A Review of the Effect of Winemaking Techniques on Phenolic Extraction in Red Wines

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Abstract: Winemaking variables and techniques are known to affect the phenolic composition of red wines. This review surveys the current literature on the impact of processing and fermentation parameters on the phenolic profile of the finished wines. Six winemaking variables and techniques have been reported to increase phenolic concentration: fermentation temperature, thermovinification, must freezing, *saignée*, pectolytic enzyme treatments, and extended maceration. In contrast, sulfur dioxide levels and cold-soak treatments have frequently been shown to have no or little lasting effect or to lead to a decrease in phenolic levels. Finally, carbonic maceration, yeast selection, and skin and juice mixing practices have produced variable results depending on the grape varieties studied.

Key words: phenol, tannin, wine composition, phenolic extraction

Phenolic compounds are important to red wine quality because they impact color, mouthfeel, and ageability. Although phenolics are present in grape juice, the amount is greatly increased in wine because of their greater solubility in ethanol than in purely aqueous solutions. Typical levels of the major grape phenolics present in red wine are shown in Table 1.

Anthocyanins, flavanols and their polymers, and polymeric pigments resulting from the reaction anthocyanins with polymeric flavanols are the phenolic compounds with the greatest sensory impact in red wine. While anthocyanins and polymeric pigments give red wines their color, flavanols and their polymers are responsible for bitterness and astringency (Joslyn and Goldstein 1964, Arnold et al. 1980, Clifford 1986, Guinard et al. 1986, Robichaud and Noble 1990, Breslin et al. 1993, Naish et al. 1993, Fischer et al. 1994, Thomas and Lawless 1995, Thorngate and Noble 1995, Kallithraka et al. 1997, Sarni-Manchado et al. 1999). The polymeric flavanols are also referred to as tannins.

Color is probably the most easily recognized aspect of red wine quality. Anthocyanins and polymeric pigments both contribute to wine color. Anthocyanins are the pigments that give black and red grapes their color. Once the grapes are crushed, polymeric pigments begin to form and their amounts continue to increase with time (Somers 1971, Nagel and Wulf 1979). By the end of the first year, 50 to 70% of the pigments will have reacted to give polymeric forms (Ribéreau-Gayon et al. 1970, Somers 1971, Nagel and Wulf 1979). Polymeric pigments are important for the long-term color stability of wine since they are more stable to pH and bisulfite bleaching (Somers 1971, Somers and Evans 1977). Anthocyanin-derived pigments, such as the vitisins, may also play a role in wine color (Vivar-Quintana et al. 2002, Schwarz et al. 2003).

Several studies have shown that anthocyanin extraction reaches a maximum early in fermentation and that the concentration drops thereafter (Nagel and Wulf 1979, Watson et al. 1995, Gao et al. 1997), whereas tannin extraction continues to increase with continued skin and seed contact (Singleton and Draper 1964, Ribéreau-Gayon 1974, Ozmianski et al. 1986). Postfermentation factors that decrease anthocyanin content increase polymeric pigment content (Dallas and Laureano 1994). These factors include time, increasing storage temperature, and lower sulfur dioxide content, with temperature appearing to have the greatest effect.

In addition, colorless phenolics such as quercetin and caffeic acid interact with anthocyanins in a phenomenon referred to as copigmentation (reviewed in Boulton 2001,

Table 1 Phenolic levels in a "typical" *Vitis vinifera* red wine.

Phenol type	Concn (mg GAE/L)
Nonflavonoids	200
Flavonoids	
Anthocyanins	150
Condensed tannin	750
Other flavonoids	250
Flavonols	50

Data from Singleton and Noble (1976).

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Manuscript submitted November 2004; revised May 2005

Berké and de Frietas 2005). Copigmentation leads to a shift in the color of wine from red to purple and accounts for 30 to 50% of the color in young red wines (Boulton 2001). Copigments may also be seen as a storage form of anthocyanin, allowing more anthocyanin to be removed from the skins and stabilized in solution until the polymeric pigments are formed.

The total phenol distribution in red grape berries has been estimated as follows: pulp, 1%; juice, 5%; skin, 50%; and the remaining 44% in the seeds (Singleton and Esau 1969). Each of these berry components contains different classes of phenolic compounds. Anthocyanins are found in the skins, specifically the hypodermal cells. Cinnamic acids and their esters are found in the juice and skins. Polymeric tannins and monomeric flavanols such as catechin are found in the seeds and skins and can be found in very low levels in the pulp, with the exception of varieties such as Alicante-Bouschet that display high levels of these compounds in the pulp. Finally, flavonols such as quercetin are found in the skins. Since the phenolics desired in red wines are found in the skins and seeds, red wines are normally made with skin contact.

Because of the large influence of phenolic compounds on red wine quality, many winemaking techniques have been developed to influence the extraction of these compounds during vinification and most have been directed at enhancing extraction. Different factors limit the extraction of different classes of phenolic compounds. The early peak and subsequent decline in anthocyanins during fermentation is inconsistent with solubility being the limiting factor for these compounds, and instead reflects their instability once released. Rather, the limiting factor for release of anthocyanins is likely a physical barrier since they are located in the vacuoles of the hypodermal cells. Extraction requires that the compounds exit both the membrane-bound vacuole and the cell itself. In order to release internal components, it is important that these membranes be ruptured rather than merely collapsed, as a collapsed membrane structure can trap internal components. During a traditional fermentation on the skins, the alcohol content, carbon dioxide, and sulfur dioxide, together with the heat of fermentation, increase the permeability of these cells and membranes. Other treatments that destroy the cell membranes may also increase release of these pigments.

For tannins, extraction appears to be hindered by limited solubility. This is supported by the saturation behavior over time (Watson et al. 1995, Gao et al. 1997). Thus far, the evidence suggests that tannins are extracted from both skins and seeds. Extraction of tannins increases with increasing alcohol content, sulfur dioxide, temperature, and skin contact time, which increase the amount of tannin soluble in wine (Berg and Akiyoshi 1958, Singleton and Draper 1964, Ribéreau-Gayon 1974, Ozmianski et al. 1986). Furthermore, there appears to be a synergistic effect with combinations of alcohol, sulfur dioxide, and temperature (Ozmianski et al. 1986).

This article reviews the literature on the impact of winemaking processes on phenolic extraction. Many of the studies have focused on Pinot noir, a variety known for poor color extraction and low pigment stability. First, the basic variables of fermentation temperature and sulfur dioxide will be covered, followed by other treatments in their order in the winemaking process: prefermentation, fermentation, and postfermentation. Finally, the effect of yeast selection is discussed.

Fermentation Temperature

Overall, higher fermentation temperatures have been reported to increase phenolic extraction. Wines produced from Pinot noir and Cabernet Sauvignon grapes were more colored (Duboscq color values) as the fermentation temperature increased for the temperatures 53°F (11.8°C), 70°F (21.3°C), and 80°F (26.9°C) (Ough and Amerine 1961). A series of papers studying the effect of winemaking practices on phenolic composition has more recently examined the effect of temperature on extraction (Gao et al. 1997, Girard et al. 1997, 2001). Initial reports with Pinot noir showed little difference in the anthocyanin content of wines over fermentation temperatures ranging from 15 to 30°C, but the total phenolics content was greater at higher fermentation temperatures (Girard et al. 1997, 2001). Since the measurements were for wine already in bottle, it is not surprising that little difference in anthocyanin content was seen, as anthocyanins are known to reach a maximum early in fermentation and then to decrease rapidly. Interestingly, a paper was published in the same time period showing the decline of anthocyanins after early fermentation in what appears to be the same grapes, with roughly a 75% decrease by bottling (Gao et al. 1997). This paper also reported that at the same time anthocyanins were decreasing, polymeric pigment was increasing, and that the formation of polymeric pigment increased greatly when the fermentation temperature was increased from 20 to 30°C. The disappearance of anthocyanins and increase in polymeric pigment over time has been confirmed in other studies (Bakker et al. 1998).

An increase in polymeric pigment with increasing fermentation temperature was also reported by Harbertson et al. (2002), who found that tannin, small polymeric pigment, and large polymeric pigment were greater at pressing for wines fermented at higher temperatures. In this study it was also observed that although tannin decreased with time between pressing and racking, the polymeric pigments increased during this time.

Another study with Shiraz showed larger, but still moderate differences in anthocyanin content with increasing fermentation temperature (Reynolds et al. 2001); however, it is unclear how long after fermentation the measurements were taken. A later study that examined the effect of several processing parameters on phenolic extraction as measured by monomeric and polymeric phenols demonstrated that increasing fermentation temperature strongly increased wine color (Monticelli et al. 1999).

The increased extraction of phenolics at higher fermentation temperatures is due to increased permeability of the hypodermal cells releasing anthocyanin and increased solubility of other phenolics in the wine solution. The increase in polymeric pigment appears to be due more to increased extraction of tannins than of anthocyanins since anthocyanin levels drop so early in fermentation. If adequate tannin is not present early in fermentation to trap anthocyanins, there will be less polymeric pigment in the wine.

Sulfur Dioxide Effects

At the levels of SO_2 and temperatures normally used for red wine fermentations, sulfur dioxide does not dramatically affect the extraction of phenolics. In an early paper, it was found that increasing SO_2 levels affected Pinot noir extraction in fermentations at 53°F (11.8°C), but not at 70°F (21.3°C) or 80°F (26.9°C) (Ough and Amerine 1961). Heatherbell et al. (1996) also observed increased extraction with higher levels of SO_2 at lower temperatures in a cold-soak trial. Watson et al. (1995) reported that for Pinot noir with initial additions of 0, 50, and 100 mg/L total SO_2, there were no differences in anthocyanin content, but that polymeric pigment and caftaric acid were lowest for the 100 mg/L addition. In a study with Roriz grapes, Bakker et al. (1998) reported that as total SO_2 levels were increased from 0 to 75 to 150 mg/L, early fermentation extraction of total pigment was increased; however, there was little difference in the finished wines. That was also true for the concentrations of total phenolics and anthocyanins in the finished wines. Sulfur dioxide was also shown to have a positive effect on pigment extraction in Cabernet franc (Amrani et al. 1995).

In addition to any effect on extraction, sulfur dioxide also impacts wine color via reversible bisulfite bleaching of anthocyanins (Jurd 1964). It has also been reported that bisulfite can bleach polymeric pigment, though to a lesser degree (Bakker et al. 1986).

Cold Soak

During cold-soak treatment the must is held at low temperatures, usually 10 to 15°C, for several days before fermentation. The rationale offered is that aqueous extraction improves wine color. However, based on the known effects of alcohol and temperature on extraction of anthocyanins and tannins, improved extraction would not be anticipated. Even if cold maceration did increase anthocyanin extraction, it would not be expected to increase the polymeric pigment formation that is necessary to have a long-term impact on color. Unfortunately, none of the studies of cold maceration have included measurement of polymeric pigment.

The results obtained with Pinot noir consistently show that a cold soak alone has either no effect or a negative effect on the phenolic composition. Heatherbell et al. (1996) observed no difference between the control and the cold-soak treatment alone; however, a cold soak in the presence of 50 mg/L or greater sulfur dioxide increased the anthocyanin content of young wines and total phenols in the finished wines. Furthermore, the differences in anthocyanin content disappeared during aging. Experiments in Burgundy also showed that cold maceration before fermentation was detrimental to the color and phenolic content of Pinot noir unless the treatment was in conjunction with high levels (250 mg/L) of sulfur dioxide (Gerbaux 1993, Feuillat 1996). The above results suggest that the effect of higher temperature is dominant and that SO_2 effects are only observed in its absence. Other experiments confirm the negative impact of cold prefermentation maceration on Pinot noir: wines made with a cold soak were lower in anthocyanin, color intensity, and flavonols (Watson et al. 1995).

In addition, a German research paper reported the rate of color extraction measured as absorbance at 520 nm (A520) to be slower for Pinot noir and Dornfelder at lower temperatures, although the effect was much greater in Dornfelder (Fischer 1997). A study with Cabernet Sauvignon also found a slower rate of color extraction and a greater maximum extraction measured as A520 in wines subjected to cold-soak treatments, but by the end of fermentation the levels were more similar (Okubo et al. 2003). Overall, greater A520 was seen in the young Cabernet Sauvignon wines subjected to cold-soak treatments, but whether this difference persisted throughout aging was not reported.

The results obtained with Pinotage and Sangiovese are similar to those for Pinot noir. Marais (2003a,b) found that cold soaks at 10 or 15°C prolonged extraction of Pinotage and that they did not produce large differences in the phenolic profile of the finished wines. Comparison of cold-soak to traditional fermentation of Sangiovese also showed little difference in the anthocyanin and tannin content of the wine (Zini et al. 2003).

Reynolds et al. (2001) reported that a cold soak increased anthocyanin extraction in Shiraz when combined with lower fermentation temperatures (15 and 20°C), but not high fermentation temperatures (30°C), but it is not clear how old the wines were at the time of measurement.

Must or Grape Freezing

While cold soak has been reported to have little effect on the phenolic composition of the resulting wines, freezing the must before fermentation potentially has a much greater effect. Must freezing causes the berry cells to burst, breaking cell membranes and releasing anthocyanins. Use of solid carbon dioxide confers the additional advantage that, as it freezes the berries, it sublimes to blanket the must, protecting the berries from oxygen before fermentation.

Dry ice was used to freeze Merlot must (Couasnon 1999). Compared to the control wines, those produced with must freezing contained 52% more tannin and 50% more anthocyanins. Similar results were also obtained with

Cabernet Sauvignon and Cabernet franc. It is interesting that this method reportedly increased tannins while heat damage to the berries only enhances anthocyanins (see thermovinification section below). Freezing may also break the tannin-containing cells of the seeds, increasing extractability, but this observation requires further study.

In a variation of must freezing, Pinot noir pomace was separated from the juice, frozen, and added back to the fermenting juice when it contained ~6% alcohol (Girard et al. 2001). In this example, the wines contained slightly more anthocyanin than wines fermented without pomace freezing, and the effect on total phenols content was variable. It is possible that a smaller effect was obtained in this study compared to the dry-ice technique because the skins were not present from the beginning of fermentation and the maximum in anthocyanin extraction usually occurs in the first few days of fermentation. In this study there were not large differences in anthocyanin concentration for any of the treatments, including high-temperature fermentation.

Thermovinification

Though there are several variations of the procedure, basic thermovinification entails heating the skins to 60 to 70°C for a short time, extracting them with the juice, pressing, and then cooling before fermentation. The heat damages the hypodermal cell membranes, releasing anthocyanins, and it also denatures polyphenol oxidase, preventing browning. Since there is no alcohol present at the time of heating, it would not be expected to increase tannin extraction. It has, in fact, been reported that compared to fermentation on the skins, thermovinification leads to improved color (anthocyanin) extraction, but much lower phenolic extraction overall (Auw et al. 1996).

Most studies of this technique have been with Pinot noir. Girard et al. (1997) reported that thermovinification resulted in more than a 2-fold increase in anthocyanins in Pinot noir from British Colombia than did fermentation on the skins at 20 and 30°C. In another paper apparently based on the same fermentations, the content of malvidin 3-glucoside was tracked over time from 0 to 225 days and revealed a unique extraction profile in thermovinification (Gao et al. 1997). The anthocyanin content of the thermally treated juice was at a maximum at the beginning of the fermentation and was three times that of the traditional fermentation. It then decreased rapidly by day 10, leveling off to roughly 1.5-fold that of the fermentations on the skins. That was in contrast with the pattern for fermentation on the skins with a peak at day 3 or 4, followed by a slow decrease. In addition, the increase in polymeric pigment over time was nearly identical to that of the traditional fermentation conducted at 20°C, consistent with the expectation that little additional tannin would be extracted during thermovinification without alcohol present.

Thermovinification and traditional fermentation were compared for three Italian varieties: Negro amaro, Primitivo, and Ibrido P (Stella et al. 1991). Thermovinification increased anthocyanin content for all three and decreased catechin and total phenols for the latter two.

The effects of fermentation on the skins, must heating, and must heating followed by fermentation on the skins were compared for Pinot noir (Spätburgunder), Lemberger, and Cabernet franc grown in Germany (Netzel et al. 2003). Must heating consisted of heating to 65°C, cooling for 24 hr, then pressing and inoculating. For all three varieties, the greatest increase in flavan-3-ols was found between the fermentation on the skins and must-heating treatments, while the difference between must heating and must heating plus fermentation on the skins was small. This pattern was also observed for anthocyanin extraction in Pinot noir, while only small increases in anthocyanin were seen for the heat treatments for Lemberger and Cabernet franc.

In this experiment, heating increased flavanol extraction unlike the above studies, perhaps because the must was heated as opposed to heating the skins separately so that liquid was present for extraction during the heating period. It is also interesting that heat had a much greater effect on anthocyanin extraction in Pinot noir than the other varieties studied since this variety is known for problematic color extraction.

Another experiment where the hot skins were pressed immediately led to lower levels of anthocyanins, catechins, and total phenols (Leone et al.1983). For heat treatments to be successful in increasing extraction, it appears to be necessary to have the skins in contact with the juice during or after heating. If the hot skins are not extracted with juice, the compounds released by destruction of cell membranes do not end up in the wine.

Carbonic Maceration

In carbonic maceration, whole berries or clusters are held under a carbon dioxide atmosphere and partial fermentation occurs because of the activity of glycolytic enzymes present in the grapes (Flanzy 1935). After a specific period of time, typically one to two weeks, the grapes are then pressed and the juice is inoculated to complete fermentation. The method is typically used to produce lighter, fruity wines that are meant to be consumed young.

It is difficult to draw general conclusions about the effect of carbonic maceration on the phenolic content of wine because conflicting results have been found with the different varieties studied. Timberlake and Bridle (1976) found that after four months of aging, wines from Cascade grapes produced by carbonic maceration contained less anthocyanin and polymeric pigment than wines produced by fermentation on the skins. Another group examined the effect of carbonic maceration on anthocyanins by analyzing the level in the skins of Carignane rather than in the wines and found that anthocyanin content decreased with time; however, there was no traditional fermentation as a control, so it is not known if extraction increased or decreased with carbonic maceration (Ramos et al. 1993). A more recent study that aimed to isolate the effect of stem contact from the other aspects of carbonic maceration in Tinta Miúda wines also found a decrease in anthocyanins, with no-stem contact wines containing the most anthocyanins, followed by stem contact, then carbonic maceration (Sun et al. 2001). Not surprisingly, the opposite trend was observed for catechins and tannins that may be extracted from stems.

A small decrease in tannin content in wines produced by carbonic maceration has also been reported (Beelman and McArdle 1974). Results of a multi-year study of Hungarian varieties indicated that carbonic maceration increased the total phenol content of the wines compared to fermentation on the skins (Lorincz et al. 1998), while a study with muscadine cultivars showed no effect or a decrease in total phenols with carbonic maceration (Carroll 1986). Finally, another paper comparing fermentation of varietal blends with and without carbonic maceration confirmed that the effect on total phenols was dependent on the varieties present (Pellegrini et al. 2000).

Prefermentation Juice Runoff

In this practice, which is also referred to as *saignée*, juice is removed before fermentation, thus increasing the skin to juice ratio. Since the anthocyanins and tannins extracted during fermentation are found in the skins and seeds, this practice should theoretically increase their concentration in the finished wine. However, if extraction were strictly solubility limited, the effect would be the opposite since there would be less liquid to dissolve the phenolics. In practice, the former is generally observed.

It has been suggested that juice runoff mimics the effect of smaller berries since this increases the skin to juice ratio, and it was used to study the effect of berry size on wine phenolic composition (Singleton 1972). For nine varieties, the flavonoid and anthocyanin content of the wines at roughly four months of age was greater, with 10% juice runoff compared to the control, and that these were greater in the control than wines made with a 10% juice addition. Later experiments over three vintages of Malbec mostly agreed with these results, although it is not known if the phenolics were measured at a comparable age (Zamora et al. 1994). The wines produced with *saignée* were characterized by more and larger tannins and by more polymeric pigment in all vintages. They also contained more anthocyanins in two of the three years. *Saignée* was also found to slightly increase the color and phenolics content of young Pinot noir (Gerbaux 1993).

The effect of 10 and 20% juice runoff was also studied for two vineyard blocks of Syrah (Gawel et al. 2001). At press, increases in anthocyanins and total phenolics with juice runoff were observed only for one of the vineyard blocks, which had a much greater level of extraction of total phenolics overall. However, after six months, the differences between the juice runoff treatments had virtually disappeared as well as the differences between vineyard blocks. This study raises questions about the long-term value of these treatments and the differences in their effects with different varieties.

Pectolytic Enzymes

Pectolytic enzymes have been used to try to increase wine color because they break down skin cell walls, releasing pigments. The purity of the enzyme preparation is important, because if ß-glucosidases are present, anthocyanins can be converted to the less stable aglycones, resulting in color loss. Use of these enzymes also generally increases juice yield. There are many studies supporting an improvement in color extraction with these enzymes (Ducruet et al. 1997).

Early reports on the effect of pectinases demonstrated an increase in color and total phenol content at the end of fermentation with Grenache, Carginane, Zinfandel, and Petite Sirah (Ough and Berg 1974, Ough et al. 1975). It was also reported that enzymes improved the color of young Merlot (Takayanagi et al. 1997) and Valpolicella (Zent and Inama 1992) wines. At the end of fermentation, increased total phenols content was seen for Cabernet Sauvignon and Isabel (Daudt and Polenta 1999) wines made with enzyme treatment, but no difference in total phenols was found with Syrah (Clare et al. 2002). Another study with Cabernet Sauvignon showed no effect of enzymes on anthocyanin extraction postmalolactic fermentation, but increased tannin extraction (Guerrand and Gervais 2002).

Five enzyme preparations were compared for their effects on Pinot noir wines and found to increase tannin and polymeric pigment with no change or a decrease in anthocyanin content (Watson et al. 2000). In another study by the same group using Pinot noir and Cabernet Sauvignon, none of the enzymes were found to increase anthocyanin content at six months of aging and some were found to decrease it (Wightman et al. 1997). In both cases, the decrease in anthocyanins was accompanied by an increase in polymeric pigment, so it may be due to polymeric pigment formation. An independent group saw no effect on anthocyanin extraction in Pinot noir at 18 months of aging, but an increase in polymeric pigment and total phenols (Parley et al. 2001).

In the majority of studies, pectinases do not seem to increase anthocyanin extraction but do seem to increase extraction of other phenolics including tannins. The fact that no effect on anthocyanins was observed may be because the wines were tested after aging, allowing losses by degradation or polymeric pigment formation. Pectinase treatment has also been found to increase polymeric pigment formation.

Pump-Overs and Punch-Downs

In fermentation on the skins, a cap develops as carbon dioxide evolution causes the grape solids to rise to the top of the fermentation vessel. This has two potentially negative consequences: heat is trapped in the cap and there is reduced contact between the bulk juice and the skins and seeds. To overcome these problems, the skins and juice are usually mixed several times a day, either by pushing the skins below the juice surface (punch-down) or by pumping juice out and spraying it over the top of the skins (pump-over). The effect of a pump-over is dependent upon the timing during fermentation, as it will be influenced by both fermentation temperature and whether skins are circulating through the pump. The temperature the cap is allowed to attain is also an important variable, but one that has not been adequately investigated. Since some type of juice mixing is always included in red fermentations, studies have focused on comparing the different methods. Generally, it has been found that the results depend on the variety.

Early work showed a small increase in the color and tannin concentration with pump-overs versus punch-downs (Ough and Amerine 1961). Recently, the different classes of phenolics were quantified and compared for manual punch-down, mechanical punch-down, and mechanical pump-over for three grape varieties: Pinot noir, Dornfelder, and Portugieser (Fisher et al. 2000). With the exception of quercetin that was greatly increased by pump-over in all cases, the results were variety dependent. The largest differences were found for Pinot noir, where mechanical punch-down or pump-over increased most classes of phenolics by 100 to 200% and mechanical punch-down gave greater extraction than pump-over. Very little polymeric pigment was found with any of the extraction methods, but that may be due to the analysis method. For Dornfelder, both mechanical methods increased extraction of the nonpolymeric phenols, with pump-over mildly favoring extraction over punch-down. The smallest effect was observed with Portugieser where only quercetin, flavanols, and caftaric acid increased with mechanical methods.

Another study comparing punch-downs with pump-overs also found a variety-dependent effect. For Negramano and Primitivo, anthocyanins, catechin, and total phenols were greater for pump-overs, but with Sangiovese there was little effect on anthocyanins and decreases in catechins and total phenols were observed (Leone et al. 1983).

A study with Pinotage comparing manual punch-down, pump-over, and rotary mixing found that extraction rates of total flavonoids, total tannins, and anthocyanins were slower for pump-overs and that lower concentrations were achieved (Marais 2003a,b). Analysis of the total phenols content of the resulting wines showed that the highest concentration was obtained with rotary mixing, followed by punch-down, then pump-over.

Pump-over has also been compared to delestage for the Italian variety Montepulciano d'Abruzzo (Bosso et al. 2001). Delestage, also referred to as rack and return, is a technique where all of the juice is drained from the tank and then poured back over the skins. Pump-overs gave higher anthocyanins, polymeric pigments, and tannins with the largest differences observed for tannins. During bottle aging, the differences in anthocyanins and tannins decreased slowly while the differences in polymeric pigment increased, with the pump-over wines showing more polymeric pigment formation. Of course these results are likely dependent on the type of pump-over operation performed and the length of time it is performed.

Maceration Time

The practice of extended maceration prolongs skin contact after the must has fermented to dryness. Based on what is known about anthocyanin and tannin extraction profiles, extended maceration would be expected to increase tannin, but not anthocyanin content. It is widely believed to increase extraction of the skins and seeds and this belief is generally supported by the literature.

It has been demonstrated that longer pomace contact during fermentation, 10 versus 4 or 5 days, increased the concentration of anthocyanins and tannin in the wines at bottling and that the wines which had longer pomace contact contained more polymeric pigment after one year in bottle (Gomez-Plaza et al. 2001). The largest increase in anthocyanins was between day 4 and day 5 of skin contact, with little increase after 10 days of skin contact.

A replicated small-scale fermentation study found more anthocyanins in young wines made with extended pomace contact and more total phenols at all stages of aging (Scudamore-Smith et al. 1990). A study that compared maceration of Cabernet Sauvignon for 7, 13, and 21 days found that total phenols, gallic acid, and flavanols all increased with skin contact time (Auw et al. 1996). Furthermore, the increases in total phenols and flavanols were greater between 13 and 21 days than between 7 and 13 days. Work with Cabernet Sauvignon has also shown that wines with extended skin contact, 44 days versus 14 or 23 days, contain more molecules of higher molecular weight after 21 months of aging, suggesting that they contain more polymeric pigment (Kudo and Sodeyama 2002).

Several experiments with Pinot noir have indicated that extraction of flavanols and polymeric phenols as well as the levels of total phenols increases with extended skin contact (Watson et al. 1994, 1995). A study with Syrah also showed an increase in total pigment with a three-day extended maceration, which likely reflects an increase in polymeric pigment because of greater tannin extraction (Reynolds et al. 2001). It has also been reported for Merlot that total phenols increase up to 36 days of pomace contact, though the increase slows around day 10, while pigments increase rapidly until day 4, then slowly decrease over time (Yokotsuka et al. 2000).

These studies support the expectation that extended maceration leads to increases in tannin, but not anthocyanin. The increased tannin extraction may also lead to greater polymeric pigment formation.

Yeast Selection

There has also been interest in the potential of yeast selection to affect the phenolic profile of red wines. Yeast lees has been demonstrated to adsorb anthocyanins (Vasserot et al. 1997, Morata et al. 2003), and though there are no reports yet, it seems plausible that macromolecules released on autolysis can bind polymeric phenols in a similar fashion to protein-fining agents. The interaction between tannins and mannoproteins released from different yeast has been examined, and it has been found that addition of mannoproteins from particular yeast led to more anthocyanin-tannin condensation and decreased astringency in wine (Escot et al. 2001).

There have been many studies looking for an effect of yeast selection on the phenolic profile of red wines; however, none of them mention sterile precautions to ensure that the inoculating strain was the only yeast present or monitoring of the microbial population during fermentation to verify that the inoculant was the dominant fermenting yeast. Further confusing the issue in many of the studies, the lots were pressed at dryness, so it is not clear if the different lots had the same skin contact time. Finally, not all of the studies were replicated, making it impossible to assess whether or not the results were statistically significant.

What appears to be the first report on the effect of yeast selection on the phenolic profile of red wines is a review by Cuinier (1988) summarizing the results of several experiments in Burgundy. The phenolic composition of wines produced by different yeast strains was compared using color intensity and total phenolics, both in absorbance units. These nonreplicated studies showed small if any effects on color intensity and total phenolics.

More recently the same author reported the results of a replicated study, comparing Gamay fermentations with 18 yeasts, including wild yeast genera, which showed differences up to 100 mg/L in anthocyanins and 10 absorbance units in total phenolics among *Saccharomyces* strains (Cuinier 1997). There was uniform skin contact time for all yeasts, although alcohol levels at pressing may have varied. Color intensity and total phenolics were measured as previously; however, there was no separate measurement of polymeric phenols such as tannins.

The initial work on the effects of yeast selection on the phenolic profile of Pinot noir suggested that differences were obtained according to yeast strain (Dumont et al. 1993); however, quantitative results were not given. This study is unique because efforts were made to demonstrate that the fermenting yeast was the inoculating strain using PCR techniques. These initial results were not confirmed by a study comparing six yeast strains in fermentation of Pinot noir that found little difference in the phenolics measured as total phenolics, color intensity, anthocyanins, and tannins (Lorenzini 2001). A later replicated study of vinification effects on the color of Pinot noir included two yeast strains, but found that yeast strain made basically no difference in total phenolics, anthocyanins, or color density (Girard et al. 2001).

This group also compared seven additional yeast strains with Pinot noir and six with Cabernet franc and Merlot (Mazza et al. 1999). Once again the yeast strain did not have a great effect on phenolic composition, although greater differences were observed than in the earlier study. However, since all fermentations were pressed at dryness, differences in skin contact time could confound yeast effects. For all three varieties the following ranges of phenolic parameters were observed: total phenolics, 150 mg/L gallic acid equivalents, and anthocyanins, 50 mg/L malvidin 3-glucoside equivalents.

Another group reported differences in the color of Syrah and Cabernet Sauvignon fermented with *Saccharomyces bayanus* strains compared to other yeasts, although the Syrah experiment was not replicated (Eglinton and Henschke 2003). In the Syrah wines, absorbance at 520 nm was greatest for uninoculated fermentations, followed by *Candida* fermentations, then *S. bayanus (S. cerevisiae* var. *bayanus)* fermentations. Cabernet Sauvignon wines fermented with *S. bayanus* were reported to have more color resistant to bleaching, greater color density, and more purple hue than wines fermented with *S. cerevisiae* var. *cerevisiae*, but the statistical significance was not reported. These studies did not standardize skin contact time, and it is mentioned that *S. bayanus* was a slower fermenter with Cabernet Sauvignon. That means *S. bayanus* wines had more skin contact and thus could contain more copigmentation cofactors, leading to the purple hue.

Three yeast strains were evaluated in Sangiovese and found to give nearly identical phenolic profiles (Zini et al. 2002). Other studies argue that particular strains give superior color (Delteil 1995, Boisson et al. 2002).

The most recent study on the effect of yeast selection on phenolic composition compared five *Saccharomyces* yeast in fermentation of four varieties: Teroldego, Lagrein, Merlot, and Enantio (Nicolini et al. 2003). Great differences were not observed and there was no information about statistical significance, as it was not a replicated study. The color intensity of the wines was affected more by variety than by yeast strain; however, most of the data was reported as an average of the four varieties with a range of 83 mg/L and 54 mg/L catechin equivalents for total phenols and anthocyanin contents, respectively.

Conclusions

Of the winemaking variables and techniques reviewed here, six have been found to increase the phenolic composition of the finished wine: fermentation temperature, thermovinification, must freezing, *saignée*, pectolytic enzyme treatments, and extended maceration. Sulfur dioxide levels and cold-soak treatments have been shown to have little to no lasting effect or to lead to a decrease in phenolics. Finally, carbonic maceration, yeast selection, and skin and juice mixing practices have produced variable results depending mainly on the grape varieties studied.

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