Cold soak not an effective means of colour extraction

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Introduction

To the consumer, wine colour is often indicative of overall quality. Anthocyanins are responsible for the colour of red wine and are present as polymeric complexes or as free anthocyanidins. Skin proanthocyanidins are considered to be softer and have a higher quality than the seed proanthocyanidins. Anthocyanins are often released by aqueous extraction from the berry during a period of maceration. Maceration not only extracts anthocyanins, but other compounds as well, including tannins, proteins and polysaccharides.

Fermentation and maceration occurs simultaneously as the skin and juice are in contact and the anthocyanins are diffusing from the skins to the juice. The cell walls of the berries are broken down and the anthocyanins found in the cell vacuole are extracted into the wine through a process of diffusion. There are several different winemaking methods that aim to break down these cell walls to extract large amounts of phenolic pigments.

A common method to induce phenolic extraction involves holding the un-inoculated must at cool temperatures of around 15°C or at cold temperatures of around 4°C for an extended period of time before fermentation. This process is what is known as a cold soak and is meant to improve wine colour after the slow aqueous extraction of the anthocyanins. The juice soaks with the skins for several days, after which the colour is theoretically darker due to an increase in optical density. There are several aspects that impact the ability of the juice to extract colour during a cold soak, including the copigmentation, temperature and ethanol.

The molecular bonds that occur between the anthocyanins, pigments and other non-coloured organic components are the results of a process called copigmentation. As much as 50% of the colour in red wine can be due to copigmentation, thus expressing a great impact on colour. This phenomenon is central to understanding how its behaviour as a dynamic equilibrium is vitally important to the extent of colour extraction. Copigmentation puts a limit on colour potential, the extraction of pigments, primarily anthocyanins, and the strength of the retention of these pigments for long term colour stability. Because colour can change over time, the initial extraction of pigments is really only the first part of the long process of colour development. Literature points to the fact that an adsorption and desorption equilibrium exists between the pigment concentration at the cellular level in the grape skins and the pigment concentration in the wine. Copigmentation will shift the anthocyanins and pigments out of the free anthocyanin pool of the adsorption equilibrium, which results in a greater potential for adsorption of pigments in the wine causing an elevated level of extraction.

Temperature plays one of the most important roles in the entire winemaking process. The temperature of the alcoholic fermentation is extremely important, as studies have shown that there is a positive correlation between increasing temperatures and greater levels of colour extraction (Gao *et al*., 1997). Therefore, it stands to reason that various temperatures of the cold soak will affect the wine, especially in conjunction with the temperature of the primary fermentation. Reynolds *et al.* (2001) found that Syrah increased levels of anthocyanins after a cold soak when it was coupled with lower fermentation temperatures, between 15°C and 10°C ($P<0.05$) but no increase was shown when the temperature of fermentation was around 30°C.

Previous research on the subject of cold soak extraction suggests that the results of such a treatment are inconsistent or do not persist in the finished wine. Much of the literature directly contradicts the notion that the wine will extract more phenolics during a cold soak (Heatherbell *et al*., 1996 & Koyama *et al*., 2007). The effects of ethanol and temperature on anthocyanins and tannins would be more likely to hinder extraction rather than promote it. Somers and Evans (1979) found a loss in anthocyanins and total phenolics in Syrah wines after a heat treatment. They attributed the loss to the effect that ethanol has on the structures of deeply coloured pigment aggregates present in the juice prior to fermentation, which suggests that this same phenomenon may also occur after a cold soak. As ethanol increases, there is a significant reduction in the equilibrium of the pigment concentration in the wine and the concentration in the grape skins, and as mentioned before, higher temperatures are correlated with pigment extraction as opposed to cold temperatures (Gao *et al*., 1997). However, this portion of literature tends to look at wine after being bottled. The polymerisation of pigments that commences during this period tends to reduce the differences seen in anthocyanin concentrations of cold soaked and control wines (Sacchi *et al.*, 2005).

Other than varietal factors, climatic factors and the condition of fruit maturity also greatly influence the anthocyanin content of the berries. Other factors limiting the intensity of extraction are the concentrations of certain cofactors present in the skins at harvest and their solubility under the conditions that the juice or wine creates. The ultimate limitation to extraction is the amount of coloured pigments present in the berry. This radically changes from variety to variety, and some varieties, such as Pinot noir, are notorious for low concentrations of anthocyanins that results in poor colour development. Syrah has proven difficult to display a colour difference in scientific experiments. One study analysed Cabernet Sauvignon, Monastrell and Syrah after a 10-day 10°C cold soak. Both the Cabernet Sauvignon and the Monastrell showed positive differences in colour extraction while Syrah showed no effect (Busse-Valverde *et al.*, 2010). Gil-Muñoz *et al*. (2001) also found no significant increase in anthocyanin extraction from Syrah which had undergone a pre-fermentative cold soak ($P>0.05$).

Materials and methods

Syrah grapes were harvested from the UC Davis vineyards in September of 2011. The fruit was crushed and de-stemmed using a Delta E1 (Bucher Vaslin). The processed fruit was then allocated to four 55 gallon research fermenters. These vessels are made of stain-

less steel, jacketed for temperature control and are designed for variable capacity. Each must was dosed with 50 ppm of SO_2. Three of the musts were then stored at 2°C, 6°C and 10°C during a period of cold soak for two weeks prior to inoculation. The control was immediately inoculated with Lalvin EC 1118 (*Saccharomyces cerevisiae bayanus*). The cold soaked tanks were also inoculated in the same fashion. The maximum fermentative temperature was 27°C and the tanks were completely pumped over twice daily.

Upon the completion of fermentation, each tank was pressed using a specially designed hydraulic press, whereafter the wine was inoculated with Viniflora Oenos (*Oenococcus oeni*). Upon completion of MLF, 50 ppm SO_2 was added to attain a free sulphur dioxide content of about 30 ppm and racked from its lees into stainless steel barrels with limited headspace. The wine was then kept in a cool room and topped biweekly with N_2 gas. The finished wines were bottled in the UC Davis winery.

After bottling, the wines were analysed for colour and phenolic concentration using a Hewlett Packard 8453 UV-Vis spectrometer. The wines were also analysed in a duo-trio test in which a panel of eight participants was asked to evaluate the colour of a reference wine and then choose the same wine from two other wines.

Results

Spectrophotometric readings were taken on all the finished wines in order to estimate phenolic content and colour. The results are summarised in Table 1.

Discussion

The cold soak at 6°C and 10°C but not 2°C resulted in an increase in anthocyanin extraction as is evident by the increase in colour intensity measurement (Table 1). This suggested that the increased extraction was most likely the extraction of anthocyanin monomers such as malvadin 3-glucoside since the monomeric anthocyanins have been implicated in colour intensity (Gao *et al*., 1997). Since monomeric anthocyanins typically polymerise during aging, leading to increased colour stability (A_{420}) but decreased intensity (A_{520}), the results of the spectrophotometric data also suggest that the polymeric anthocyanins may have increased as is evident by the increase in hue since polymeric anthocyanins absorb at 420 nm. These results contradicted results in the literature. Gardner *et al*. (2010) found that a cold soak treatment of Cabernet Sauvignon musts increased the polymeric anthocyanins, but had no effect on the monomeric anthocyanins as a function of colour intensity. It is possible that the extraction of anthocyanins is dependent upon variety and can be influenced by small differences in the vinification process.

The spectrophotometric data did not match the data obtained from the sensory trial. The only treatments that participants were able to correctly match to the reference with any statistical significance was the 10°C treatment compared to the 6°C as well as the 10°C treatment compared to the 2°C treatment (Table 2). The expectation is that if there was a true difference between the higher temperature cold soak treatments and this difference was interpreted by the panel as either colour intensity or hue, then the most dissimilar wines would be the 10°C cold soak and the control, as there was the greatest difference in both categories (Table 2). However, when analysed by the sensory panel, there was no significant difference between the colours of these two wines. Furthermore, one would expect that the wine produced by the 6°C cold soak would also be too similar to the wine produced by the 10°C to be differentiated when the spectrophotometric data are used exclusively, but this difference was detected by the sensory analysis. Since the wines were not filtered, it is possible that there was a difference in turbidity from the residual yeast lees carried over during racking, that may have been perceived by the panellists.

Since the cold soak treatment is supposed to increase overall phenolic extraction, the A_{280} was measured in an attempt to quantify the total phenolics (Table 1). The A_{280} was measured and four absorbance units subtracted from it to account for the flavonoids naturally present as non-desirable phenolics. The A_{280}-4 measurement is a gross approximation for estimated total phenolic content by subtracting out what a study by Somers and Ziemelis (1985) found to be the average value for flavonoid concentration in commercial wines. It is likely that these wines had low flavonoid content that resulted in the low total A_{280}, and not because of a complete absence of anthocyanins and other phenolics. The utility of this estimation is that it is a good comparison to use between wines produced in a similar manner from the same lot of must. It is surprising however that in this case the 10°C cold soak treatment wine did not have the highest phenolic content as determined by the methods employed in this study, even though the wine had the highest colour intensity. It is possible that they were simply not extracted, however this is anomalous, as tem-

TABLE 1. Spectrophotometric data of varying cold soak treatment temperatures normalised to a 1 cm path length.

Sample	$A_{280\ nm}$	A_{280}-4	$A_{420\ nm}$	$A_{520\ nm}$	Hue (A_{420}/A_{520})	Colour Intensity ($A_{420} + A_{520}$)
Control	3.973	35.73	1.02	1.67	0.611	2.69
2°C	3.821	34.21	1.08	1.59	0.679	2.67
6°C	4.331	39.31	1.40	2.02	0.693	3.42
10°C	3.798	33.98	1.45	2.09	0.694	3.54

TABLE 2. Duo-trio sensory analysis of treatments analysed for colour. Temperatures incubated for cold soak indicated as well as the sample used for the reference (R).

Comparison	% correct	Significant (P<0.05)
Control (R) vs 2°C	33%	No
10°C (R) vs 2°C	87.5%	Yes
Control (R) vs 6°C	75%	No
10°C (R) vs 6°C	87.5%	Yes
Control (R) vs 10°C	37.5%	No
2°C (R) vs 6°C	75%	No

peratures for cold soak and fermentation were high and temperature has been shown to be the most important variable for phenolic extraction (Gao *et al*., 1997). The value of the A_{280} was low for a commercial wine as was established in a study by Somers and Ziemelis (1985) that analysed 400 commercial red wines from Australia and found that the range in A_{280}-4 values was between 23 and 100 with an average of 50. The wines produced here were substantially lower, most likely as a result of immature fruit. Wines with values greater than 40 may benefit from aging, suggesting the wines produced here would likely need to be consumed early.

The current study demonstrated some consistencies as well as discrepancies with previous research but the literature on cold soak itself is quite contradictory. A study by Busse-Valverde *et al*. (2010) found that a cold soak treatment was ineffective in extracting anthocyanins from Syrah seeds and skins but it was effective with Cabernet Sauvignon and Monastrell varieties. Gardner *et al*. (2010) found that a cold soak treatment for Cabernet Sauvignon was ineffective in extracting higher amounts of phenolics, but was successful in producing a wine with a higher hue but with no significant change in intensity. The results of our trial coupled with the results from previous research suggest that a cold soak treatment may be effective in increasing total anthocyanin levels in young wines, but this phenomenon is most likely dependent on a number of variables including temperature and duration of cold soak, grape variety, the vinification process and aging regimen.

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