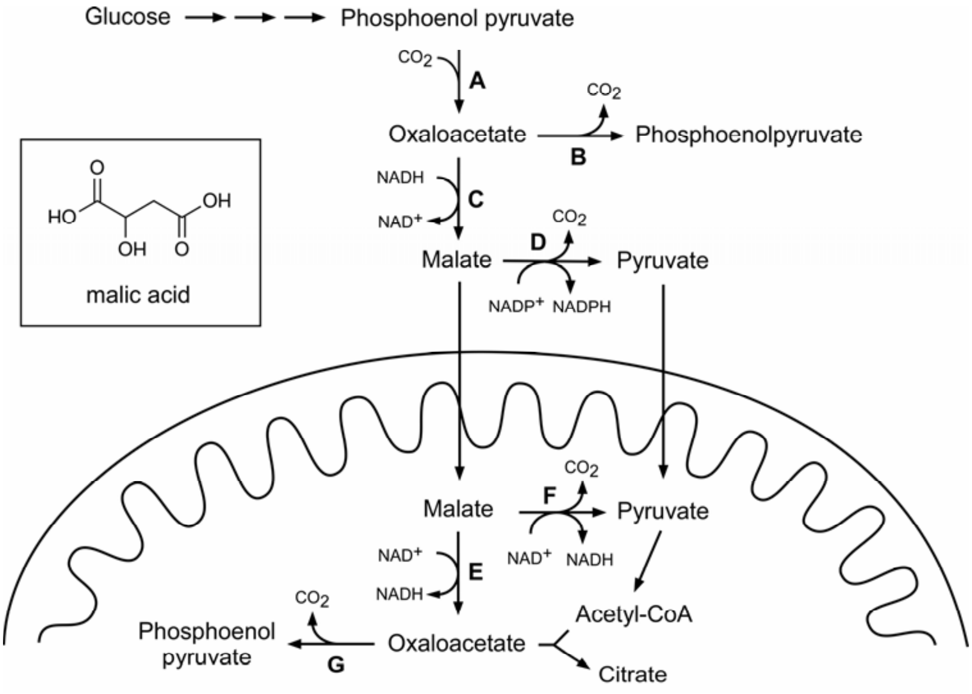


Fig. 7 Malic acid metabolism in grapevine. (A) Phosphoenolpyruvate carboxylase; (B) Cytosolic phosphoenolpyruvate carboxykinase; (C) Cytosolic malate dehydrogenase; (D) Cytosolic malic enzyme; (E) Mitochondrial malate dehydrogenase; (F) Mitochondrial malic enzyme; (G) Mitochondrial phosphoenolpyruvate carboxykinase. (See text for references).

chondrial malate dehydrogenase participates in malate degradation in response to summer growth conditions. In contrast, the cytosolic isoform operates at lower temperatures, mainly reducing oxaloacetate to malate. Temperature responses of phosphoenolpyruvate carboxylase and malic enzymes were studied by Lakso and Kliwer (1975). The activity of malic enzyme increases with increasing temperature up to 46ºC, indicating high thermal stability. In contrast, phosphoenolpyruvate carboxylase has a temperature optimum at about 38ºC, with a rapid heat inactivation above this temperature. The different temperature responses of these enzymes are involved in regulating malic acid levels in grape berries, with a maximal value for malic acid accumulation between 20 and 25ºC. These physiological data show that the decrease in malic acid during maturation is the result of reduced malate synthesis in combination with an accelerated catabolic rate. However, other pathways such as decreased translocation from the leaves to the berries may also play an important role. Additionally, accumulation of potassium into the berries results in the formation of tartrate and malate salts, accounting also for a reduction of the total acid concentration. Attempts to use malate/tartrate ratio as an index of ripeness do not bear a lack of a precise correlation with the development of grape flavour. Additionally, the ratio itself is quite variable across different varieties and growing conditions and seasons and is of little true predictive value. Despite their structural similarity, tartaric and malic acids are synthesised and degraded by markedly different biochemical processes.

Organic acids in wine

In addition to flavour and colour, the pH of wine grapes is one of the most important and controversial quality parameters for grape quality and the wine-making industry. The pH value of the wine reflects the amount and the strength of the acids, and the effects of minerals and other materials present in the wine. Although many factors are involved, wine pH depends upon three major factors: the total amount of acid present, the ratio of malic acid to tartaric acid, and the quantity of potassium present. If a wine is too low in acid, it tastes flat and dull. If a wine is too high in acid, it tastes too tart and sour. Also, acidity allows wine to maintain its freshness while also molding its flavour components and its colours. In addition, acid content in the berries and then in the must affect vinification and wine stability. High acidity (pH 3-4) avoids the development of contaminating bacteria but also impairs the main processes of alcoholic fermentation, since the optimal pH for yeast action is 4-6.

As referred, at harvest time, the main acids found in the berries are tartaric and malic, and, in minor amounts, citric acid, and they are carried over through the fermentation process into the finished wine. Although in minor amounts in grape berry, succinic, lactic and acetic acids present in wine mainly result from alcoholic or malolatic fermentation. Other acids can be found in the wine, appearing as small quantities of secondary products (for example, gluconic acid) resulting from vinification procedures or bacterial attack. The desirable acid content depends on wine style and on how much residual sugar is left in the wine. Ideally, the acid content of grapes should fall in the range from 0.65 to 0.85 g/100 ml (%). One of the most important winemaking tasks consists in adjusting the initial acid content of the grapes before starting fermentation in order to produce a balanced wine. Important and instructive information about wine acids and their influence on wine quality and stability can be found in Blouin and Guimberteau (2000), Blouin and Cruège (2003), Eisenman (1999) and Dominé *et al*. (2004).

Berry taste as well as the organoleptic properties and aging potential of wines are intimately linked to the levels of L-tartaric acid present in the fruit and to those added during vinification for flavouring and antioxidant purposes (de Bolt *et al*. 2006). In fact, tartaric acid is the strongest of grape acids and is responsible for much of the tart taste of wine, and it contributes to both the biological stability and the longevity of wine. The quantity of tartaric acid slowly decreases to small amounts after fermentation. Both potassium and calcium combine readily with tartaric acid and form potassium bitartrate and calcium tartrate crystals that precipitate during fermentation. Aged wine usually contains about two-thirds as much tartaric acid as the starting grapes because of tartrate precipitation. Bacteria can attack tartaric acid causing devasting losses. Malic acid is the most fragile wine acid and confers a green taste to the fruit and to the wine. During alcoholic fermentation, yeasts consume some of the malic acid present. Further decrease in malic acid content is achieved during malolactic fermentation (ML), when bacteria convert malic acid into lactic acid. ML fermentation is a standard procedure to reduce the acidity of wines made from grapes grown in cool regions. In addition, some byproducts produced during the ML fermentation can contribute positively to the complexity of the wine. However, when malic acid is deliberately retained to improve the acid balance of the wine, special steps may be needed to prevent occurrence of ML fermentation after the wine is bottled. The ecology, growth, and survival of lactic acid bacteria in wines, along with the changes they cause to wine composition, have been reviewed by Wibowo *et al.* (1985).

Although present in relatively small amounts, citric acid confers a fresh and slightly acid taste to wines. Like malic acid, citric acid is easily converted into other compounds by wine microorganisms. For example, citric acid can be fermented into lactic acid, and some types of lactic bacteria can ferment citric acid into acetic acid. Excessive amounts of acetic acid are never desirable in wine, which is why the citric to acetic acid fermentation can be a serious problem. Minor amounts of succinic acid come from the grape berry but small quantities of this acid are always produced by yeasts during the primary fermentation. The flavour of succinic acid is a complex mixture of sour, salty and bitter tastes, and succinic acid is responsible for the special taste characterising all fermented beverages. Once formed, succinic acid is very stable and it is seldom affected by bacterial action. Lactic acid in wines confers a slightly sourish taste as it does in yogurts. Very little lactic acid comes from the berry, but all wines contain some. During primary fermentation a small amount of lactic (L-(+)-lactic) acid is formed from sugar by yeast, but large amounts (D-(-)-lactic) are formed from malic acid during malo-lactic fermentation. In addition, some lactic bacteria can produce both lactic and acetic acid from sugars (“lactic souring”), glycerol and tartaric acid present in the wine. The right-hand form of lactic acid can be distinguished from the left-hand form in the laboratory very easily, so winemakers have a sensitive way of monitoring bacterial activity in wine simply by measuring the two forms of lactic acid. Contrarily to the acids referred above that are nearly odourless, the high volatility of acetic acid makes wines less appealing to consumers, with unpleasant aroma and palate. Very little acetic acid come from the grapes, but during alcoholic fermentation yeasts form small amounts of acetic acid and some is always formed during malolactic fermentation. Bacteria fermenting citric acid in the wine produce much of this acetic acid. Lactic bacteria often convert residual sugar into acetic acid in stuck fermentations. Vinegar bacteria (*Acetobacter*) can produce large quantities of acetic acid from ethyl alcohol by an oxidation process, in the presence of large quantities of air. The ecology, growth, and metabolic behaviour of acetic acid bacteria on the grape and in musts and wine, along with changes they cause to wine composition has been reviewed by Drysdale and Fleet (1988).

High levels of organic acids and ethanol, together with minute amounts of sugars, make wine an unpropitious environment for most microorganisms; however the yeast species *Zygosaccharomyces bailli* and those of the genus *Dekkera*/*Brettanomyces* are able to grow and survive in the harsh conditions of the wine causing high economic losses. *Z. bailii* is highly resistant to ethanol and can metabolize the acetic acid produced during wine fermentation, either in the presence or absence of residual sugar concentrations (Sousa *et al.* 1996, 1998). Due to these features, it is able to grow at the final stages of wine fermentation and maturation and is considered one of the most dangerous wine spoilage yeasts. In fact, *Z. bailii* is often responsible for causing re-fermentation in bottled wines leading to the formation of deposit and turbidity and hampering commercialisation. Conversely, the yeasts of the genus *Dekkera*/*Brettanomyces* are well known for their capacity to produce obnoxious flavours and odours (Heresztyn 1986; Fugelsang *et al.* 1993). In particular, these species are reportedly involved in the development of phenolic character in red wines. Ethyl-phenols, which may range from a few micrograms to several milligrams per liter, affect wine quality, even in quite small quantities, giving it “animal” phenolic odours or even barnyard and stable smells at higher concentrations (Chatonnet *et al.* 1995). In addition, species of both *Brettanomyces* and *Dekkera* are strongly acidogenic, being able to produce large amounts of acetic acid (Gerós *et al.* 2000a). These yeast species are not among the dominant organisms in grape juice during fermentation probably due to their low growth rate, which makes them unable to compete with *S. cerevisiae*; however they seem to be much better adapted to survive in wine being able to use acetic acid, ethanol or glucose as the only carbon and energy sources (Gerós *et al.* 1999, 2000b; Silva *et al.* 2004).

NITROGEN IN GRAPE BERRY AND WINE

Like every living organism, the vine tree needs nitrogen to grow. Part of this nitrogen is recovered in the berry, the must and also the wine, after being metabolised by yeast and bacteria. Lack of nitrogen can halt growth and development of the vine tree, whereas an excess can increase the vigour, prolong growth, delay maturation, favour the development of mildew and rot and decrease the level of anthocyanins and tannins in the berry. Nitrogen excess is detrimental to sugar accumulation in the berries during ripening (Delgado *et al.* 2004).

In the berry and the must, nitrogen can be found under mineral (NH_4^+, NO_3^-, and NO_2^-) and organic (free amino acids, proteins and other nitrogenated organic compounds such as urea, ethyl carbamate and nucleic acids) forms. Total nitrogen in the must can vary from 100 to 1200 mg/L, and usually red wines possess higher nitrogen content than white wines. This nitrogen, called fermentable nitrogen, is used by yeast to carry on normal alcoholic fermentation of the must. When fermentable nitrogen is below 150-200 mg/L, ammonium (in the form of phosphate, sulphate or sulphite salts) is added to the must to avoid “stuck” fermentations and formation of hydrogen sulfide and other sulphur odours (Kunkee 1991; Jiranek *et al.* 1995).

Mineral nitrogen in the form of NH_4^+ can represent up to 80% of the total nitrogen before *véraison* but it decreases to 5-10% after maturation and decreases even further after fermentation of the must. The content in NO_3^- and NO_2^- can be considered negligible, about 0.5-2 mg/L and 5-40 µg/L, respectively (Blouin and Cruège 2003).

The must contains about thirty amino acids, but only around seven are present in quantities above 100 mg/L: proline, arginine, glutamine, alanine, glutamate, serine and threonine. Amino acids in wines originate from various sources, such as indigenous compounds in grapes metabolised by yeasts during the growth phase, excreted by living yeasts or released by proteolysis during the autolysis of dead yeasts or by enzymatic degradation of grape proteins (Cataldi *et al.* 2003). Total amino acid content can vary widely, from 300 to 5000 mg/L, and represents about 20-50% of the total nitrogen in the must. The amino acid profile of wine can be used to differentiate wines according to vine variety, geographical origin, and year of production (Huang and Ough 1991; Holland *et al.* 1995).

Yeast and bacteria use these amino acids to grow and ferment the must and can release some of these amino acids back into the wine after fermentation; however, wine is generally less rich in amino acids than the initial must it is derived from. The role of amino acids in wine taste was previously considered as being small or non-existent; however, recent work by Dartiguenave and co-workers (2000) showed that they can interfere with the sensation of acidity due to their buffering capacity, and work performed by Torija *et al.* (2003) revealed that ammonium consumption by yeasts results in a greater acidification of the media than amino acids consumption. They can also serve as precursors for the synthesis of aromatic compounds, such as isoamyl acetate, isovaleric acid and isobutyric acid and their ethyl esters, as well fusel alcohols and methionol, all of which are by-products of yeast amino acid metabolism (Ferreira *et al.* 2000).

Amino acids can also be present in polymerised form, such as small oligopeptides of 2-4 amino acids to large proteins of up to 150,000 Da in size. The peptides can be used by bacteria as nutrients and can also have a role in the oxidation of the must: glutathione can react with quinones resulting from the oxidation of phenolic compounds. Oligopeptides can also play a role in the perception of sweetness (Desportes *et al*. 2001).

Proteins are present in widely varying quantities in the must and wines, and furthermore, the determination of the quantity of proteins can vary depending on the methods used. Total protein content of must and wine is increased by nitrogen fertilisation of the vine tree, high fermentation temperatures, and prolonged contact with bacteria and yeasts. Typically, they are present in concentrations ranging from 15 to 230 mg/L (Ferreira *et al*. 2002). Proteins can also contribute to the development of problems in the fermentation of white wines due to exposure to cold, heat or cold-heat temperature variations. These problems are largely absent in red wines due to the precipitation of proteins with tannins (Somers and Ziemelis 1973). Compared to other compounds such as sugars and phenolics, the organoleptic effect of proteins on wine aroma can be considered negligible, but it has been shown that proteins can bind to volatile compounds, increasing the volatility of certain aromatic compounds (such as ethyl octanoate), and reduce it in other cases (such as ethyl hexanoate) (Blouin and Cruège 2003). The protein content is also of economical importance because it greatly affect the clarity (translucency) of the wine (Ferreira *et al*. 2002). Because of this, proteins are usually precipitated from the wine by addition of bentonite. In sparkling wines, a correlation has been found between protein concentration and foam quality (Brissonnet and Maujean 1993).

Other nitrogenated compounds are also present in the wine, such as urea, which originates from arginine and is a precursor of ethyl carbamate (Monteiro *et al*. 1989). This compound is a known carcinogen (Ough 1976), and some countries impose a limit on the amount that can be found in wine. However, the amount of ethyl carbamate present in wine is normally well below the maximum allowed.

Wine also contains biogenic amines, such as histamine, tyramine, putrescine, cadaverine, spermidine and spermine, which are formed mainly during the malolactic fermentation from amino acids present in the must (Vidal-Carou *et al*. 1990; Soufleros *et al*. 1998; Lonvaud-Funel 2001) but can also be present in the berries. High amounts of these biogenic amines can cause problems for some consumers: for instance, histamine can cause headaches, hypertension and digestive problems while tyramine can be associated with migraine and hypertension (Sillas-Santos 1996). Nucleic acids (DNA and RNA) from the berries, yeast and bacteria are also present in minute quantities in the must and wine and can sometimes be used to identify the vine variety, bacteria and yeast used, based on molecular biology techniques.

PHENOLICS IN GRAPE BERRY AND WINE

Grape phenolics

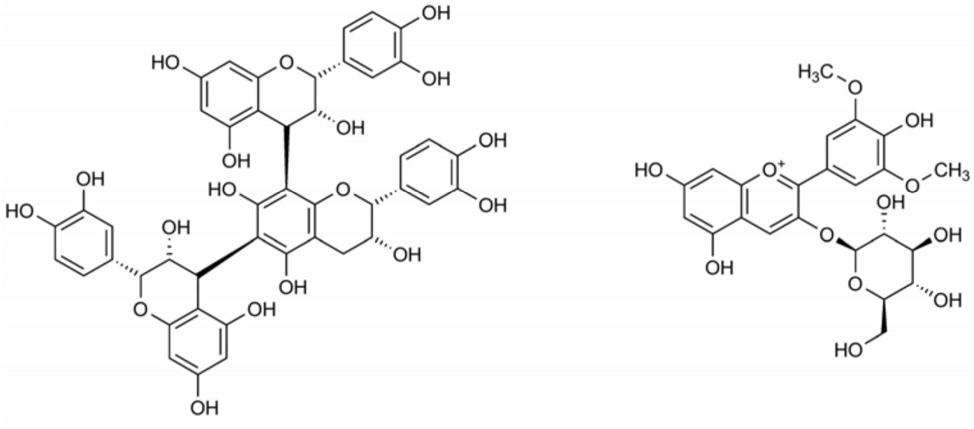
Wine phenolic compounds can be defined as molecules naturally derived from plants or microbes, consisting of a phenyl ring backbone with many substitutive possibilities. Some phenolics like coumaric, caffeic, ferulic and vanillic acids are relatively simple, while others are more complex polymeric structures such as the tannins, which strongly contribute to the mouthfeel, quality and palatability of red wines. Many are involved in plant protection as biologically active growth inhibitors of other living systems. In addition, they contribute to colour and flavour of food items, including wine. Since many have strong antioxidant activities, they also play important beneficial roles in the mammalian systems (reviewed by Weston 2005). Catechins, tannins, and anthocyanins are the most concentrated natural antioxidants present in red grape and wine (Mattivi *et al*. 2002). The understanding of grape and wine phenolics is an increasingly important requirement for managing wine styles efficiently. Given the inquisitiveness of humans and the importance of wine colour, flavour, and astringency, the management of phenolic compounds predates their structural determination. A wide research body on grape and wine phenolics has been published. Two recent reviews summarize the advances on the understanding of phenolic structures and diversity and enlighten the synthesis and distribution of these compounds in grape berry tissue types (Adams 2006; Kennedy *et al*. 2006).

The berry skin contains tannins and pigments, the pulp contains juice but no pigments, and the seeds contain tannins. From a biological perspective, the insoluble cutin of the epidermis and the insoluble lignin of the hard seed coat are phenolics, which are as important as the skin tannins and pigments and seed tannins. Nevertheless, only the soluble phenolics of the grape berry are important in winema-king. Phenolic compounds of the grape are divided between the flavonoids and the non-flavonoids group. Flavonoids make up a significant portion of the phenolic material in grapes and include several classes, such as proanthocyanidins (tannins), anthocyanins and flavan-3-ol monomers (**Fig. 8**). Tannins or proanthocyanidins are polymers of flavan-3-ols and are the most abundant class of soluble polyphenolics in grape berries. Tannins confer astringency to red wines and are extracted from the hypodermal layers of the skin and the soft parenchyma of the seed between the cuticle and the hard seed coat, as well as, from the peduncle of the grape berry. They are a very diverse set of biomolecules varying in size from dimers and trimers up to oligomers with more than 30 subunits (Adams 2006; Kennedy *et al*. 2006).

Anthocyanins are responsible for red wine colour and are co-located with tannins in the thick-walled hypodermal cells of skin. These compounds absorb visible light and are responsible for many of the colours seen in plant tissues, ranging from red to blue. Anthocyanins are anthocyanidins covalently associated with one or more sugar molecules. Free anthocyanins are absent from grapes and wine. Anthocyanins can be esterificated by acids, such as acetic and coumaric acid, and substituted with hydroxyl or methyl groups that will confer a specific hue at light (Blouin and Cruège 2003; Adams 2006). The structures of the common anthocyanins in *V. vinifera* grapes and wine were determined in 1959 (Ribéreau-Gayon 1959). Malvidin-3-*O*-glucoside was found to be the major anthocyanin present along with its acylated forms. The total anthocyanin content in fresh skin of De Chaumac grape was determined to be 2.88 μmol/g, and its concentration into the vacuoles was 97 mM (Moskowitz and Hrazdina 1981).

Flavan-3-ol monomers (catechins) are responsible for bitterness in wine and may also have some associated astringency. The major flavan-3-ol monomers found in grapes and wine include (+)-catechin, (-)-epicatechin, and (-)-epicatechin-3-*O*-gallate (Su and Singleton 1969). The chemical structure of the flavan-3-ols, catechin and epicatechin, compared with the known structures of tannin polymers, suggests a precursor product relationship. It is generally agreed that much of the flavan-3-ol monomers originate from seed material, although they are also present in grape skin hypodermal cells (Adams 2006; Kennedy *et al*. 2006). Catechins, tannins, and anthocyanins are the most concentrated natural antioxidants present in red grape and wine (Mattivi *et al*. 2002).

Nonflavonoid phenolics (with a simple C6 backbone) are found in grapes and wine, but with the exception of hydroxycinnamic acids, they are present at low concentrations (Kennedy *et al*. 2006). Volatile phenolics, such as benzaldehyde (bitter almond taste in wine), phenylacetaldehyde, benzyl alcohol, 2-phenylethanol (rose) and vanilline (vanilla), are found mainly in berry skin and are involved in the primary aromas that developed during ripening (Garcia *et al*. 2003). The hydroxycinnamates, like coutaric acid (**Fig. 8**), are the third most abundant class of soluble phenolics in grape berries, after tannins and anthocyanins (Lima *et al*. 2004). They are present in hypodermal cells along with tannins and anthocyanins and in mesocarp and placental cells of the pulp. Thus, while they are found in

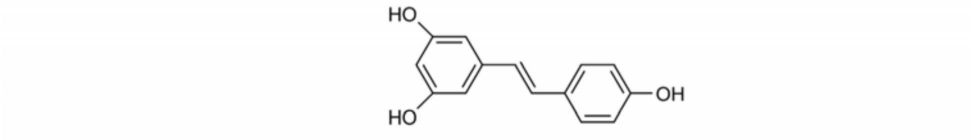


Tannin structure

Anthocyanin structure (malvidin 3-*O*-glucoside)

Flavan-3-ol monomer (catechin)

Hydroxycinnamic acid (coutaric acid)



Resveratrol

Fig. 8 Example of the structure of some phenolic compounds found in grape berries.

red wines, they are usually the most abundant class of phenolics in free-run juice and consequently in white wines (Adams 2006), where they contribute for white wine colour browning under oxydation, in association with non phenolic molecules (Blouin and Cruège 2003; Kennedy *et al.* 2006). In the sub-epidermal tissue of DeChaumac grape two major hydroxycinnamic acid esters, tartrate esters of *p*-coumaric and caffeic acids are found; their concentrations in fresh grape skins being 0.222 and 0.757 µmol/g, respectively (Moskowitz and Hrazdina 1981).

A nonflavonoid compound class that, although present in trace quantities in wine, has been drawing attention are the stillbenes, and more specifically resveratrol (**Fig. 8**). This molecule attracted little interest when it was discovered in 1940, but, after it was postulated to be responsible for some of the cardioprotective effects of wine in 1992, the number of papers testing its effects both *in vitro* and *in vivo* has been increasing exponentially, and it is now linked to many of the beneficial effects of wine (reviewed by Baur and Sinclair 2006). This compound is found in the skin of the grape berry in response to attack from fungi, mainly *Botrytis cinerea* (Ebel 1986), and can be present in its free form or as a glucoside, piceid. Its concentration in red wine can vary from 0.1 to 12 mg/L (Goldberg *et al.* 1994) and, despite being much lower than the concentration of other phenolics present in wine, it is the only known source of dietary resveratrol, thus justifying the attention it has been receiving recently.

Biosynthesis of phenolic compounds in grape berry

Fig. 9 outlines the most common biosynthetic routes of phenolics present in grape. The biosynthesis of soluble phenolic begins with the aromatic amino acid phenylalanine, a product of the shikimate pathway. The early precursors of the shikimate pathway are erythrose-4-phosphate and phosphoenol pyruvate. This pathway is responsible for producing, besides phenylalanine, the other aromatic amino acids tyrosine and tryptophan. Cells that accumulate these soluble phenolics must allocate carbon to the shikimate pathway, in proportion to the total amount of polyphenolics they produce and accumulate. The first enzyme responsible for the phenolic syntesis is PAL (phenyl ammonia lysase), which converts phenylalanine into cinnamic acid. This compound undergoes a series of transformations resulting in the formation of precursors of several simple phenolics, like phenolic acids, lignin precursors, etc. The incorporation of 3 molecules of malonyl-CoA, produced via the acetate pathway, with the 4-coumaroyl-CoA starts the phenylpropanoid pathway (Dias 2003). These precursors generate complex phenolic compounds, like the flavonoids or the stilbenes, depending on the intervening enzyme, chalcone synthase (CHS) or the stilbene synthase (SS), respectively. Skin anthocyanins, which show little turnover once formed, appear to behave like typical end products. The other phenolic classes exhibit patterns of accumulation and subsequent decline during ripening, suggesting their degradation and utilization for the biosynthesis of other compounds, or the covalent as-

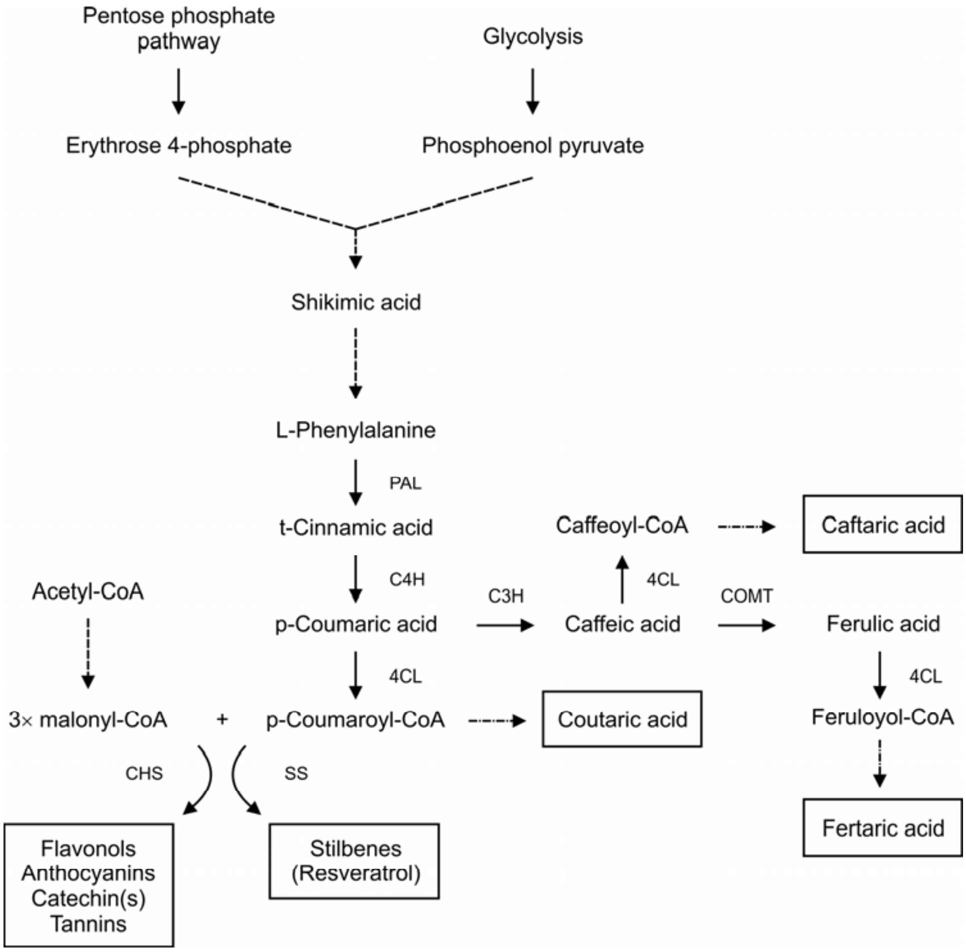


Fig. 9 Biosynthetic pathway of the major soluble phenolic classes found in grape berries. Compounds or classes that accumulate in the fruit are outlined by rectangles. Other classes are present at very low levels (adapted from Dias 2003 and Adams 2006). PAL, phenylalanine ammonia lyase; C4H, cinnamate-4-hydroxylase; C3H, coumarate-3-hydroxylase; 4CL, 4-hydroxycinnamate:CoA ligase; COMT, caffeic acid *O*-methyltransferase; SS, stilbene synthase; CHS, chalcone synthase. Full lines represent direct enzymatic conversion, dashed lines represent omitted intermediates and dashed and dotted lines represent a yet unknown pathway.

sociation with other cellular compounds. In all cases, much more is known about the anabolism than the catabolism of polyphenolics during berry development and ripening. Skin tannins, which are synthesized very early in berry development, change very little on a per berry basis from *véraison* to harvest, although their concentration declines along berry growth. Qualitative changes, such as the increase of the polymerization degree, can also take place from *véraison* to harvest (Kennedy *et al.* 2001). Generally, there is a decline in seed tannins during ripening that accompanies seed brow-ning, possibly due to tannin oxidation (Adams 2006).

Acquisition of the red/blue colour of red varieties along ripening is a visual indicator of the biochemical processes that occur in grape berries. This reflects more precisely the accumulation of anthocyanin pigments in the vacuoles of skin cells, absent in the white grape varieties. Studies of pigmentation mutants have resulted in the characterization of some genes involved in anthocyanin synthesis. There are two phases for anthocyanin pathway gene expression in grape berry skins during development. At early stages, most of the genes are expressed and then repressed during the lag phase of development. After *véraison*, a coordinate induction of all the genes of this pathway coincides with the accumulation of anthocyanins in the skin (Boss *et al.* 1996; Boss and Davies 2001).

On a per berry basis, total hydroxycinnamates in mesocarp tissues peak prior to *véraison* and then decline, leading to a constant amount (per berry) as the fruit ripens. In ripe fruit, the most abundant hydroxycinnamates are caftaric and coutaric acids (Romeyer *et al.* 1983). Singlenton *et al.* (1986) showed that the level of hydroxycinnamates in the juice of different *vinifera* varieties is highly variable, ranging from 16 to 430 mg/L.

Phenolic ripeness and sugar ripeness

Many factors influence the chemical composition of grapes and, consequently, the quality of wines, including maturity stage, genetic makeup, climatic conditions and management practices. To make a high quality wine, grapes must contain an adequate amount of sugars and acids, but also balanced levels of phenols (Jackson and Lombard 1993; Jones and Davies 2000; Musingo *et al.* 2005). As sugar concentration is an indicator of berry maturity ("sugar ripeness"), changes in tannins content are used by the growers to control harvest, and this process is called "phenolic ripeness". In addition to being a major parameter which determines the ethanol content of the wine, "sugar ripeness" may also affect "phenolic ripeness", because the expression of several enzymes of the phenylpropanoid pathway may be controlled by sugars. Sugar regulates the expression of chalcone synthase genes of petunia (Tsukaya *et al.* 1991) and of camellia (Takeuchi *et al.* 1994). In addition, it has been shown that sugars enhance anthocyanins production in grape suspension cells (Larronde *et al.* 1998; Vitrac *et al.* 2000). The synergic effect of sucrose and ABA on polyphenols and anthocyanins accumulation in grape was also demonstrated, despite the possible contradiction that exists in the literature (Pirie and Mullins 1976; Hiratsuka *et al.* 2001). "Phenolic ripeness" allows the selection of flavoured grapes and thus avoids the elaboration of unpleasant unripe tastes in the wine. In the same way, the total colour in the berries is a measurement of quality, so any means of increasing the amount of anthocyanins accumulated in the berries would be an advantage to both the grape grower and the winemaker. Viticultural practices have been developed to try and increase the amount of colour in berries (Boss and Davies 2001). However, to be able to establish a defined and common phenolic maturity is a matter of some subjectivity, since each vine has its own flavour, and diverse factors can influence the polyphenol berry content at harvest. Increased sunlight and moderate fertility support vine growth and cluster formation and lead to an increased formation of secondary plant products, including phenolics such as quercetin, which is an excellent indicator of sunlight available to the development of grape clusters. Ample, but not excessive moisture, as well as adequate, but not excessive fertility, will also result in higher concentrations of phenolics and tannins in red wine grapes (reviewed by Weston 2005). As referred to in the previous section, nitrogen, present in the soil as nitrite and nitrate, affects polyophenols content and, consequently, the wine taste and flavour (Delgado *et al.* 2004). Disease pressures can result in reduced berry quality, leading to reduced phenolic content over time in harvested grapes. For instance, the enzyme laccase produced by *B. cinerea* can degrade complex polyphenols and thus modify wine flavour and colour (Slomczynski *et al.* 1995). Esca disease results in significant changes in the phenolic composition of grapevine leaves (Lima *et al.* 2006). As a whole, although the biochemical networks responsible for phenolics production in grapes are reasonably well understood, the knowledge of their regulation and coordination in different varieties, tissues, and environments remains a challenge for the future.

Phenolics in wine

Today, much research is focused on how phenolic compounds are structurally modified during the wine extraction, fermentation and aging, changing their sensory properties (reviewed by Weston 2005; Kennedy *et al.* 2006). Since the greatest concentration of phenolics occurs in the seeds and stems, if one could destem and even remove seeds, one could minimize the concentration of complex green tannins and astringency, which results from excessive extraction of these plant parts. Since it is difficult to remove seeds, destemming is generally practiced when one desires a fruitier wine with less complex astringency. By macerating before fermentation, one can extract additional colour and tannin from the pomace. Fermentation conditions, in particular the temperature, and the choice of the yeast strain will confer different chemical and flavour characteristics to wine. As previously mentioned, the presence of yeast and bacteria or other fungal organisms in wine directly impacts the wine quality and flavour and influences the metabolism of phenolics in wine. The influence of oak on aroma and flavour of wine has also been a matter of interest. Among the 250 species of the genus *Quercus*, two European species, *Q. petraea* (sessile oak) and *Q. robur* (pedunculate oak) and one American species, *Q. alba* (white oak) have been used in the past and continue to be widely employed in barrel-making (Chatonnet and Dubourdieu 1998). In french oak forests, sessile and pedunculate oaks usually stand side by side. Research into the composition of the wood has shown that sessile oaks are richer in aromatic substances such as vanillin and methyl octalactone (characterized by a distinct odour of coconut, celery, and fresh wood; Masson *et al.* 2000), whereas pedunculate oaks primarily contain phenolic compounds such as ellagitannins or catechol tannins. American oak contains fewer tannins than French oak, but more aromatic compounds, particularly methyl octalactone. The quantity of extractable methyl octalactones in American white oak is sometimes excessive and would be likely to have a negative influence on the wine's aroma. The differences in the composition of American white oak and European sessile oak indicate that wines are likely to develop differently according to the origin of the barrels used for aging. European sessile oak and American white oak are perfectly suitable for aging fine wine, whereas the low aromatic potential and, in particular, the high ellagitannins content of pedunculate oak indicate that it should only be used for aging brandies (Chatonnet and Dubourdieu 1998). Wine character is also influenced by the age and preparation of the oak. For instance, proper control and toasting operations in barrel-making could facilitate the use of American white oak, by modelling the release of volatile and aromatic substances from the wood (Chatonnet and Dubourdieu 1998). Smaller barrels result in more phenolic and tannin content, due to the increased surface exposure area and extraction.

Aging of red and white wines results in altered and reduced phenolic contents in the wine product. Anthocyanin-tannin complexes can be formed stabilizing the colour of red wines and resulting in wines that taste less fruity and less astringent after aging (Scudamore-Smith *et al.* 1990). Oxidation reactions involving phenolics might also change the chemical and sensory profile of wines (reviewed by Waterhouse and Laurie 2006). Oxygen in the air is always ready to react with unprotected juice or wine and many of the substances present are adversely affected by oxidation, producing unpleasant, bitter, off-odours and off-tastes. However, it is recognized that some degree of oxygenation may be beneficial for red wine development, but the quality of white wines is generally impaired by excessive air exposure. As tannins and anthocyanins interact with oxygen, which diffuses during barrel storage, these compounds further polymerize and become less astringent. Red wines become lighter in colour, and proanthocyanidins and other polyphenolics eventually aggregate in larger molecules which accumulate as sediment over time at the base of the bottle. In contrast, white wines often deepen in colour, turning darker honey colours as they oxidize and age. There is also some degree of continuous oxygen exposure during wine ageing in the bottle. A range of alternative closure techniques, which might vary in such aspects as degree of market acceptability, oxygen permeation rate, ability to carry out "flavour scalping" and their propensity to contribute "taints" of varying character are now available and used by the industry (reviewed by Jones *et al.* 2004). The widespread existence of cork taint is now universally acknowledged, however its prevalence is a matter of intense debate. Cork taint is mainly due to the presence of 2,4,6-trichloroanisole (TCA) in the wine. The unpleasant, musty off-flavour can render an otherwise excellent wine completely useless and may cause economic losses (Buser *et al.* 1982). Roll-on tamper evident screw cap closures (ROTE) have recently become a popular alternative for Australian and New Zealand white wines. The performance of different closures on the composition and sensory properties of bottled Semillon wine was recently evaluated (Godden *et al.* 2001). Wine under the screw cap retained the highest concentration of SO_2 and ascorbic acid and showed the lowest degree of browning together with low levels of oxidised aroma characters, suggesting that ROTE closures are less permeable to oxygen than natural cork. However, ROTE closure produced a rubber-like flavour/aroma in wine after 18 months, probably as a consequence of the lack of oxygen. The choice of the ideal closure technique is a controversial topic in the modern wine industry and a truthful debate is being damaged by commercial interests. High temperature during wine ageing affects negatively its sensory properties and visual stability. Besides the effect of temperature on the formation of ethyl carbamate (accelerating the reaction between urea and ethanol) (Tegmo-Larsson and Spittler 1990), exposure of wine to heat increases the loss of individual and total anthocyanins, reducing colour density (Dallas and Laureano 1994). As mentioned in the section "Potassium in grape berry and wine", wine pH also influences red wine colour. Only a small proportion of the anthocyanins in red wine are in the red flavylium form at wine pH and this equilibrium is modified upon the addition of sulphur dioxide (reviewed by Kennedy *et al.* 2006).

ODORIFEROUS COMPOUNDS IN GRAPE BERRY AND WINE – VARIETAL AROMAS

Given the importance of the aroma on the quality of the wine it has been object of numerous studies and the chemical basis and diversity of grape and wine odoriferous compounds has been reviewed in detail (Bayonove *et al.* 1998; Oliveira 2000; Ribéreau-Gayon *et al.* 2000; Mateo and Jiménez 2000; Blouin and Cruège 2003; Swiegers *et al.* 2005). Wine aromas are made up of several hundreds of volatile compounds, at concentrations ranging from several mg/l to a few ng/l, or even less. The complexity of wine aromas, which makes them particularly difficult to study, is due to

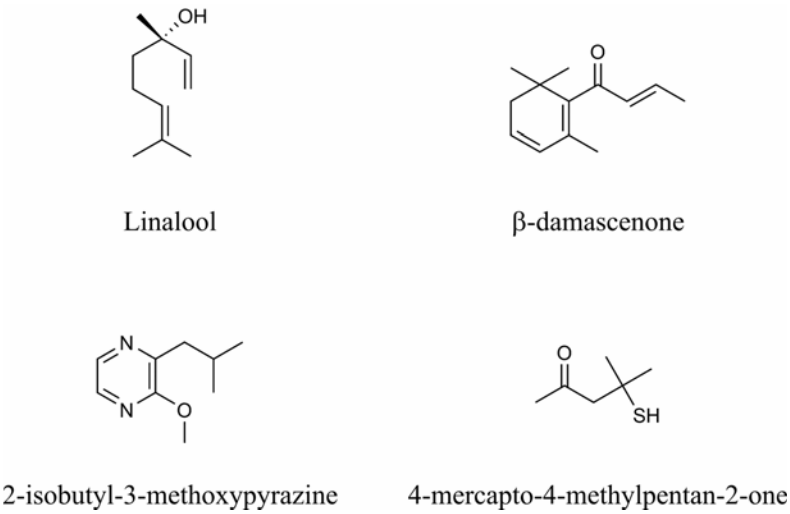


Fig. 10 Example of the structure of some important aroma compounds found in grape berries and wine.

the diversity of the mechanisms involved in their development: i) grape metabolism, depending on the variety, as well as climate, soil and vineyard management techniques; ii) biochemical phenomena (oxidation and hydrolysis) occurring prior to fermentation, triggered during extraction of the juice and maceration; iii) the fermentation metabolisms of the microorganisms responsible for alcoholic and malolactic fermentations, and iv) chemical or enzymatic reactions occurring after fermentation, during aging of the wine in vat, barrel and bottle. The volatile metabolites produced by yeast fermentation are derived from sugar and amino acid metabolism and comprise esters, higher alcohols, carbonyls, volatile fatty acids and sulfur compounds, giving wine its vinous character. As referred above, the many odoriferous compounds released into barrel-aged wine by the oak also have an impact on aroma. However, odoriferous compounds from grapes (reflecting the particular grape variety) play a more decisive role in the quality and regional character of wines than any other aroma component. These include mainly monoterpenes, C_{13}-norisoprenoids, methoxypyrazines, and sulfur compounds, and are responsible for the varietal aroma of wines. Six-carbon alcohols, derived from grape lipids during vintage processing, may also act as varietal markers (Oliveira *et al.* 2006).

The odoriferous compounds in *V. vinifera* which have been studied in the greatest detail belong to the terpene family. Numerous studies have shown that the terpenoid compounds form the basis of the sensory expression of the wine bouquet, and they can be used to differentiate grape varieties (Oliveira *et al.* 2004). These compounds are responsible for the characteristic aroma in Muscat grapes and wines, although they are also present (at low concentrations) in simple-flavoured varieties. Both free forms and odourless precursors, mainly glycosylated, have been identified in grapes and wines. Terpene compounds belong to secondary plant constituents, whose biosynthesis begins with acetyl-coenzyme A (CoA), and are located in the berry skin. At present, about 50 monoterpene compounds are known. The dominating monoterpene alcohols, particularly from Muscat varieties, are linalool (**Fig. 10**), geraniol, nerol, citronellol, α-terpineol and hotrienol. A general classification of grape varieties based on monoterpene concentration allows division into (1) intensely-flavoured muscats, in which total free monoterpene concentrations can be as high as 6 mg/L; (2) non-muscat but aromatic varieties with total monoterpene concentration of 1-4 mg/L, including Traminer, Huxel and Riesling varieties; and (3) more neutral varieties not dependent upon monoterpenes for their flavour, including numerous cultivars such as Cabernet Sauvignon, Sauvignon Blanc, Merlot, Shiraz and Chardonnay. In these cultivars monoterpenes are at such low concentration, generally below the perception threshold, that these compounds can only play a minimal role in determining varietal flavour. In contrast, Loureiro variety may be classified among aromatic ones as linalool is always above its perception threshold in grapes (Oliveira *et al.* 2000).

There are three categories of monoterpenes with some interrelationships between them. On the top of the complex are the free aroma compounds, commonly dominated by linalool, geraniol, and nerol, together with the pyran and furan forms of the linalool oxides. However, depending on how juice has been treated and on other factors, which may include climate, many additional monoterpenes can be found in this group, i.e. citronellol, α-terpineol, hotrienol, nerol oxide, myrcenol, the ocimenols plus several other oxides, aldehydes and hydrocarbons. In wines, several monoterpene ethyl ethers and acetate esters have also been found among free aroma compounds. The most odoriferous of the monoterpene alcohols are citronellol and linalool. Furthermore, olfactory impact of terpene compounds is synergistic. They play a major role in the aromas of grapes and wines from the Muscat family, as concentrations are often well above the olfactory perception thresholds (**Table 1**). The second category of monoterpenes consists of the free odourless polyols (diols and tiols). A most significant feature of the polyols is that some of them are reactive and can break down easily to give pleasant and potent volatiles, i.e. diendiol (3,7-dimethylocta-1,5-diene-3,7-diol) which can give hotrienol and nerol oxide. These monoterpene polyols are present in grapes at concentrations up to 1 mg/L.

Table 1 Characteristics of the main monoterpenols and examples of concentrations in wines made from different varieties (adapted from Ribéreau-Gayon *et al.* 2000).

Monoterpene	**Olfactory description**	**Olfactory perception threshold (µg/L)**	**Content (µg/l) in wine**						
			Muscat of Alexandria	**Muscat of Frontignan**	**Gewürz traminer**	**Albariño**	**Riesling**	**Muscadelle**	**Sauvignon Blanc**
Linalool	Rose	50	455	473	6	80	40	50	17
α-Terpineol	Lily of the Valley	400	78	87	3	37	25	12	9
Citronellol	Citronella	18	ND	ND	12	ND	4	3	2
Nerol	Rose	400	94	135	43	97	23	4	5
Geraniol	Rose	130	506	327	218	58	35	16	5
Hotrienol	Linden	110	ND	ND	ND	127	25	ND	ND

ND – not detected

The third category of monoterpenes, the glycosidically conjugated forms, also make no direct contribution to the aroma of the grape. Glycosides are, in most cases, more abundant than the unglycosilated forms of individual monoterpenes and polyols. The glycon moiety is composed by glucose or by a disaccharide constituted by glucose and rhamnose or arabinose or apiose. In flavoured grapes, glycoside precursors are evaluated between 6.5 and 28 mg/L juice.

Terpene glycosides can be hydrolysed in acidic medium or by enzymes; under the former conditions, rearrangements of the monoterpenol may occur, whereas under the latter conditions the changes in the natural monoterpenol distribution are minimal. Nevertheless, even in diminutive extent, acid hydrolysis occurs always in the wine pH conditions (≈3.0). Enzyme hydrolysis has been used to enrich flavour by release of free aromatic compounds from natural glycoside precursors producing a more "natural" flavour in the wine. The application of exogenous enzyme activities (β-glucosidase, α-rhamnosidase, α-arabinosidase and β-apiosidase) bring out the aromas of young wines made from "Muscat-flavoured" grape varieties. The enzymatic mechanism occurs in two successive steps: firstly, an α-rhamnosidase, an α-arabinosidase or β-apiosidase hydrolyse the corresponding sugars and then an β-glucosidase liberates the odoriferous aglycon. But glycosidase preparations have less effect on simple-flavoured grape varieties. In addition, the potential use β-glucosidase activity of grapes to liberate terpenes from grape juices or wines has been studied. In the same way, alcoholic fermentation has little effect on the hydrolysis of glycosylated potential of grapes, mainly due to enzymatic activity inhibition by glucose. However, yeast species of the genus *Hansenula* isolated from the fermenting must were reported to have an inducible β-glucosidase activity. The activity of this enzyme was also reported in different *Saccharomyces* strains, whose activity is inhibited by ethanol but not by glucose.

The oxidative degradation of carotenoids, terpenes with 40 carbons (tetraterpenos) produces derivatives with 9, 10, 11 or 13 carbon atoms (Enzell 1985). Among these compounds, norisoprenoid derivatives with 13 carbon atoms (C_{13}-norisoprenoids) have interesting odoriferous properties. Megastigmane forms (benzene circle substituted on carbons 1, 5 and 6, and an unsaturated aliphatic chain with four carbon atoms attached to C6) of these norisoprenoid derivatives include compounds like β-damascenone (**Fig. 10**), with a complex smell of flowers, tropical fruit and stewed apple, and β-ionone, with a characteristic aroma of violets, probably present in all grape varieties.

The role of methoxypyrazines in the herbaceous aroma of certain grape varieties, such as Cabernet Sauvignon, is now well established. These compounds exist in a free state in grapes and no precursor forms have been identified. Methoxypyrazines are nitrogenated heterocycles produced by the metabolism of amino acids. The compounds 2-isopropyl-3-methoxypyrazine, 2-*sec*-butyl-3-methoxypyrazine and 2-isobutyl-3-methoxypyrazine (**Fig. 10**), have odours reminiscent of green pepper and asparagus, or even earthy overtones. These highly odoriferous compounds have extremely low perception thresholds in water, on the order of one nanogram per liter, and have been identified in many grape varieties and their wines; however, their concentrations are only significant above the recognition threshold in Sauvignon Blanc, Cabernet Sauvignon (Bayonove *et al.* 1975) and Cabernet Franc grapes and wines, and sometimes in Merlot. This herbaceous aroma, is usually most apparent when the grapes are unripe.

More recently, some highly odoriferous sulfur compounds with thiol functions have been shown to participate in the aromas of certain grape varieties, especially Sauvignon Blanc. These compounds occur in grapes in *S*-cysteine conjugate form. The first molecule found to be a characteristic component of the aroma of Sauvignon Blanc wines was 4-mercapto-4-methyl-pentan-2-one (**Fig. 10**) (Darriet *et al.* 1993). This molecule is extremely odoriferous with a marked smell of boxwood and broom and concentrations may reach 40 ng/l in Sauvignon Blanc wines with typical varietal character. It is now well established that some sulfur compounds are present in must in *S*-cysteine conjugate form in much larger quantities than the aromas they generate in wine. The corresponding aromas are revealed during alcoholic fermentation, probably due to the action of a specific enzyme. These aromas vary in intensity depending on the *S. cerevisiae* yeast strain used for fermentation. Therefore, fermentation brings out the primary aroma hidden in the fruit. The characteristic aroma of grape varieties such as Sauvignon Blanc, and other grape varieties with relatively simple aromas, appears during alcoholic fermentation.

Free and bonded forms of terpenols accumulate in ripening grapes from the colour change onwards. Some authors report a continuous accumulation of monoterpenes, even in overripe grapes, while others share the more widespread opinion that the free monoterpenes start to decrease before maximum sugar level is reached. Vineyard conditions during ripening, such as temperature and water supply, may influence aroma development during ripening. C_{13}-norisoprenoid derivatives develop according to similar pattern. The carotenoid concentration decreases following colour change that correlates with increased concentrations of C_{13}-norisoprenoids, mainly in glycosylated forms. Exposure of the grapes to sunlight during ripening accelerates carotenoid breakdown (Razungles *et al.* 1998). It has been demonstrated that the excessive hydrocarbon smells (produced by the non-megastigmane C_{13}-norisoprenoid derivative TDN, 1,1,6-trimethyl-1,2-dihydronaphtalene) that sometimes develop as Riesling wines age are related to extremely high temperatures, especially during the grape ripening periods. Therefore, although hot climates are favourable for the accumulation of sugar, they are not necessarily best in terms of wine quality. The concentrations of methoxypyrazines, which are highest in unripe grapes, gradually decrease during ripening. Increasing the grape's exposure to the sun during ripening has a positive effect on the reduction of methoxypyrazines (Heymann *et al.* 1986); low temperatures have an opposite effect (Lacey *et al.* 1991) and soil has a decisive influence. Grapes grown in well-drained, gravel soils have the lowest concentrations of methoxypyrazines.

CONCLUSIONS AND PERSPECTIVES

Grape berries function as a sophisticated biochemical factory. They import and accumulate water, minerals, sugar, amino acids, organic acids, and synthesize flavour and aroma compounds. Many scientific advances have been achieved in understanding physiological, biochemical, and molecular aspects of grape berry maturation. Some have led to the improvement of wine quality through better grape-growing practices; but this area of basic research is still wide open. For instance, although the biochemical networks responsible for polyphenols biosynthesis in grapes are reasonably well understood, how the networks are regulated and coordinated in different varieties, tissues and environments remains problematic. These secondary metabolites are of major importance for wine quality influencing wine colour, taste, odour and resistance to pathogens. Sensory analysis is of paramount importance to understand the numerous variables that interact to build up the sensory perception of wine and may, ultimately, influence production decisions. The complex biochemical machinery of the fermenting yeast turns grape sugar into alcohol and modifies wine style. The improvement of the quality of certain wines achieved by reducing acidity, or the development of a low-alcohol beverage, are just some of the possibilities resulting from the construction of genetically modified yeasts. However, the general usage of genetically modified organisms in human diet remains a theme of controversy. Wine industry is nowadays a global activity tailored to meet consumer expectations and the French paradox has alerted consumers to the potential beneficial effects of moderate wine consumption on health and life span. Perspectives raised by health benefit associated with the non-alcoholic components of wines may, however, endanger its diversity. Growers and viticulturists may be tempted to produce standard "healthy" wine, favouring for instance red and polyphenol-rich wines, instead of white and polyphenol-poor wines. During the past decades, the wine industry was deeply restructured by replacing outdated equipment and modernizing wine-making methods. Viticultural techniques were improved and the exchange of information increased considerably among wineries and winemakers around the world. The phenomenon of wine globalization led to the production of high quality standardized wines all over the world. In this context, a challenge for the future consists in educating consumers to resist the current tendency of homogenizing wine styles (The New York Times, "For the next big thing look to Portugal"; January 2006): «[These wines] did not try to imitate flavors and styles that are popular elsewhere. They are not what marketers call "fruit forward", essentially one step removed from soft drinks. (…) They were decidedly Old World in style, with tannins, mineral flavors and good acidity, excellent with food but not wines for people milling around in a banquet hall. (…) What makes these wines so distinctive? Look no further than the grapes: not a cabernet, merlot or syrah among them.» Another challenge is also to adapt the wine production to the consumer's taste in environmental conditions that are changing (global warming) and to be increasingly cautious about phytochemical treatments.

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