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Evolution of Polyphenols during Vinification and Wine Storage

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ABSTRACT

Beginning with the 'French Paradox' observations in the 1990's much interest has been devoted to the profile and contents of plant secondary metabolites, not only of grapes and wines, but also of numerous other plant foods, due to their assumed health-beneficial properties. Grapes belong to the most important fruit crops, and some 80% of this crop is processed into red and white wines. Anthocyanins are the most important phenolic compounds of grapes imparting color to red wines. Together with non-colored phenolic co-pigments they are further characterized by their diverse bioactive attributes, which still need unequivocal proof in most cases. For these reasons, comprehensive efforts have been undertaken to optimize vinification technology in order to maximize extraction rates of polyphenols from the grapes. The present review provides a survey of process strategies applied to achieve this aim, also taking into consideration innovative technologies for the production of both red and white wines. Furthermore, novel findings with regard to the evolution of phenolic compounds in the course of wine aging and storage and to the potential of winemaking by-products as a source for the recovery of phenolic compounds are considered.

Keywords: aging, anthocyanin, extraction, grape, grapevine, maceration

Abbreviations: **ABTS**, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate); **DMPD**, *N*,*N*-dimethyl-*p*-phenylenediamine; **DNA**, deoxyribonucleic acid; **DPPH**, 2,2-diphenyl-1-picrylhydrazyl; **FRAP**, ferric reducing antioxidant power; **ORAC**, oxygen radical absorbance capacity

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INTRODUCTION

Polyphenols are ubiquitously found in the plant kingdom as plant secondary metabolites and, thus, are an integral part of the human diet (Bravo 1998). They exert numerous functions in plants, e.g. they may act as attractants to pollinators and seed dispersers by producing appealing colors as in the case of anthocyanins, but they may also function as antifeedants (Delgado-Vargas *et al.* 2000; Stintzing and Carle 2004). Furthermore, phenolic compounds may serve as light screens against damaging radiation, which can also be seen from the fact that their biosynthesis is upregulated upon light exposure, especially UV-B rays (Pan *et al.* 2009). This also explains why most phenolic compounds are mainly found in external and aerial tissues. Polyphenols might also function as transport vectors for monosaccharides or as osmoregulators during periods of drought and low temperatures (Chalker-Scott 1999). From a plant physiological viewpoint the antioxidant activity, which is common to all phenolic compounds, is probably more important since they are assumed to increase the plant response to oxidative damage in order to maintain the regular physiological status in tissues affected by biotic or abiotic stress factors. Thus, plant phenolics also directly and indirectly act against infections and aggression by microorganisms and as protection against herbivorous insects and mammals as feeding deterrents (Robards and Antolovich 1997; Harborne and Williams 2000; Aherne and O'Brien 2002).

Due to their localization in external parts of the plant material food preparation techniques such as peeling, skinning and trimming may reduce total phenolic contents of processed foods. The preparation of liquid foods from fruits and vegetables, as in the case of winemaking, is also associated with significant losses of phenolic compounds due to poor extraction yields from the skins and peels. In processed foods, flavonoid amounts can, thus, be significantly lower compared to fresh products as a consequence of thermal treatment, leaching effects or incomplete extraction.

Received: 5 March, 2009. Accepted: 27 June, 2009.

Invited Review

However, due to some adverse effects of phenolic compounds on product quality, this has not always been considered a drawback. Among other things, polyphenols may cause discoloration of plant foods as a result of enzymatic browning reactions (Friedman 1996; Robards *et al.* 1999). Fermentation products such as acetaldehyde or further compounds such as glyoxylic acid and furfural were shown to induce the polymerization of flavanols and anthocyanins, thus, affecting color during processing and aging of fruit-derived foods (Es-Safi *et al.* 2003). Depending on their structural features and concentrations polyphenols are also responsible for astringency and bitterness (Llaudy *et al.* 2004; Mateus *et al.* 2004). Phenolic compounds also contribute to sediment formation by polyphenol-protein interactions resulting in undesirable hazes in products such as wine, beer and fruit juices (Siebert 1999). Since proteins are effectively precipitated by highly polymerized tannins, by-products of plant food processing containing high amounts of phenolics, e.g. grape pomace, are considered inferior when used as a feed because of their negative effect on protein digestibility (Robards and Antolovich 1997; Bravo 1998). Furthermore, polyphenols were shown to negatively affect mineral absorption due to complexation reactions (Bravo 1998).

More than 15 years ago, the MONICA project, a worldwide monitoring system for cardiovascular diseases organized by the World Health Organization, led to the term 'French Paradox', an apparent discrepancy of a high fat diet with a low incidence of coronary atherosclerosis. These findings were attributed to the regular consumption of red wine (Renaud and De Lorgeril 1992; Frankel *et al.* 1993) and have led to a significant change in the evaluation of phenolic compounds, because in search for the active principles of wine, much interest has been paid to polyphenols, and wine is considered an important contributor of antioxidant activity to the human diet, even though *in vitro* antioxidant potency does not necessarily prove *in vivo* biological activity (Seeram *et al.* 2008). Beginning with these observations, research interest has focused on plant phenolics, since numerous epidemiological studies revealed an inverse correlation between the dietary uptake of phenolic compounds and risk of certain diseases, such as coronary heart diseases, stroke and certain forms of cancer (e.g. Gupta *et al.* 2008; Liu *et al.* 2008), which has mainly been attributed to their antioxidant activity and radical scavenging capacity (Conde *et al.* 2007), even though studies on absorption, metabolism and *in vivo* effects of polyphenolics and their metabolites are still scarce. Beyond these properties numerous other effects have been revealed in more recent studies which may explain the putative health-beneficial properties of phenolic compounds, such as an impact on signalling pathways regulating cellular growth (Kern *et al.* 2005, 2006; Sparwel *et al.* 2009) or the enhanced synthesis of detoxifying enzymes of phase II metabolism (Veeriah *et al.* 2006). Furthermore, the protection of DNA by polyphenols from oxidative stress may play a role in disease prevention (Schaefer *et al.* 2006a, 2006b). However, the complex profile of phenolic compounds in plant extracts and the limited availability of reference compounds hampers studies on the bioavailability and physiological and nutritional effects of individual compounds. Problems also arise from the fact that many experiments have been performed with animals and that extrapolation to humans is not straightforward.

Despite these difficulties in unravelling the mechanisms of health-beneficial effects *in vivo* plant food rich in polyphenols and the dietary uptake of high amounts of phenolic components are generally considered helpful for disease prevention and desirable in terms of a healthy diet. Therefore, regular consumption of plant food has also been propagated by health authorities, such as the '5 a day campaign' (Kammerer *et al.* 2005a), which advises the consumption of three portions of vegetables (400 g) and two portions of fruit (250 g) per day. For this reason, food producers aim at delivering products high in antioxidant activity either by optimizing process conditions or by exploiting novel plant materials with desirable profiles and contents of plant secondary metabolites, which can be seen e.g. from the discussion of 'superfruits', a marketing term, which has been introduced by the food and beverage industry.

Grapes are an important crop both with regard to their complex polyphenolic profile and their market share of all fruit crops. Most of the grapes are used for winemaking, thus, much interest has been devoted to optimizing polyphenol recovery during vinification yielding wines with high amounts of antioxidant compounds. This review aims at providing an overview of the techniques and processes in the course of red and white winemaking and storage and their impact on polyphenols, also taking into consideration novel technologies, which have been developed in recent years.

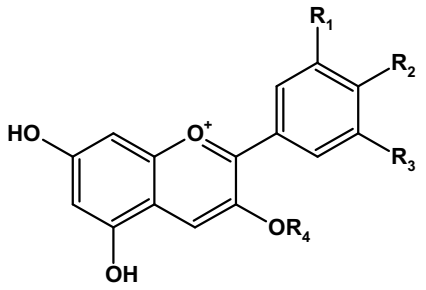
Impact of grape phenolic profile and contents on wine quality

The phenolic contents and color of red and white wines mainly depend on the grape variety, because grapes of different varieties are known to significantly differ both in the profile and contents of phenolic compounds. Comprehensive data are available on anthocyanins, phenolic acids, stilbenes and non-anthocyanin flavonoids of grapes, and their fingerprints have been used for regional and cultivar-related differentiation (e.g. Mazza 1995; Goldberg *et al.* 1996; Carreno *et al.* 1997; Soleas *et al.* 1997; Goldberg *et al.* 1998; Otteneder *et al.* 2004). Thus, the phenolic profiles of wines have also been used for grape cultivar identification (Nikfardjam *et al.* 2007). Basic structures of the most important phenolic compounds found in grapes and grape-derived products and by-products are illustrated in **Fig. 1**, revealing a highly complex polyphenolic profile.

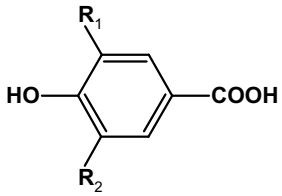
Furthermore, vine pruning and training system were also shown to affect polyphenol contents of grapes. Cluster thinning has a marked impact on setting and veraison and also affects the phenolic composition of the grapes and their respective wines. Thus, depending on the grape variety, it is possible to increase anthocyanin contents up to 70% applying particular training systems (Gonzáles-Neves *et al.* 2002; Pérez-Lamela *et al.* 2007). Additionally, the phytosanitary conditions of the grapes and their maturity as well as cultivation conditions have been shown to affect the phenolic contents (Kennedy *et al.* 2002; Cortell and Kennedy 2006; Nikfardjam *et al.* 2006; Pastor del Rio and Kennedy 2006; Cortell *et al.* 2007a). This is of particular interest, since the color and phenolic contents of wines are correlated with the polyphenol contents of the grapes they are originating from (González-Neves *et al.* 2004; Cortell *et al.* 2007b). As an example, **Fig. 2** illustrates the differences in anthocyanin contents in the skins of different grape pomace samples of different cultivars and vintages, which do not only depend on the vinification techniques but also mainly on the characteristics of the respective grape cultivars.

Due to the high impact of phenolic compounds on wine quality and characteristics, such as color, astringency and bitterness, much effort has been devoted to the optimization of polyphenol extraction using different vinification techniques. Among the factors influencing polyphenol extraction, fermentation temperature and must or grape freezing play a predominant role, even though the latter method puts additional costs to the technology and, thus, is not commonly applied. In contrast, thermovinification is often performed as an alternative to skin maceration. This technology allows to damage hypodermal cell membranes and to denature polyphenol oxidase, which enables enhanced release of phenolic compounds and prevents browning. Furthermore, carbonic maceration, the fermentation of whole berries or clusters under a CO_2 atmosphere, prefermentation juice runoff (saignée), the application of pectinolytic enzymes, pumping-over and punching-down of the cap, which is developed as CO_2 causes the grape solids to rise to the top of the fermentation vessel, the maceration time and

Anthocyanins

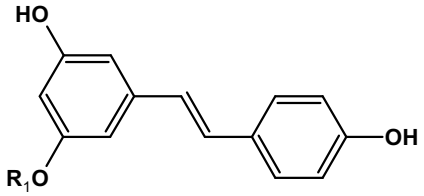


Compound	R_1	R_2	R_3	R_4
Cyanidin 3-*O*-glucoside	OH	OH	H	glc
Cyanidin 3-*O*-*p*-coumaroylglucoside	OH	OH	H	*p*-coumaroyl-glc
Delphinidin 3-*O*-glucoside	OH	OH	OH	glc
Delphinidin 3-*O*-acetylglucoside	OH	OH	OH	acetyl-glc
Malvidin 3-*O*-glucoside	OCH_3	OH	OCH_3	glc
Malvidin 3-*O*-acetylglucoside	OCH_3	OH	OCH_3	acetyl-glc
Malvidin 3-*O*-*p*-coumaroylglucoside	OCH_3	OH	OCH_3	*p*-coumaroyl-glc
Peonidin 3-*O*-glucoside	OCH_3	OH	H	glc
Peonidin 3-*O*-acetylglucoside	OCH_3	OH	H	acetyl-glc
Peonidin 3-*O*-*p*-coumaroylglucoside	OCH_3	OH	H	*p*-coumaroyl-glc
Petunidin 3-*O*-glucoside	OCH_3	OH	OH	glc
Petunidin 3-*O*-acetylglucoside	OCH_3	OH	OH	acetyl-glc
Petunidin 3-*O*-*p*-coumaroylglucoside	OCH_3	OH	OH	*p*-coumaroyl-glc



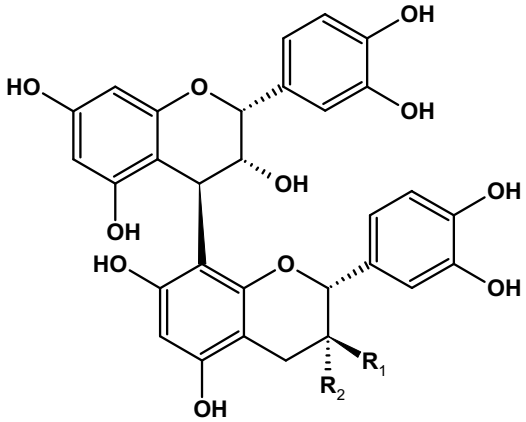
Hydroxybenzoic acids

Compound	R_1	R_2
Gallic acid	OH	OH
p-Hydroxybenzoic acid	H	H
Protocatechuic acid	OH	H
Syringic acid	OCH_3	OCH_3



Stilbenes

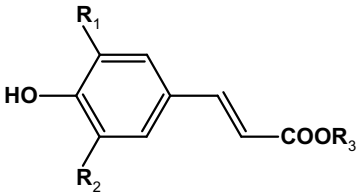
Compound	R_1
trans-Polydatin	glc
trans-Resveratrol	H



Dimeric procyanidins

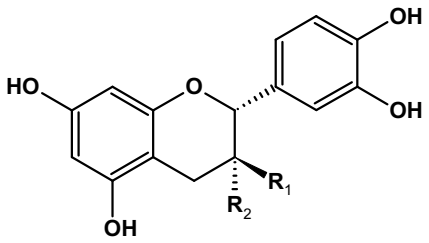
Compound	R_1	R_2
Procyanidin B1	OH	H
Procyanidin B2	H	OH

Abbreviations: gal, galactose; glc, glucose; glcA, glucuronic acid; rham, rhamnose



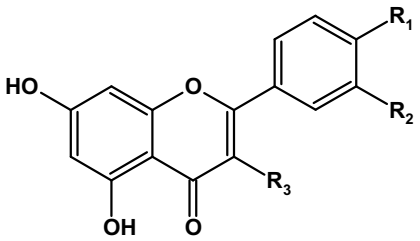
Hydroxycinnamic acids

Compound	R_1	R_2	R_3
Caffeic acid	OH	H	H
Caftaric acid	OH	H	tartaric acid
p-Coumaric acid	H	H	H
Coutaric acid	H	H	tartaric acid
Fertaric acid	OCH_3	H	tartaric acid
Ferulic acid	OCH_3	H	H
Sinapic acid	OCH_3	OCH_3	H



Monomeric flavan 3-ols

Compound	R_1	R_2
Catechin	OH	H
Epicatechin	H	OH
Epicatechingallat	H	O-gallic acid



Flavonols

Compound	R_1	R_2	R_3
Isorhamnetin 3-O-glucoside	OH	OCH_3	glc
Kaempferol	OH	H	H
Kaempferol 3-O-glucoside	OH	H	glc
Quercetin	OH	OH	H
Quercetin 3-O-galactoside	OH	OH	gal
Quercetin 3-O-glucoside	OH	OH	glc
Quercetin 3-O-glucuronide	OH	OH	glcA
Quercetin 3-O-rhamnoside	OH	OH	rham

Fig. 1 Structures of important phenolic compounds in grapes and grape-derived products and by-products. Cited from **Kammerer DR, Schieber A, Carle R** (2005) Characterization and recovery of phenolic compounds from grape pomace – A review. *Journal of Applied Botany and Food Quality* **79**, 189-196.

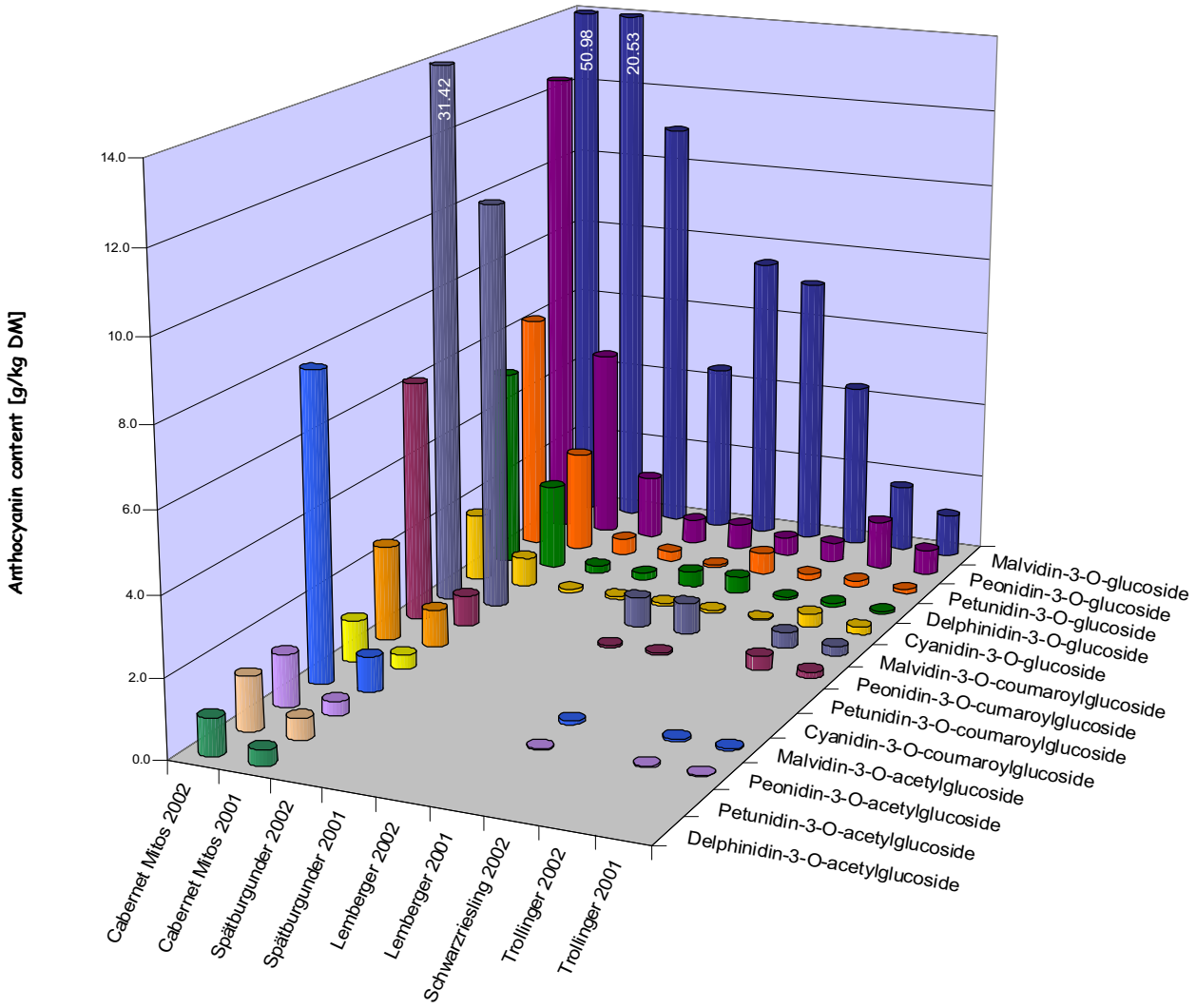


Fig. 2 Contents of individual anthocyanins in the skins of grape pomace samples of different cultivars and vintages. Data according to Kammerer *et al.* (2004).

yeast selection have been described to potentially affect anthocyanin and polyphenol extraction from the grapes (Sacchi *et al.* 2005).

Maceration techniques and their influence on the phenolic profile and antioxidant properties of wines

The type of maceration, the steeping of grape skins and seeds in the must allowing the extraction of grape components into the liquid phase, is known to be a key step during vinification determining the phenolic profile and contents and, thus, the antioxidant potential of the resulting musts and wines. Prolonged skin contact during mashing was shown to considerably enhance mass transfer and polyphenol extraction into the musts of white grapes, which goes along with an increase in the antioxidative capacity of the wines as evaluated by the inhibition of human LDL oxidation as compared to wines produced by immediate pressing of the grapes after crushing (Hurtado *et al.* 1997). In the course of fermentation of red grape mash individual phenolic compounds were shown to be released into the musts at different times, which both reflects their extractability and localization within different parts of the grape. Most hydroxycinnamates are released at an early stage of fermentation, prior to ethanol formation, such as caftaric and coutaric acids, followed by skin anthocyanins and flavonol glycosides. In contrast, the contents of flavan 3-ols and gallic acid from the seeds steadily increased with fermentation time, especially in the presence of ethanol (Nemanic *et al.* 2002; Zou *et al.* 2002). Expectedly, maceration times do not only affect polyphenol yields but also sensory characteristics of the resulting wines, which are closely interrelated (Budic-Leto *et al.* 2008). Wines prepared by skin maceration for several days have been shown to exhibit higher concentrations of antioxidant polyphenolics as compared to wines produced by thermovinification, which is often applied due to limited capacity of tanks and containers needed for fermentation (Talcott and Lee 2002).

Generally, the cell wall is the major barrier to be overcome in the course of polyphenol extraction during winemaking. For this reason, researchers have tried to correlate the differences in cell wall composition between different varieties with anthocyanin and polyphenol extractability. As an example, 'Monastrell' grapes proved to be more difficult with regard to anthocyanin extraction. Major differences between the cell wall composition of different varieties as deduced from multiple regression analyses were found in the pectin and cellulose contents, but differences in the arabinoxylan, arabinogalactan and xyloglucan fractions might also be of importance. However, it must also be kept in mind that the thickness or density of the cell walls might affect pigment extraction as well (Ortega-Regules *et al.* 2006).

Prior to grape crushing and maceration stems are mostly removed. The decision whether to destem the grapes or to ferment the mash consisting of skins, seeds and stems, has a pronounced effect on the phenolic contents of the resulting wines. Grape stems contain significant amounts of polyphenols. Their removal before grape crushing prevents exces-

sive uptake of polyphenols, which is undesired, since stem phenolics contribute to bitter and astringent tastes (Benítez *et al.* 2005). Accordingly, red wines, which were made from non-destemmed grapes, exhibited higher levels of phenolic compounds (Sun *et al.* 2001). In contrast, the wines obtained from ´Palomino fino´ grapes with different degrees of destemming did not significantly differ in their polyphenolic contents (Benítez *et al.* 2005). Thus, when evaluating the effects of destemming on polyphenol release during maceration, the grape cultivar needs to be taken into consideration.

A systematic comparison of 'Monastrell' wines produced by applying maceration times of 4, 5 and 10 days, respectively, clearly revealed that long maceration times resulted in wines with higher anthocyanin and polymeric phenolic contents, greater color density and an overall improved color quality. However, it has also been stated that not all grape varieties, e.g. 'Muscadine' grapes, are suitable for extended skin fermentation, because of greater astringency upon longer skin contact. Furthermore, SO_2 addition at the moment of grape crushing enhanced the transfer of polyphenols from the skins to the must. Together with storage temperatures below 20°C, early SO_2 addition and long maceration times produced wines with highest anthocyanin contents and color retention (Gómez-Plaza *et al.* 2001, 2002). However, it has also been reported that, in contrast to the release of non-colored phenolic compounds, the amounts of which steadily increase with the length of maceration, anthocyanin contents were lowered after a few days of maceration (Budic-Leto *et al.* 2003).

The grape temperature during crushing and maceration also significantly affects the quantitative composition and evolution of phenolic compounds, since the temperature strongly influences the rate of polyphenol extraction from grape skins during the first days and also has an impact on the reactions occurring during the very first moments of vinification, such as enzymatic oxidation and polymerization reactions. Upon further steps of vinification temperature effects become less predominant, since the fermentation process itself raises the temperature of the must, and differences are then less pronounced as vinification progresses (Gil-Muñoz *et al.* 1999).

Cold maceration techniques have now often been evaluated as a further tool to improve polyphenol extraction, not only of red but also of white grapes, and to produce high-quality wines suitable for aging, and such a process was shown to increase anthocyanin concentrations and color intensity (Nemanic *et al.* 2002). These general trends were also observed in a study evaluating the cold maceration of 'Sangiovese' grapes at temperatures ranging from -5 to +5°C by adding either liquid nitrogen or solid CO_2. With CO_2 the improvement of wine quality was directly proportional to the decrease in temperature, whereas for liquid nitrogen differences between maceration at +5 and 0°C were insignificant (Parenti *et al.* 2004). Thus, this effect is not only dependent on temperature. Some contradictory results concerning the effects of cold maceration have been reported in the literature. It has been generally found that the improvement in quality, which is attributed to cold maceration, varies with different grapes and vintages and that the choice of cold maceration temperature and duration is of utmost importance with regard to sensory and chemical characteristics of the resulting wines allowing to obtain different qualitative results (Parenti *et al.* 2004). When dry ice is added to the grape mash, freezing causes lysis of the grape skin cells, which is caused by the increase of the volume of the intracellular liquids, thus disrupting the cell membranes. This may serve as an explanation for the enhanced release of anthocyanins and non-colored phenolic compounds as well as of aromatic volatiles, which significantly affects must and wine quality (Álvarez *et al.* 2006). However, it still needs to be assessed whether the improvement in wine quality will justify the additional costs caused by cold maceration.

Carbonic maceration is an alternative to traditional winemaking techniques and is mainly applied to obtain very young, fruity red wines. For this purpose, whole grapes are fermented in a CO_2-rich environment prior to crushing. Carbon dioxide permeates through the skins and stimulates alcoholic fermentation in the intact grape berries. Significant differences in the color and phenolic composition of wines obtained by carbonic maceration and by traditional skin contact fermentation are known to differ, which is mainly due to the anaerobic fermentation in the case of carbonic maceration. When compared to mash fermentation processes with or without stem contact, the wines resulting from carbonic maceration were characterized by lower color intensities and lower phenolic contents (Spranger *et al.* 2004). Similar results were obtained in another study comparing carbonic maceration with traditional skin contact fermentation of 'Syrah' grapes. From the former process red wines with lower anthocyanidin monoglucoside contents and lower amounts of total phenolics were obtained. These exhibited higher lightness values, and the color was less saturated, whereas hue angle values were comparable to the traditionally produced wines (Gómez-Míguez and Heredia 2004). In contrast to these reports, carbonic maceration was also shown to enhance extraction of some particular compounds, namely catechins and oligomeric and polymeric procyanidins (Sun *et al.* 2001).

The release of polyphenols during maceration may further be enhanced by using horizontal or vertical rototanks or macerators, which allow to automatically homogenize the grape mashes throughout maceration, thus improving the transfer of phenolic compounds into the must (Budic-Leto *et al.* 2005), or of other sophisticated maceration containers making use of CO_2 formed during fermentation to effectively agitate the mash and, thus, to improve the extraction of solid grape parts (Garde-Cerdán *et al.* 2008a), since otherwise the contact between juice and the skins and seeds is significantly reduced. The effects of different techniques on polyphenol release have recently been reviewed (Sacchi *et al.* 2005).

The steady increase of phenolic contents in the course of maceration goes along with an increase of the antioxidant capacity of the musts and wines, which has been evaluated using *in vitro* test systems, such as the DPPH, ABTS, DMPD, FRAP or ORAC assays, by electron spin resonance spectroscopy, by a chemiluminescence assay and by methods evaluating biomarkers of oxidative stress, such as lipid peroxidation inhibition and inhibition of damage to DNA, respectively, also trying to correlate antioxidative capacity with the contents of individual phenolic compounds (Brenna and Pagliarini 2001; Burns *et al.* 2001; Fernández-Pachón *et al.* 2004; Girotti *et al.* 2006; Lachman *et al.* 2007; Rivero-Pérez *et al.* 2007; Noguer *et al.* 2008).

Technical enzyme preparations used for enhancing polyphenol release in the course of vinification

Pectinolytic enzyme preparations are commonly applied in the winemaking industry for several reasons. Inter alia, they allow a faster start of fermentation, an increase in must yield and easier pressing, and an improved clarification of the wines. One of the most important reasons for the application of pectinases is the enhanced release of polyphenols from the solid parts of the grapes, which may result in higher color densities, anthocyanin and total phenolic contents when applied to red grape mashes. Thus, pectinolytic enzymes are commonly used for improving red wine color (Pardo *et al.* 1999), which also enhances visual color intensity (Guadalupe *et al.* 2007). However, treatment with pectinases is also performed in white winemaking processes, mainly to improve clarification and stabilization. As demonstrated for red winemaking, white grape mashes treated with cell wall degrading enzymes were also shown to produce wines with significantly higher polyphenol amounts (Pérez-Magarino and González-San José 2001). Obviously, enzymatic cell wall hydrolysis does not negatively affect

polyphenol and anthocyanin stability, which might be expected from the loss of colloidal polyphenol stabilization by hydrocolloids, since studies with mannoprotein addition to red wines did not reveal neither any effect on anthocyanin and non-colored polyphenol contents nor on color (Guadalupe and Ayestarán 2008). However, there are also reports in the literature showing that pectinolytic enzymes do not necessarily improve anthocyanin extraction or that they may even cause decreased pigment levels (Sacchi *et al.* 2005). This may be due to glycosidase side activities of technical enzyme preparations, which may cause hydrolysis of anthocyanins (Kammerer *et al.* 2005b).

Effects of pressing parameters on grape juice composition

The composition of grape musts and wines also depends on the type of press and the pressures applied to the mash. The press type may have an impact, because different systems may differ in the way and the homogeneity the pressure is exerted on the mash. A systematic comparison of different pressures revealed the free run juices to contain highest concentrations of glutathione, while they were low in polyphenolic contents, thus, also exhibiting a low browning potential. Moderate pressures yielded juices with high concentrations of proteins and polyphenol oxidase activity, and polyphenol oxidase activity in the course of vinification was shown to be highest immediately after pressing (Wissemann and Lee 1980). Maximum polyphenol concentrations were found in juices from high pressure pressing (Valero *et al.* 1989; Yokotsuka 1990). This might also be partially attributed to shorter pressing times, which go along with a reduced exposure to oxygen. In particular those phenolic compounds, which are easily oxidized by grape polyphenol oxidases, such as hydroxycinnamic acids, have been studied in detail with regard to their transfer into the must as a function of pressing pressures due to their contribution to the browning potential of white and red wines. In a study on the composition of 'Sauvignon blanc' musts the duration of skin contact and the level of pressure during pressing were shown to be important factors determining the composition of the grape musts (Maggu *et al.* 2007). Longer skin contact times resulted in a greater release of varietal aroma compounds into the must. At the same time and with increasing pressures exerted on the mashes this enhanced aroma release was offset by an increase of the oxidative potential, as could be deduced from a decline in the glutathione content and an increase of easily oxidizable phenolic compounds, which favor browning reactions and lead to a loss of varietal aroma components (Maggu *et al.* 2007). The localization of phenolic compounds within the grape plays an important role with regard to the transfer into the musts during pressing (Barroso *et al.* 1987).

The application of a decanter centrifuge after pressing has been demonstrated in the case of white wines to decrease the need for fining agents and to increase the overall quality of the wines by reducing the browning quality (Foster and Cox 1984).

Alcoholic and malolactic fermentation and their effects on the evolution of phenolic compounds

Yeasts may significantly affect the polyphenol contents and color of wine due to surface adsorption (Vasserot *et al.* 1997, 2007), the formation of metabolites such as pyruvic acid and acetaldehyde that further react with phenolic compounds, or yeast glycosidase activities. Such differences have been demonstrated with several *Saccharomyces* strains producing wines, which significantly differed in their color intensity, total phenolic and monomeric anthocyanin contents and antioxidant capacity, which was also reflected by the wine composition after aging (Caridi *et al.* 2002, 2004; Brandolini *et al.* 2007; Bautista-Ortín *et al.* 2007; Sidari *et al.* 2007; Romano *et al.* 2008). More detailed studies were also performed on the effects of yeast on the evolution of individual phenolic compounds. As an example, yeasts were shown to significantly affect the amounts of *cis*- and *trans*-resveratrol and the respective glucoside isomers in wines. Differences may be due to adsorption onto the yeast surface and hydrolysis catalyzed by yeast glucosidases (Poussier *et al.* 2003; Clare *et al.* 2005). Different potential of various *Saccharomyces* strains to adsorb phenolic compounds was also shown for anthocyanins and tannins (Sidari *et al.* 2007). More detailed investigations into the adsorption of anthocyanins by yeast cell walls revealed pronounced differences of individual pigments with regard to their potential to be bound by the yeasts. Acetylated and coumaroylated anthocyanins were more prone to adsorption than their non-acylated counterparts (Vasserot *et al.* 1997, 2007). Among the non-acylated monoglucosides malvidin 3-glucoside was most adsorbed, indicating that more hydrophobic compounds are preferably bound. However, significant differences were also found in the adsorption capacity of different yeast strains with some of them adsorbing more than twice the amounts as compared to other strains (Morata *et al.* 2003). In some studies, the impact of yeasts on the evolution of anthocyanins and non-colored phenolic compounds of wines appeared of minor relevance (Nikfardjam and Pickering 2008). The effects of yeasts on the polyphenolic profile always need to be associated with the presence of oxygen. A certain amount of oxygen is needed for optimal yeast growth and lipid and sterol synthesis by the yeasts. When oxygen is abundant, reactive oxygen species (ROS) are produced. Thus, yeasts also play a dominant role in wine aging, since they can consume oxygen for at least three years if the wines are not clarified or lees are added back to the wine. The release of ROS by yeast cells may favor the oxidation of wine phenolics. On the other hand, yeasts may compete with polyphenols for oxygen, thus, hindering microoxygenation, however, this interrelation between yeasts, oxygen and wine aging has not been studied so far (Salmon 2006).

Whenever yeast assimilable nitrogen contents are low, diammonium phosphate is commonly added to grape musts to avoid the risk of slow and stuck fermentation. Such a supplementation was shown to affect wine color and phenolic profile as well. Whereas no difference was observed for polymeric anthocyanins and tannins as compared to control samples, diammonium phosphate supplementation caused a significant increase in malvidin 3-glucoside levels of the respective wines, which was possibly due to higher ethanol contents in the supplemented samples enhancing anthocyanin extraction or protecting the pigments against degradation reactions. This went along with higher color intensities and total color differences, which were even perceivable by the human eye (Ugliano *et al.* 2008).

One step of traditional enological practice is wine aging on yeast lees, which is applied for the production of some white wines and which has gained increasing popularity in recent years for the aging of red wines. Lees consist of dead or residual yeasts which are deposited at the bottom of wine vats. Normally, the lees are removed in the course of vinification by transferring the wine to another container, a process which is known as racking. In a study on the interaction of yeast lees and red wine polyphenols adsorption of phenolic compounds onto the yeast surface followed a biphasic kinetics. The first phase (~0.2 h) was characterized by a rapid binding of phenolic compounds followed by a slow, constant adsorption, which reached its saturation after about one week, where about one third of the total free anthocyanins were bound to the yeast surface. This went along with a noticeable decrease of absorbance at 420 and 520 nm. Significant differences between the relative adsorption rates of individual pigments were not observed (Mazauric and Salmon 2005).

Malolactic fermentation, the conversion of malic acid into lactic acid, which is usually performed after alcoholic fermentation, is an additional step during vinification reducing wine acidity and providing additional flavors for the wines through metabolic reactions, mainly of *Oenococcus*

oeni. Expectedly, polyphenol amounts of the wines are affected by the fermentation conditions, such as aeration and temperature (Reguant *et al.* 2005). More detailed comparisons of different lactic acid bacteria revealed differences in the malolactic reactions with regard to the wine composition, with wild populations showing a greater impact on the contents of phenolic compounds as compared to selected monocultures. Malolactic fermentation caused the formation of novel compounds, which were not detected in the wines prior to this fermentation step, such as *trans*-ferulic acid and several flavanols. Furthermore, the contents of catechin, epicatechin, *trans*-resveratrol, tyrosol and tryptophol, which were already present after alcoholic fermentation, significantly increased. Fermentation with wild lactic acid bacteria strains caused significant hydrolysis of the hydroxycinnamoyl derivatives *trans*-caftaric and *trans*-coutaric acids releasing free *trans*-caffeic and *trans*-coumaric acids, whereas other strains showed little or no effect on these compounds. Accordingly, the contents of the myricetin and quercetin aglycones were increased after spontaneous malolactic fermentation, which is also probably due to hydrolytic activities (Hernández *et al.* 2007). A significant decrease in caftaric and coutaric acid contents upon malolactic fermentation and the concomitant increase in caffeic and coumaric acid levels was also observed in another study concluding that an alteration of the phenolic profile due to fermentative processes will also have an impact on color evolution and stability (Hernández *et al.* 2006). Accordingly, *trans*- and *cis*-piceid levels of wines showed a significant decrease, which went along with an increase of the *trans*- and *cis*-resveratrol amounts. This observation was attributed to *ß*-glucosidase activities of microorganisms involved in malolactic fermentation, especially *Oenococcus oeni* (Yunoki *et al.* 2001; Poussier *et al.* 2003).

The fining of wines – impact of process parameters on wine composition

Wine clarification using fining agents is performed to produce clear, bright wines devoid of suspended or colloidal compounds. Clarification processes go along with a reduction of phenolic contents, especially of compounds involved in unwanted oxidation reactions and particularly contributing to excessive astringency, however, they should have only little or no effect on essential aromatic and flavor compounds of wine. Thus, fining aims at improving the organoleptic characteristics of wine. By lowering polyphenol contents, fining may also be applied to minimize browning reactions, especially of white wines, during vinification and storage. Polyvinylpolypyrrolidone (PVPP) was more effective than casein and gelatin regarding the color of wines (Puig-Deu *et al.* 1996; Vrhovsek and Wendelin 1998; Puig-Deu *et al.* 1999; Marti *et al.* 2001). However, decreased polyphenolic contents do not always go along with a lower susceptibility towards browning (López *et al.* 2001). In addition to the aforementioned fining agents egg albumin, microcrystalline cellulose, activated charcoal and bentonite may also be used. Wines produced from 'Vinhão' grapes and fined with the aforementioned agents did not show any significant change of the hues as compared to unfined wines. However, the color density and anthocyanin concentrations in fined wines were lower, especially when PVPP was used for clarification (Castillo-Sánchez *et al.* 2008). In contrast to these findings, investigations into the effects of winemaking techniques on the color of 'Monastrell' wines revealed best color characteristics when low-temperature maceration wines were clarified with PVPP as compared to a bentonite/gelatin fining (Gómez-Plaza *et al.* 2000). Such contrasting results illustrate the complex interdependencies of various vinification treatments and also show that these processes may not be assessed separately and that different varieties and wines may behave differently.

Oenological gelatins are also mainly used for clarification and stabilization in order to reduce the turbidity and to decrease the astringency of musts and wines, which may be caused by certain phenolic compounds. At the pH value of wines, oenological gelatins are positively charged, thus interacting with the negatively charged colloids, such as tannins. When a critical size is reached, the colloid complex precipitates. Five gelatins differing in their net charge density and molecular weight distribution were compared with regard to their effects on wine composition. They showed different sedimentation behavior. The net charge density seemed to be correlated to the minimum active dosage producing an appreciable flocculation. The treatments with these gelatins significantly reduced turbidity, total polyphenol contents, color intensity and the amount of brown polymers. The extent to which these parameters were modified, depended on the type of gelatin used for clarification (Versari *et al.* 1998). More detailed studies on the clarification of different wines with a commercial gelatin and two fractions derived from it, which differed in their molecular weights, revealed a different precipitation potential of gelatin, depending on the evaluated wine. Gelatin did not precipitate low-molecular phenolics but was selective for highly polymerized and galloylated tannins. The gelatin preparation with a molecular weight of 16,000 Da precipitated more polymerized tannins as compared to a gelatin with a molecular weight of 190,000 Da, thus, providing the opportunity to selectively remove specific high-molecular phenolic compounds by choosing the appropriate fining agent (Maury *et al.* 2001). In contrast, PVPP binds and precipitates also low-molecular phenolics.

The haze-forming potential of wines may also be lowered by bringing it into contact with macromolecular hydrophilic compounds capable of forming hydrogen bonds with phenolic compounds, thus removing those components, which may otherwise cause protein-polyphenol precipitates during storage (Katzke *et al.* 2008) or by micro- or ultrafiltration (Goncalves and Norberta de Pinho 2003). However, the latter method is also known to significantly reduce pigment amounts, even when cut-off sizes of > 10.000 Da are chosen. It is important to note that bovine spongiform encephalopathy caused a situation of crisis leading the public and winemakers to loose their confidence in the use of gelatins and animal proteins in general for wine fining. Studies have therefore been performed to search substitutes for gelatins and egg proteins. In this context, plant proteins were successfully tested, namely showing that fining using plant proteins such as glutens did not alter the color of red and white wines (Marchal *et al.* 2002a, 2002b, 2003).

Co-pigmentation phenomena and their role in the vinification process

Much interest has been devoted to the color of red wines and ways to stabilize the color by technological means. Co-pigmentation is one important effect contributing to pigment and color stabilization in wines. Co-pigments such as caffeic acid and ferulic acid are effective in stabilizing anthocyanins, whereas phenolics such as epicatechin and catechin are weak co-pigments. Since there are grape varieties which are rich in phenolic co-pigments effectively stabilizing wine anthocyanins, whereas other cultivars are lacking these stabilizing principles, the co-winemaking, which means co-maceration and co-fermentation of different grape varieties, might be a helpful tool for obtaining color-stable red wines. The co-winemaking of 'Monastrell' grapes together with 'Cabernet Sauvignon' and 'Merlot' grapes was assessed in terms of phenolic content, pigment stabilization and color. The resulting wines showed hyperchromic effects at 530 and 620 nm. Furthermore, the addition of 'Cabernet Sauvignon' or 'Merlot' grapes during vinification increased the total phenolic contents and, thus, favored co-pigmentation and stabilized anthocyanins, which gave rise to enhanced formation of polymerized pigments, which are characterized by higher color stability as compared to monomeric anthocyanins (Lorenzo *et al.* 2005). Even white grapes may be added during the production of red wines. However, in a study on the addition of white

grapes to red grape mash only minor differences with regard to the phenolic profile and color evolution were observed (Etaio *et al.* 2008).

Co-winemaking may not be mixed up with blending or "coupage". Wines are usually blended to improve color, taste, alcohol content, body and aroma, thus, to enhance product quality. Expectedly, the polyphenolic profile of blended wines is depending on the wines used for blending, which may significantly differ in their phenolic profile and contents. This also modifies the reactions occurring during wine aging, which depends on the initial phenolic profile (Monagas *et al*. 2006a).

In another approach, isolated co-pigments, namely caffeic acid, rutin, catechin and tannins from white grape skins and seeds, were added to musts obtained from 'Tempranillo' grapes prior to fermentation. Again, co-pigment supplementation favored co-pigmentation reactions, thus, producing wines characterized by more intense colors, higher anthocyanin retention, superior contribution of anthocyanins to the color of the wine and improved sensory characteristics with regard to astringency (Álvarez *et al.* 2009). In contrast, rutin enhanced co-pigmentation when it was added prior to fermentation of 'Tempranillo' or 'Cabernet Sauvignon' mashes, whereas hydroxycinnamic acids showed even converse results (Schwarz *et al.* 2005).

Wine aging and microoxygenation as a means to stabilize wines

Wine aging is an important process in the course of winemaking significantly affecting the aroma and phenolic profile, because compounds genuinely found in grapes may be degraded or transformed into novel products. As an example, numerous aldehydes and ketones are formed from alcohols in wine, which may further react with both anthocyanins and non-anthocyanin phenolic compounds (Waterhouse and Laurie 2006). This is most apparent for anthocyanins, showing a progressive loss, which is mainly due to their participation in numerous chemical reactions, also causing color change from the bright red of young red wines to brick red hues of aged wines. These reactions have been shown to be highly complex and to produce a wide range of novel anthocyanin derivatives, which are stable under the conditions prevailing in wine, thus imparting a range of red colors to wine, which are retained for up to several decades. Among these derivatives pyranoanthocyanins, such as the vitisins, carboxypyranoanthocyanins and pyranoanthocyanin-phenol pigments, and reaction products between anthocyanins and flavanols mediated by aldehydes, such as anthocyanin-alkyl-flavanol-pigments, pyranoanthocyanin-flavanol pigments and vinylpyranoanthocyanin-flavanol pigments, as well as direct condensation products between anthocyanins and flavanols have been characterized (Brouillard and George 1997; Hayasaka and Kennedy 2003; Schwarz *et al.* 2004, 2003; de Freitas and Mateus 2006; Rentzsch *et al.* 2007a, 2007b). However, wine aging may also be monitored by measuring the concentrations of further phenolic compounds, such as gallic acid, catechin, *p*-coumaric acid and *trans*-resveratrol (Brenna *et al.* 2005). When red wines were stored in bottles under non-oxidative conditions, the decrease of monomeric anthocyanins showed a first-order kinetics, revealing up to 66% loss of initial total monomeric anthocyanins. Interestingly, degradation rates were significantly different depending on the grape cultivar. Acylated anthocyanins revealed higher losses as compared to their non-acylated counterparts, which is possibly due to their hydrolysis releasing non-acylated anthocyanins. Compounds with different anthocyanidin backbones did not show significant differences in their aging behavior. Pyranoanthocyanins formed upon reaction of genuine grape anthocyanins with further wine constituents, such as anthocyanin-pyruvic acid adducts showed similar or lower disappearance rates than the respective precursors in the first months of aging, whereas anthocyanin-vinylphenol and anthocyanin-vinylflavanol adducts did not show any significant variation in their contents during the whole 26 month storage period (Monagas *et al.* 2005a, 2006b). In contrast, the non-anthocyanin phenolics of these wines differed in their evolution patterns during aging. As an example, *trans*-caftaric and coutaric acids showed a significant decrease in their contents with a concomitant increase in *trans*-caffeic and *p*-coumaric acids. Flavanol contents also exhibited a major decrease, being greater for dimeric procyanidins as compared to the monomeric compounds. These changes are also attributed to the formation of novel colored and uncolored oligomeric and polymeric compounds, which in turn is responsible for a change of the sensory properties of the wines (Monagas *et al.* 2005b).

The extent of aging and oxidation reactions of wines stored in bottles also depends on the type of closures. Wines sealed with screwcaps exhibited a lower drop of SO_2 contents as compared to cork closures, which went along with lower browning of the wines as a result of decreased browning reactions (Brajkovich *et al.* 2005). The fact that oxygen availability plays a predominant role in wine aging becomes evident from a study performed with wines stored in both glass and polyethylenterephthalate (PET) bottles with and without an oxygen scavenger. Expectedly, degradation and oxidation rates were highest in wines stored in PET bottles due to the high oxygen permeation rate of this material, whereas an oxygen scavenger in the PET material significantly reduced oxidation reactions, which could be deduced e.g. from a less pronounced decline of the antioxidant potential of wines stored in the latter material (Giovanelli and Brenna 2007).

Oxygen content during aging is the predominant factor determining sensorial characteristics of the wines, because it significantly affects the profile and contents of phenolic and aromatic compounds and, thus, also color, astringency and aroma. The changes during aging are mainly reflected by oxidation, condensation and polymerization reactions, forming novel pigments and polymerized compounds. Furthermore, acetaldehyde, which is formed from ethanol upon oxidation, serves as a bridging agent between phenols, among others between anthocyanins and non-colored phenolics. Microoxygenation, which is increasingly applied in the wine industry since the nineties, implies the continuous and controlled addition of small amounts of oxygen during wine aging, bringing about high-quality wines. When microoxygenation was performed prior to malolactic fermentation, a general decrease in total phenolic contents, but also the stabilization of wine color and better retention of color intensity were observed. Therefore, a significant color loss, characteristic of non-oxygenated wines after malolactic fermentation, could be avoided (Pérez-Magarino *et al.* 2007). Depending on storage time and oxygen supply the color of red wines changes significantly, and the concentrations of anthocyanin derivatives present in grapes, such as pyranoanthocyanins, ethyl-briged compounds and products resulting from cycloaddition reactions between anthocyanins and flavanols, which are mediated by acetaldehyde, increased (Atanasova *et al.* 2002). Preliminary one-dimensional ^{1}H NMR experiments with oxygenated wines also revealed an increase in the amount of oxygenated compounds, which was attributed to the wine aging process (Conte 2008).

Furthermore, the determination of the wine redox potential may be a valuable tool to monitor the aging process. Besides being affected by the pH value, the redox potential is influenced by numerous components of wines, which can be present both in oxidized and reduced forms. Variations of the redox potential throughout wine storage have been shown to reflect oxidation reactions occurring during the aging period. This parameter also proved helpful for discriminating between wines which have been aged in barrels or using oak chips and oak staves, respectively (del Álamo *et al.* 2006).

With this background it is not astonishing that the addition of SO_2 markedly affects such aging reactions. The decrease of monomeric pigment contents and the increase

of polymeric compounds were largely suppressed in wines treated with 200 mg/L SO_2. This is due to the fact that SO_2 can reduce oxidized polyphenol structures and that it is likely to hinder pathways involving the formation of carbocations at the C4 position of proanthocyanidins. When the respective wines were stored in bottles devoid of oxygen, oxidation and polymerization reactions were slowed down (Tao *et al.* 2007).

Several attempts have been made to accelerate wine aging, e.g. by electrochemical oxidation of wine components, however, these have not been put into practice so far (Bertuccioli *et al.* 2007; Fell *et al.* 2007).

Wine lees also play an important role with regard to aging reactions, since yeasts have been shown to be responsible for considerable oxygen consumption. Experiments simulating wine aging revealed strong interaction of wine lees and polyphenols with regard to reactivity towards oxygen. With increasing contact times, oxygen consumption capacity of polyphenols increased, whereas that of the yeast lees was strongly lowered resulting in a total decrease of reactivity towards oxygen as compared to the reactivity of both components studied separately. Such interactions are probably due to adsorption of phenolic compounds onto the yeast surface (Salmon *et al.* 2002; Mazauric and Salmon 2005).

Must oxygenation has also been proposed for the production of white wines rich in phenolic compounds, which are susceptible to browning reactions during storage. Controlled oxygenation leads to a significant decrease in total phenolic contents, thus, bringing about low browning capacity of the resulting wines (Guerzoni *et al.* 1981; Vaimakis and Roussis 1993; Schneider 1998), and the S-glutathionyl caftaric to caftaric acid ratio has been suggested as an index of must oxidation even in finished wines (Singleton *et al.* 1985). The susceptibility of wines towards browning during storage is a highly important attribute for wine producers. Thus, accelerated browning tests have been developed to evaluate this parameter, such as the electrochemical oxidation of the wines and monitoring of the absorbance at 420 nm, yielding results comparable to those of real-time storage experiments (Palma and Barroso 2002).

Maturation of red wines in wood barrels

Aging of wines, which is increasingly performed in wood barrels, is of particular importance for improving color stability and the organoleptic properties of the products. This aging process significantly alters the phenolic profiles of the wines due to extraction of wood phenolics into the wines or adsorption of polyphenols derived from the grapes onto the barrel material as well as because of oxidation and polymerization reactions of phenolic compounds. For this reason, the barrel material, *i.e.* the oak species and the geographical origin, and its treatment, i.e. the type and length of seasoning and the degree of oak toasting, are important determinants of the phenolic profile and contents of the wines. The toasting has the highest impact on the profile and quantity of oak wood compounds which are likely to be extracted in the wines, thus, also affecting their organoleptic properties. Thorough studies of the evolution of phenolics from oak woods revealed a time-dependent increase of the concentrations of most benzoic and cinnamic acids of Spanish, French and American oak woods. The toasting was more important with regard to the formation of extractable phenolics, since significant increases especially of sinapic and coniferyl aldehydes, of syringaldehyde, vanillin, syringic and vanillic acids were observed. The woods of different origins behaved similarly, however, quantitative differences were found regarding these compounds, also enabling a differentiation between the woods of different origins (Cadahía *et al.* 2001; Gougeon *et al.* 2009). A Rioja wine aged in barrels made of wood from these three origins exhibited significant differences in their chromatic parameters and total anthocyanin contents. Additionally, the evolution of non-colored low-molecular phenolic compounds also depended on the type of barrel wood, which allowed the production of wines with different characteristics. Apart from the barrel material the changes also depend on the conditions and duration of the winemaking process (Fernández de Simón *et al.* 2003). Such differences were also found in another study comparing the effects of oak origin, the barrel volumes and the age of the barrel used for wine aging. The latter parameter is an important issue to be considered by enologists, since extended use of oak barrels is known to cause a progressive colmatation of the wood pores reducing oxygen diffusion rates and with it reduced oxygen contents of the wines, which significantly lowers oxidation and aging reactions. Thus, the barrels can only be used a very limited number of times. The barrel size is of particular importance because it determines the surface/volume ratio and, thus, the permeation of oxygen per volume of wine. Expectedly, the study showed that polymerization of wine anthocyanins is favored in small and new barrels, which is crucial for enhancing color stability. Furthermore, the sensory analysis also revealed the wines aged in smaller barrels to reach higher scores (Perez-Prieto *et al.* 2003). Even though oak is commonly used for producing wine barrels, futher woods, e.g. from acacia, cherry, chestnut and mulberry have been evaluated with regard to their potential for wine aging. The comparison of these materials demonstrated mulberry wood to be unsuitable for the aging of red wines due to a significant decrease of fruity notes, producing wines which do not meet consumer expectations from a sensory point of view. Furthermore, cherry wood barrels proved to provide an environment favoring oxidative reactions and, thus, making it less suitable for longer aging periods (de Rosso *et al.* 2009).

Since aging in barrels is a time-consuming process and also adds high costs due to the limited reusability of the barrels, alternatives for wine aging have been searched, such as the application of oak chips or oak staves. The comparison with barrel aging revealed oak chips to accelerate the aging process and trigger polymerization reactions. Differences between the aging systems as deduced from discriminant analysis were observed with regard to the contents of low-molecular phenolic compounds, some of which were lost when oak chips were used (del Alamo Sanza *et al.* 2004). Several further attempts have been made to accelerate wine aging, e.g. by the application of macerates from oak shavings, however, such processes have not been implemented in modern vinification practices (Monedero *et al.* 1998).

The decrease of total polyphenols during aging in wood barrels may not necessarily be ascribed only to polymerization reactions but also to sorption of the compounds onto the barrel surface. The latter phenomenon is characterized by a two-step kinetics. The first step is probably due to a surface sorption mechanism, whereas the slower second step might result from a diffusion mechanism. The proportion of phenolic compounds adsorbed onto the wood surface and that polymerized in the course of wine aging depends on the chemical structure. Whereas only minor parts of monomeric anthocyanins are adsorbed by the wood and the predominant part is polymerized, up to 50% of compounds such as *trans*-resveratrol may be adsorbed by the barrel material (Barrera-García *et al.* 2007).

Innovative vinification technologies to improve grape polyphenol extraction into the must

Among the more sophisticated technologies pulsed electric field (PEF) treatment as a non-thermal treatment method has also recently been applied to improve the quality of red wine. Three different field strengths (2, 5 and 10 kV/cm) were applied to treat the mashes of three grape varieties, 'Garnacha', 'Graciano' and 'Mazuelo', in order to optimize maceration and, thus, enhance the transfer of polyphenols into the must and improve the color of the resulting wines. PEF treatment caused a significant increase in color intensity, total anthocyanin and total phenolic contents as compared to the control wines from non-treated musts. The

study also revealed that the effect of an increase in field strength on the release of phenolic compounds was cultivar-dependent. Higher field strengths did not always imply a further increase in mass transfer rates (López *et al.* 2008a, 2008b). Alternatively, PEF treatments may also be applied to preserve grape musts before inoculation with yeasts allowing to reduce SO_2 concentration, which is usually added to control undesirable microorganisms and enzyme activities, without significantly changing the sensory properties of the resulting wines (Garde-Cerdán *et al.* 2008b).

The flash release treatment is another technique, which may be applied to produce polyphenol-enriched grape juices and musts. This process comprises rapid heating of the grapes at high temperatures (> 95°C) with vapor at atmospheric pressure and subsequent exposure of the grapes to a strong vacuum, causing instantaneous vaporization, which itself results in a cooling of the grapes and cell wall rupture, again enabling enhanced polyphenol release into the must. This treatment was shown to significantly raise polyphenol levels in the must, but it also modified the phenolic profile as compared to control musts, which can probably be attributed to different extraction kinetics of tannins from seeds and skins. Both heating and cell wall rupture are responsible for the enhanced release of phenolic compounds, and thermovinification itself without pressure release was shown to be only partly responsible for increased mass transfer rates (Morel-Salmi *et al.* 2006).

The potential of winemaking by-products for the recovery of phenolic antioxidants

Vinification may generally be characterized as an aqueous or hydro-alcoholic extraction of the grape skins and seeds, where most phenolics are located. The grape berries are only squeezed to obtain a mash, and a significant reduction of the grape skin particle size does not occur. For this reason, polyphenol extraction from the skins and seeds in the course of winemaking is usually poor. Grapes are among the world's largest fruit crops with an annual world production of around 66 million tons in 2007 (FAO 2009). The vinification by-products represent some 13-20% of the weight of grapes processed and consist of skins, seeds and stems. Annual grape pomace amounts reported in the literature are estimated values taking into account that about 80% of the crop is used in winemaking (Mazza and Miniati 1993). Accordingly, amounts reported in the literature differ significantly, ranging from 5-7 million tons to 14.5 million tons solely in Europe (Hang 1988; Meyer *et al.* 1998; Schieber *et al.* 2001; Torres and Bobet 2001). Given these figures, ways have been searched to exploit the press residues, which are characterized by high moisture contents and, thus, are susceptible to rapid microbial spoilage. The production of organic fertilizers from grape pomace is limited due to germination problems caused by the resulting soils containing high amounts of phenolic compounds (Bonilla *et al.* 1999; Negro *et al.* 2003), whereas the use as animal feed is limited due to poor digestibility as a consequence of high amounts of polymeric polyphenols, which inhibit cellulolytic and proteolytic enzymes and the growth of some rumen bacteria (Famuyiwa and Ough 1982; Schurg *et al.* 1980).

Based on the 'French Paradox' observations revealing a positive impact of grape or wine phenolics on human health, from an economic viewpoint the polyphenol fraction of grape by-products is more interesting than the aforementioned options. Anthocyanins are considered the most valuable phenolic compounds of grape pomace, which is characterized by a highly complex phenolic profile, similar to that of grapes consisting of hydroxybenzoic and hydroxycinnamic acids, monomeric, oligomeric and polymeric flavan 3-ols, stilbenes and flavonols and flavonol glycosides. Total polyphenol amounts have been reported to range up to 4% of the dried pomace (Lu and Foo 1999). Storage of the pomace without drying does not only affect the microbiological state but also the phenolic profile, since the formation of novel compounds may be observed under oxidative conditions (Fan *et al.* 2004).

To assess the potential of grape pomace for the industrial recovery of polyphenols, the phenolic profile and contents of individual compounds were determined in 14 pomace samples originating from red and white winemaking and, thus, allowing to compare polyphenol amounts in by-products originating from different grape cultivars and different vinification techniques. Up to 40 individual compounds were identified and quantified in the skins and seeds, revealing very high polyphenol contents in most samples demonstrating grape pomace to be a valuable raw material for polyphenol extraction (Kammerer *et al.* 2004). The study also revealed great differences in the contents of individual compounds depending on cultivar and vintage.

Anthocyanins have often been extracted from winery by-products using sulfite-containing water or alcohols to yield pigment preparations ("oenocyanin"), which can be applied as natural colorants (Bocevska and Stevcevska 1997; Ayed *et al.* 1999). Since sulfite cannot be removed quantitatively from the extracts and due to its pseudoallergenic potential, alternatives to sulfite-assisted extraction procedures have been studied. Among these the application of cell wall degrading enzymes, such as pectinases and cellulases, has been demonstrated to enhance polyphenol yields during grape pomace extraction (Meyer *et al.* 1998). More detailed studies of the yields of individual phenolics upon enzymatic digestion of grape skin cell wall polysaccharides showed significantly improved extraction rates for most polyphenols compared to aqueous extraction. The monitoring of individual compounds revealed that technical enzyme preparations may contain side activities detrimental to the release of phenolics, such as glycosidase activities, which underlines the necessity to carefully screen the enzymes used for cell wall degradation (Kammerer *et al.* 2005b). Systematic optimization of cell wall polysaccharide hydrolysis using a D-optimal design and analysis by response surface methodology allowed to improve polyphenol yields and to reduce enzyme dosages required to hydrolyze the polysaccharide matrix and improve the release of phenolic antioxidants, thus enhancing the economic feasibility of the process. The yields obtained were comparable to those from sulfite-assisted extraction. Therefore, such a process may be considered a suitable alternative to the application of sulfite (Maier *et al.* 2008).

The crude pomace extracts obtained by enzymatic hydrolysis of grape cell wall polysaccharides may either be directly spray-dried to yield a stable product or further purified and concentrated. For this purpose, adsorption technology using apolar macromolecular resins, which is applied in the food industry e.g. for the debittering of citrus juices, is a suitable tool. Anthocyanins extracted from red wine grape pomace samples were adsorbed onto a styrene-divinylbenzene copolymer, and the analyses revealed the pigment losses during sample loading and washing out of co-extracted non-phenolics from the adsorbent column to be negligible. Elution with acidified alcohols resulted in recovery rates ranging up to 96-100 %, thus facilitating pigment concentration without any loss, since highly concentrated alcoholic eluates were obtained, which allow concentration to dryness under mild conditions (Kammerer *et al.* 2005c). Further purification and fractionation of phenolic compounds from grape pomace may be achieved using chromatographic techniques, such as high-speed counter-current chromatography. This sophisticated method even allows to isolate individual compounds from crude mixtures, which may then be used for *in vitro* and *in vivo* studies or as reference substances for analytical purposes (Maier *et al.* 2006).

CONCLUSIONS

A comprehensive overview of currently applied technologies to enhance polyphenol release in the course of vinification is given. Furthermore, the fate of phenolic compounds during wine aging and storage is considered. There

are efficient ways to produce wines with stable colors and high antioxidant potential. However, it also becomes obvious that the effects of vinification treatments are strongly cultivar-dependent. Thus, the particular grape used for winemaking with its distinctive phenolic profile and contents is one of the most important factors determining the phenolic contents of the wines derived from it. Grape cultivar and vinification techniques further determine the polyphenol contents of winemaking by-products, which may serve as a source for the recovery of phenolic antioxidants.

The development of sophisticated analytical techniques in recent years has significantly increased the knowledge of the phenolic profile and contents of grapes and products derived therefrom, which is of utmost importance both from a technological and biofunctional point of view. With even more powerful analytical tools we will be able in the future to thoroughly assess the health effects of wines and trace back these properties to individual compounds or classes of compounds or probably to synergistic effects of complex mixtures of these components. Based on this knowledge, vinification techniques may further be developed and improved to obtain wines with optimized sensory attributes and desired health-related properties.

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