INVESTIGATING THE EFFECT OF SELECTED WINEMAKING TECHNIQUES ON PHENOLIC EXTRACTION DURING CABERNET SAUVIGNON FERMENTATION

BY

SIRIWAN PANPRIVECH

ID. 551-9871

A Dissertation submitted to the Biotechnology of Biotechnology, Assumption University in part of fulfillment of the requirement for the degree of Doctor of Philosophy in Food Biotechnology

In collaboration with University of California at Davis

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Title: Investigating the Effect of Selected Winemaking techniques on Phenolic Extraction during Cabernet Sauvignon Fermentation

By: Siriwan Panprivech

Advisor: Dr. Anita Oberholster

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The manuscript has been read and in satisfaction of the thesis requirements for the Doctor of Philosophy Program in Food Biotechnology (Ph.D. FB).

Dr. Anita Oberholster

Dr. Churdchai Cheowtirakul

Prof. Dr. Glenn M. Young
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ABSTRACT

Winemaking processes or techniques are known to impact wine composition and thus quality. Because of the significant influence of phenolic compounds on red wine quality, many winemaking processes have been developed to enhance the extraction of these compounds during the fermentation process. This dissertation focuses on the potential impact of different cold soak (CS) and pump-over techniques on phenolic composition and thus quality. The impact of different CS durations (0-10 days) at 10 °C on phenolic extraction was examined during CS and fermentation as well as in the finished wines after bottling. Our study indicate that in the case of Cabernet Sauvignon and the CS and winemaking conditions used in this study, CS only had small effect on some phenolics (gallic acid, (+)-catechin, and (-)-epicatechin) with increasing CS duration when compared to non-CS wine for two harvests using Cabernet Sauvignon grapes with different phenolic content. Trends of increased seed tannin contribution showed with increasing CS duration, although there were no significant differences among wine treatments in the bottled wines. Similarly, sensory analysis indicated very little difference among the wines made with different CS duration. Furthermore, similar trends in phenolic extraction with different CS duration in research-scale wines were also seen in commercial-scale wines.

Different of pump-over conditions were studied in research-scale Cabernet Sauvignon fermentations to understand the effect of the different pump-over volumes (two, one, and half volume twice a day), pump-over frequencies (one, two, four, and eight times per day), and low extremes of pump-over volume and frequency (half volume two times, half volume one time, quarter volume two times, and no pump-over) on phenolic extraction during fermentation. The results showed that initial pump-over volumes and frequencies investigated had no significant impact on the
extraction of phenolics during fermentation. Furthermore, when low extremes of pump-over volume and frequency were studied there was a trend of increased phenolic extraction when comparing pump-over wine treatments with no pump-over, although it was not significant.

To understand the kinetics of phenolic release during fermentation, pilot-scale (2000 L) Cabernet Sauvignon fermentations were performed using a tank modified with a custom sampling grid that allowed the fermentations to be sampled at four layers (two in the cap and two in the liquid portion). Chemical gradients for skin phenolics, such as the anthocyanins, were observed to develop early during fermentation whereas phenolics located more predominantly in the seed, such as (+)-catechin, extract later. Seed extraction trends were confirmed using phloroglucinolysis. Interestingly, phenolic gradients were eliminated following a pump-over event, but were re-established during the subsequent few hours and reached a saturation point approximately 8 hours post-pump-over with no further extraction.

Thus for Cabernet Sauvignon in the winemaking conditions studied, CS had no impact on color but could lead to increase extraction of seed flavanols if applied for more than four days of CS. Additionally, different pump-over volumes and frequencies did not have a significant influence on phenolic extraction, although it was determined that pump-overs twice a day with as little as half the liquid volume is enough to ensure temperature control of the cap and fermentation homogeneity.

Siriwan Panprivech
TABLE OF CONTENTS

Acknowledgements ............................................................................................................................ iv
Abstract ............................................................................................................................................... v
Table of contents ............................................................................................................................. vii
List of tables ..................................................................................................................................... x
List of figures ................................................................................................................................... xii
Overview of dissertation .................................................................................................................. 1

Chapter 1: Literature review

Phenolic composition of grape and wine ......................................................................................... 3
  Non-flavonoids ................................................................................................................................. 4
    Hydroxycinnamic acids ............................................................................................................... 4
    Hydroxybenzoic acids .............................................................................................................. 5
  Flavonoids ..................................................................................................................................... 5
    Flavanols .................................................................................................................................... 6
    Anthocyanins ............................................................................................................................ 7
    Flavonols .................................................................................................................................... 8
Phenolic extraction from grape into wine during the winemaking process ..................................... 9
Winemaking techniques .................................................................................................................. 10
  Cold soak .................................................................................................................................... 11
  Pump-over .................................................................................................................................... 12
References ....................................................................................................................................... 13
Chapter 2: Investigating the Effect of Cold Soak Duration on Phenolic Extraction During Cabernet Sauvignon Fermentation

Abstract
Introduction
Materials and Methods
Results and Discussion
Conclusions
References

Chapter 3: Impact of Different Cold Soak Durations on Cabernet Sauvignon Fermentation and Phenolic Composition

Abstract
Introduction
Materials and Methods
Results and Discussion
Conclusions
References

Chapter 4: The Effect of Pump-over Conditions on the Extraction Rates of Phenolic Compounds and Their Compositions during Cabernet Sauvignon Fermentation

Abstract
Introduction
Materials and Methods
Results and Discussion
Chapter 5: Chemical Gradients in Pilot Scale Cabernet Sauvignon Fermentations and Their Effect on Phenolic Extraction

Abstract

Introduction

Materials and Methods

Results

Discussion

Conclusions

References
LIST OF TABLES

Chapter 1

Table 1 – Distribution of total phenols (mg/kg FW) in berry component .................. 4

Chapter 2

Table 1 – Chemical composition of finished wines made with different CS durations .................................................. 29

Table 2 – Color density and hue of wines made with different CS durations at the end of CS, the end of alcoholic fermentation (AF), and in bottled wines ........ 32

Table 3 – Concentration of phenolic compounds (mg/L ± SD) in finished wines made with different CS durations .................................................. 37

Chapter 3

Table 1 – A list of red wine attributes ................................................................. 56

Table 2 – The chemical composition of finished wines made with different cold soak durations at the time of bottling ........................................... 57

Table 3 – Color density and hue of wines made with different CS durations at the end of CS, the end of alcoholic fermentation, and after three months of bottle aging .................................................. 61

Table 4 – Concentration of phenolic compounds (mg/L) in finished wines made with different CS durations after three months of bottle aging ........ 72

Table 5 – Anthocyanin composition (mg/L) in finished wines made with different CS durations after three months of bottle aging ..................... 73

Table 6 – Proanthocyanidin composition of wines made with different CS durations at the end of CS, the end of alcoholic fermentation, and after three
months of bottle aging..............................................76

Table 7 – Significant descriptive analysis attributes for difference CS duration wines after six months of bottle aging..................................................80

Chapter 4

Table 1 – Summary of experimental conditions...............................................96

Table 2 – Concentration of phenolic compound (mg/L) in finished wines (five months post-treatment) made with different pump-over conditions in the 2012, 2013, and 2014 harvest.........................................................111

Table 3 – Proantocyanidins composition in finished wines (five months post-treatment) made with different pump-over conditions in the 2012, 2013, and 2014 harvest.........................................................112

Chapter 6

Table 1 – Initial juice panel analysis..............................................................123
LIST OF FIGURES

Chapter 1

Figure 1 – Distribution of phenolic compounds within the grape berry
(image courtesy of Teixeira, et al.) .................................................. 3

Figure 2 – Chemical structures of caftaric acid and coutaric acid present in grapes
and wine................................................................. 5

Figure 3 – Chemical structure of gallic acid........................................ 5

Figure 4 – The flavonoid ring system.................................................. 6

Figure 5 – Chemical structures of flavan-3-ols subunits.......................... 7

Figure 6 – Chemical structure of anthocyanins...................................... 8

Figure 7 – Chemical structure of quercetin........................................ 9

Figure 8 – Different layers of the grape skin (image courtesy of Pinelo, et al.)...10

Chapter 2

Figure 1 – Color density evolution at different CS durations during CS and active
fermentation as determined by UV-vis (n = 3). The end of CS for 1, 4, 7,
and 10 days of CS treatment are marked as a dashed line at 39, 111, 183,
and 255 h, respectively................................................................. 30

Figure 2 – Total anthocyanin concentration in treatments with different CS durations
during CS period and active fermentation, as determined by RP-HPLC
(n = 3). The end of CS for 1, 4, 7, and 10 days of CS treatment are marked
by a dashed line at 39, 111, 183, and 255 h, respectively.........................33

Figure 3 – Tannin concentrations in treatments with different CS durations during
CS period and active fermentation, as determined by the Skogerson–Boulton
model (n = 3). The end of CS for 1, 4, 7, and 10 days of CS
treatments are marked as a dash line at 39, 111, 183, and 255 h, respectively.

Figure 4 – The percentage of skin tannin contribution with different CS durations at the end of CS, the end of fermentation and in finished wines. Means with different letters are significantly different (p < 0.05, n = 3).

Chapter 3

Figure 1 – Evolution of color density of wines made with different CS duration during CS and alcoholic fermentation as determined by analyzing the morning samples of each treatment using UV-vis (n=3). The end of CS for 1, 2, 4, 7, and 10 days of CS treatment are marked as a dashed line at 33, 57, 105, 177, and 249 h, respectively.

Figure 2 – Evolution of (+)-catechin concentration of wines made with different CS duration during CS and alcoholic fermentation as determined by analyzing the morning samples of each treatment using RP-HPLC (n=3). The end of CS for 1, 2, 4, 7, and 10 days of CS treatment are marked as a dashed line at 33, 57, 105, 177, and 249 h, respectively.

Figure 3 – Evolution of total hydroxycinnamate concentration of wines made with different CS duration during CS and alcoholic fermentation as determined by analyzing the morning samples of each treatment using RP-HPLC (n=3). The end of CS for 1, 2, 4, 7, and 10 days of CS treatment are marked as a dashed line at 33, 57, 105, 177, and 249 h, respectively.

Figure 4 – Evolution of total flavonols concentration of wines made with different CS duration during CS and alcoholic fermentation as

xiii
determined by analyzing the morning samples of each treatment using RP-HPLC (n=3). The end of CS for 1, 2, 4, 7, and 10 days of CS treatment are marked as a dashed line at 33, 57, 105, 177, and 249 h, respectively.

Figure 5 – Evolution of total anthocyanin concentration of wines made with different CS duration during CS and alcoholic fermentation as determined by analyzing the morning samples of each treatment using RP-HPLC (n=3). The end of CS for 1, 2, 4, 7, and 10 days of CS treatment are marked as a dashed line at 33, 57, 105, 177, and 249 h, respectively.

Figure 6 – Evolution of non-acylated (a), acetylated (b), and p-coumaroylated (c) anthocyanin concentration of wines made with different CS duration during CS and alcoholic fermentation as determined by analyzing the morning samples of each treatment using RP-HPLC (n=3). The end of CS for 1, 2, 4, 7, and 10 days of CS treatment are marked as a dashed line at 33, 57, 105, 177, and 249 h, respectively.

Figure 7 – Evolution of tannin concentration of wines made with different CS duration during CS and alcoholic fermentation as determined by analyzing the morning samples of each treatment using the Skogerson-Boulton model (n=3). The end of CS for 1, 2, 4, 7, and 10 days of CS treatment are marked as a dashed line at 33, 57, 105, 177, and 249 h, respectively.

Figure 8 – Evolution of anthocyanin (a) and tannin (b) concentrations of wines made with different CS duration during CS and alcoholic fermentation in research- and commercial-scale fermentation as determined by analyzing
the morning samples of each treatment using the Skogerson-Boulton model. For commercial-scale the end of CS for 1, 2, and 7 days of CS treatments are marked as a drop line (solid line) at 24, 48 and 168 h, respectively. For research-scale the end of CS for 1, 2, and 7 days of CS treatments are marked as a drop line (dashed line) at 33, 57 and 177 h, respectively.

Figure 9 - Significant attribute intensities for the different CS duration wines after six months of bottle aging.

Chapter 4

Figure 1 - The extraction profile of malvidin-3-O-glucoside (a), quercetin-glycoside (b), (+)-catechin (c), and tannins (d) of wine made with different pump-over volume during fermentation as determined by RP-HPLC (n=3).

Figure 2 - The extraction profile of caftaric acid wine made with different pump-over volume during fermentation as determined by RP-HPLC (n=3).

Figure 3 - The extraction profile of malvidin-3-O-glucoside (a), quercetin-glycoside (b), (+)-catechin (c), and tannins (d) of wine made with different pump-over frequency during fermentation as determined by RP-HPLC (n=3).

Figure 4 - The extraction profile of malvidin-3-O-glucoside (a), quercetin-glycoside (b), (+)-catechin (c), and tannins (d) of wine made with different low extremes in pump-over volume and frequency as determined by RP-HPLC (n=3).

Figure 5 - The extraction profile of caftaric acid (a), (-)-epicatechin (b), and gallic acid (c) of wine made with different low extremes in pump-over
volume and frequency as determined by RP-HPLC (n=3)..................108

Chapter 5

Figure 1 – (a) Diagram showing the approximate locations of the twelve sampling ports. Each layer of sampling ports (1-4) is labeled. The upper layer of sampling ports (those lying above layer 1) was not used in either the 2011 or 2012 experiments. (b) Photo of a single sampling port showing the stainless steel sieve. (c) Photo of the sampling array strung in the 2000 L fermentor.................................................................123

Figure 2 – Spatial distribution of total anthocyanins during the 2011 Cabernet Sauvignon fermentation as measured by UV-VIS spectrophotometry. (a) Contour plots showing anthocyanin concentrations in mg/L malvidin-3-O-glucoside equivalents throughout the fermentor just prior to a pump-over. Fermentation progress is shown in hours post-inoculation for each contour plot. (b) Anthocyanin concentrations averaged by sampling layers. Layers are color coded to the diagram in Figure 1a.................................128

Figure 3 – Extraction profiles of representative phenolics averaged by sampling layer for the 2011 fermentation as measured by RP-HPLC. Extraction profiles for (a) gallic acid, (b) caftaric acid, (c) (+)-catechin, and (d) malvidin-3-O-glucoside are shown. Extraction profiles are color coded to the diagram in Figure 1a..................................................130

Figure 4 – Spatial distribution of tannins during the 2011 Cabernet Sauvignon fermentation as measured by RP-HPLC. (a) Contour plots of tannin concentration in mg/L (+)-catechin equivalents at various points in the fermentation just prior to pump-over. Fermentation progress is given as
hours post-inoculation. (b) Tannin concentration averaged by level and
color-coded to match the schematic in Figure 1a

Figure 5 – Phloroglucinolysis results from selected time points during the 2011
Cabernet Sauvignon fermentation showing gradients in (a) the mean
degree of polymerization, (b) the percent galloylation, and (c) the
percent seed tannin in the fermenting wine. Layers are color coded
according to the diagram in Figure 1a

Figure 6 – Extraction profiles for malvidin-3-O-glucoside during the 2012
Cabernet Sauvignon fermentation, showing the development of
concentration gradients over a twenty-hour period during (a) period 1
and (b) period 2 as measured by RP-HPLC. Pump-overs are marked
with a dashed vertical line. Coloring corresponds to the diagram shown in
Figure 1a

Figure 7 – Extraction profiles of selected phenolics during the period of extended
maceration (105-321 hours) for the 2012 experiment as measured by
RP-HPLC. The phenolics shown are (a) caftaric acid, (b) malvidin-3-O-
glucoside, (c) (+)-catechin, and (d) tannins. Each averaged sampling layer
is color coded to the diagram shown in Figure 1a
OVERVIEW OF DISSERTATION

In a study reported by the Wine Institute, ninety percent of all U.S. wine is from California. The number of California wineries increased 119 percent from 1870 wineries in 2003 to 4100 wineries in 2013. Therefore, the wine industry is becoming more competitive and it an important issue for wineries to increase the efficiency and quality of their wine production. Winemaking processes or techniques are known to impact wine composition and thus quality. Phenolic compounds are important to red wine quality because they are responsible for the color of wine, and play an important role in flavor specifically bitterness and astringency as well as other mouthfeel attributes. Because of the significant influence of phenolic compounds on red wine quality, many winemaking processes have been developed to enhance the extraction of these compounds during the fermentation process. This dissertation focuses on the potential impact of different cold soak and pump-over techniques on phenolic composition and thus quality. The ultimate aim of this research study is to understand how the selected winemaking processes including cold soak duration and pump-over volume/frequency affect the extraction of phenolics during Cabernet Sauvignon fermentation and the impact on the final bottled wine. This dissertation is divided into six chapters with four main studies.

Chapter 1 provides the background and literature review for the overall study and comprises of two main sections. The first section focuses on phenolic composition of grape and wine and the extraction of phenolics from grape during fermentation. The second section reviews the selected winemaking techniques, cold soak and pump-over.

The first study on the effect of cold soak duration on phenolic extraction is presented in chapter 2. This chapter had been written as a paper entitled, “Investigating the effect of cold soak durations on phenolic extraction during Cabernet Sauvignon
fermentation” which was published in the journal Molecules. The objective of this study is to investigate the effect of cold soak duration on phenolic extraction during cold soak period and fermentation as well as in the final wines.

The second study is presented in chapter 3 and is a continuation of the research described in chapter 2. This study was performed in order to confirm our findings from chapter 2 as well as to determine the potential influence of grape composition on the impact of cold soak on Cabernet Sauvignon composition. Additionally sensory evaluation was performed in the latter to determine the potential impact of cold soak duration on the sensory properties of the wine. Furthermore, a sub-selection of the research-scale experiments was repeated on a commercial scale for a comparison to confirm the impact of cold soak on Cabernet Sauvignon in large-scale fermentations.

The third study presented in chapter 4 discusses the effect of pump-over conditions on phenolic extraction during Cabernet Sauvignon fermentation. This study focuses on both the impact of pump-over volume and frequency. These together with the fourth study were collaborative projects with post-doctoral fellow Dr. Larry A. Lerno and Viticulture and Enology graduate students and undergraduate interns. Dr. Lerno was the lead researcher on the project and first author on the attached draft paper (chapter 4 and 5). Fifty percent of the sample collection and data analysis were completed by myself.

The fourth study investigated the gradients of phenolic composition from the cap and liquid in large-scale fermentation. This study also studied the importance of cap management techniques such as pump-over during alcoholic fermentation to prevent chemical gradients during fermentation and ensure homogenous temperature.

Lastly, chapter 6 provides the summary and conclusion of the research completed and discussed in the previous chapters.
CHAPTER 1

Literature review

Phenolic composition of grape and wine

As previously stated, phenolic compounds are important to the quality of red wine because they are responsible for color, mouthfeel and ageability of wine. Wine phenolic composition depends on the grapes used and winemaking processes that influence their extraction into the must and subsequent reactions. The main phenolic compounds from a wine quality perspective are the anthocyanins, flavanols and their oligomers the proanthocyanidins (generally referred to as tannins), hydroxcinnamic acids, hydroxybenzoic acid, and flavonols. In grapes, the skin and seed are the primary sources of phenolic compounds, with free-run juice containing relatively low concentrations of phenols. The distribution of phenols within the grape berry has been well documented (see Figure 1 and Table 1).

Figure 1. Distribution of phenolic compounds within the grape berry (image courtesy of Teixeira, et al.).
Table 1. Distribution of total phenols (mg/kg FW) in berry component.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% Fresh weight</th>
<th>Red</th>
<th>White</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>15</td>
<td>1800</td>
<td>900</td>
</tr>
<tr>
<td>Pulp</td>
<td>1</td>
<td>40</td>
<td>35</td>
</tr>
<tr>
<td>Juice</td>
<td>78</td>
<td>210</td>
<td>175</td>
</tr>
<tr>
<td>Seed</td>
<td>6</td>
<td>3500</td>
<td>2800</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>5600</td>
<td>3900</td>
</tr>
</tbody>
</table>

All phenolics are based on the primary chemical structure of hydroxybenzene (phenol). Two groups of phenolic compounds in grapes and wine are classically distinguished as non-flavonoids and flavonoids.

**Non-flavonoids**

The main non-flavonoids are phenolic acids, which are usually divided in two main groups: cinnamic acids and benzoic acids. These compounds exist predominantly as hydroxycinnamic acids and hydroxybenzoic acids that may occur either in their free or conjugated form in grape and wine. Although non-colored, the non-flavonoid constituents are known to enhance and stabilize the color of red wines by intra- and intermolecular reactions.

- **Hydroxycinnamic acids**

Hydroxycinnamic acids are based on a C6-C3 structure, meaning that three carbons are attached to the primary benzene ring (six carbons). Hydroxycinnamic acids are mainly present in mesocarp cells of the pulp and also in hypodermal skin cells, and react with anthocyanins as co-pigments, thereby stabilizing color. The most abundant of hydroxycinnamic acids found in grapes as tartaric acid esters are caftaric acid and coutaric acid (Figure 2). Due to the fact that they are mainly localized in the pulp, during crushing they are quickly released into the juice. During the fermentation process, the partial hydrolysis of tartaric esters takes place yielding free.
hydroxycinnamic acids, caffeic and \( p \)-coumaric acids \( ^{10} \). Hydroxycinnamic acids are also potent antioxidants but have no sensory impact except when oxidized, resulting in brown pigments (major effect on white wine color) \( ^{5} \).

Figure 2. Chemical structures of caftaric acid and coutaric acid present in grapes and wine.

**Hydroxybenzoic acids**

Hydroxybenzoic acids are characterized by a C6-C1 structure, which have been identified in both grapes and wine. The most abundant are gallic, \( p \)-hydroxybenzoic, protocatechuic, syringic, and vanillic acids \( ^{8} \). Gallic acid is described as the most important phenolic compound, it does not only originate from the grape itself but is also formed by hydrolysis of condensed tannins (gallic acid esters of flavan-3-ol) \( ^{5,7,8} \). Ethyl esters of gallic acids have also been identified in wine such as epicatechin gallate.

Figure 3. Chemical structure of gallic acid.

**Flavonoids**

Flavonoids have a specific three-ring (C6-C3-C6) structure. A central oxygen containing pyran ring (C-ring) of different oxidation states, is fused to an aromatic ring
(A-ring) along one bond and attached to another aromatic ring with a single bond (B-ring) as seen in Figure 4. The flavonoids found in grapes and wine all have the same hydroxyl substitution groups on the A-ring at position 5 and 7. Differences in the oxidation state and substitution on the C-ring defines the different classes of flavonoids. The substitution pattern on the B-ring defines the member of the class $^5,^7$. There are three main classes of flavonoids; flavanols also known as flavan-3-ols, which are the building blocks of grape tannins; anthocyanins are the red-colored phenols; and flavonols.

![Figure 4. The flavonoid ring system.](image)

- **Flavanols**

  Flavanols are benzopyran that have a saturated carbon chain between carbon position between 2 and 3, a hydroxyl function at position 3 and no carbonyl group at position 4 (Figure 4) $^7$. Flavanols are present in grapes and wines as monomers as well as oligomers and polymers, which are known as proanthocyanidins or condensed tannins. Proanthocyanidins are grape-derived compounds of great importance to red wine quality, which contribute to the bitterness and astringency of the wines. Flavanols appear mainly as four monomeric units: (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, and (-)-epicatechin-3-O-gallate (Figure 5). They are found primarily in the grape skins (epidermal and hypodermal cell layers) and seeds (parenchyma cell layer) $^9$ but the four monomeric units are distributed differently within the berry components. Grape seed proanthocyanidins comprise (+)-catechin, (-)-epicatechin, and
(-)-epicatechin-3-0-gallate, whereas grape skin proanthocyanidins additionally contain
(-)-epigallocatechin \(^\text{11-14}\). Skin proanthocyanidins have a higher mean degree of
polymerization (mDP), typically about 20-40 subunits than seed proanthocyanidins
(around 8-12 subunits). Skin proanthocyanidins have a lower proportion of galloylated
subunits (epicatechin gallate) than those in the seeds \(^\text{15,16}\). Flavanols are released from
both grape skins and seeds during winemaking. However, the amount of flavanols
extracted will be affected by the maceration practices used.

\[
\begin{align*}
\text{(-)-Epicatechin-3-O-gallate} & \quad \text{(-)-Epigallocatechin} \\
\text{(-)-Epicatechin} & \quad \text{(-)-Catechin}
\end{align*}
\]

**Figure 5.** Chemical structures of flavan-3-0ls subunits.

- **Anthocyanins**

Anthocyanins are co-located with tannins in the hypodermal cells of grape skin
for most *Vitis vinifera* varieties including Cabernet Sauvignon. Teinturier cultivars
(e.g., Alicante Bouchet) also contain anthocyanins in the pulp \(^\text{17}\). Anthocyanins are
responsible for the color of grapes and wine, a characteristic that is determined by their
chemical structure, their degree of hydroxylation, methylation, and/or glucosylation \(^\text{7,18}\).
There are five anthocyanins in grape and wine (Figure 6): cyanidin (orange red),
peonidin (red), delphinidin (bluish red), petunidin (bluish red), and malvidin (bluish
red). Anthocyanins are usually found as glycosylated anthocyanins, a sugar molecule bound to the anthocyanin moiety. These base compounds are also found as acylated anthocyanins with acetic, p-coumaric, and caffeic acids, which linked to the sugar molecule 5, 17.

![Chemical structure of anthocyanins.](image)

**Figure 6.** Chemical structure of anthocyanins.

The degradation of grape cell walls starts with crushing. The diffusion process is favored by the water-soluble nature of anthocyanins, resulting in a peak of extraction within the third to fifth day of maceration and fermentation. Following the peak of anthocyanin extraction, a drop in their concentration is typically observed 19-21. The loss of anthocyanins during the latter stage of maceration and fermentation has been attributed to many factors, including adsorption onto grape solids or yeast cell walls, incorporation into polymeric pigments, formation of pyranoanthocyanins, and oxidative cleavage of the heterocyclic C ring leading to direct anthocyanin degradation 13, 15, 18, 19, 21-23.

- **Flavonols**

Flavonols are characterized by the presence of a double bond between atoms C2 and C3, and a hydroxyl group at position 3. Flavonols are yellow pigments, as they strongly absorb light in the visible range (360 – 400 nm) 5. They are important co-factors for the color-enhancing phenomenon known as copigmentation. Flavonols are always found in a glycosidic form (glucoside, glucoronide, and galactoside) in grape berries. Flavonols in the glycosidic form are co-located with the anthocyanins in the
grape skin (epidermal cells layer). During winemaking and aging glycosylated form of flavonols is hydrolyzed and the aglycone form is released such as quercetin from quercetin-glycoside (Figure 7). Whereas the glycosylated flavonols are partially water soluble and more soluble in ethanol wine, the flavonols aglycones are poorly soluble in wine.\textsuperscript{5, 7, 9, 13}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{quercetin.png}
\caption{Chemical structure of quercetin.}
\end{figure}

**Phenolic extraction from grape into wine during the winemaking process**

During the red winemaking process, the transfer of phenolic compounds from the red grapes to the must mainly takes place from the grape skins during the maceration step, which for red wines takes place directly on the crushed berries.\textsuperscript{24} The grape skin can be divided into three layers (Figure 8): the outermost layer, the cuticle is composed of hydroxylated fatty acid called cutin, and is covered by hydrophobic waxes; the intermediate epidermis; and the inner layer, the hypodermis, which is composed of several cell layers that contain most of the phenolics in grape skin (anthocyanins, flavonols and tannins).\textsuperscript{25} During winemaking process, juice is released from the broken grape cells (mesocarp cells). Phenolic are extracted from skins directly through the epidermal cell layer, but the extraction cannot be accomplished. Diffusion is required for extraction into the inner layer of hypodermal cell where anthocyanins, flavonols and tannins are located.\textsuperscript{9} The releases of phenolic compounds from seeds occur after the seeds have attained a certain hydration level. Once seeds have reached
hydration, the leakiness of parenchyma cells outside the seed coat allows the effective release of phenolics into the wine independent of the ethanol concentration \(^{13, 19, 26, 27}\).

However, the concentration of phenolic extracted in red wines is dependent on the grape (original concentration) and the impact of different vinification factors on extraction. The extraction rate of phenolics during winemaking is primarily a function of the following: grape maturity, temperature, contact time, and alcohol content \(^{3, 7, 9}\).

![Figure 8.](image) Different layers of the grape skin (image courtesy of Pinelo, et al. \(^{24}\)).

**Winemaking techniques**

Winemaking variables and techniques are known to affect the phenolic composition of red wines. Fermentation temperature, thermovinification, must freezing, saignée, pectolytic enzyme treatments, and extended maceration have been reported to increase phenolic concentration. Sulfur dioxide additions, cold soak, cabonic maceration, yeast selection, and skin and juice mixing practices have produced variable results with respect to phenolic extraction \(^{3}\). The following techniques were selected to study on the impact of winemaking techniques and variables on phenolic extraction in this dissertation: cold soak and pump-over.
Cold soak

The application of low temperature (10-15°C) maceration prior to fermentation is known as pre-fermentative cold maceration or cold soak. This technique consists of keeping the crushed grapes at low temperature for several days prior to primary fermentation to improve the extraction of pigments, tannins and aromas from the grape skins to the wine. Thus, extraction takes place in the absence of ethanol because these low maceration temperatures prevent yeasts from starting the fermentation process. Gómez-Míguez, et al. showed that pre-fermentative cold maceration of Syrah grapes at 15 °C for seven days was a useful technique to increase the extraction of anthocyanins and other phenolic compounds, to obtain darker and less brown wines. De Santis and Frangipane proved that a Merlot wine produced with cold soak (8 °C for four days) had a higher concentration of phenolic compounds than wines produced with traditional maceration. Gil-Muñoz, et al. found that the cold soak (10°C for seven days) promoted a high level of anthocyanins extraction in Cabernet Sauvignon wines, whereas in Syrah greater results were obtained using dry ice and frozen grapes techniques. Busse-Valverde, et al. reported that cold soak (10°C for 10 days) increased the proanthocyanidin concentration in Monastrell and Cabernet Sauvignon wines but had no effect on Syrah wines.

A study with Pinotage also found that cold soak at 10°C can improve the quality of wine but they did not produce large difference in phenolic compounds of the finished wine, and decreased concentration of acetate and ethyl esters with an increase in cold soak skin contact time at 15°C for 2 and 4 days. Reynolds, et al. found that Shiraz wine displayed increase in anthocyanin extraction when combining cold soak (2°C for 10 days) with lower fermentation temperature (15 and 20°C), but not high fermentation temperature (30°C). Moreover, Cejudo - Bastante, et al. studied
the effect of the time of cold soak on the evolution of phenolic compounds and color of Syrah wine. They demonstrated that 12 days cold maceration time resulted in wines with higher phenolic content and also more stable color with more red bluish tonalities than shorter cold maceration time (8 days) and traditional maceration. Another study with cold soak duration showed 4 and 10 days cold soak had no significant effect on proanthocyanidin concentration of Pinot noir wine. In addition, the effect of different temperature maceration on sensory properties was studied by Damijanić, et al. The results showed that the prefermentative cryomaceration (5 °C for five days with dry ice) increased red color, fruity flavor and good body/finish in Teran wines. Parenti, et al. also reported that Sangiovese wines obtained with cold maceration at 5 °C with solid CO2 and at 0°C and 5 °C with liquid nitrogen showed greater intensity of all the descriptors (color, flavor, and balance) than traditional wine.

Cold soak studies have produced variable results, depending mainly on the cold soak conditions (duration and temperature) and the grape varieties studied. More research is needed on this subject.

Pump-over

During fermentation, the cap rises to the top due to carbon dioxide production, which reduces contact between the skins and seeds and the juice and affects extraction from the skins and seeds. Additional heat from fermentation is trapped in the cap, as it is a poor conductor of heat. High temperature in the cap could inactivate the yeast there impacting fermentation rate. To overcome these problems, the cap and juice are mixed by doing pump-overs, a cap management techniques used in red winemaking. Essentially it is a technique that improves mixing of the cap and the juice by spraying the liquid below over the top of the cap in order to promote
extraction of color, and flavor constituents in addition to cooling the cap \(^{40}\). Fischer, et al. \(^{41}\) compared the effect of manual punch-down, mechanical punch-down and pump-over treatments on wine phenolic compounds for three grape varieties Pinot noir, Dornfelder and Portugieser. Both the mechanical punch-down and pump-over enhanced the extraction of all phenolic compounds in comparison to the manual punch-down. The pump-over gave higher quercetin level in all varieties of wines. A study with Pinotage comparing manual punch-down, pump-over and rotary mixing found that lower content of total flavonoids, total tannins and anthocyanins were achieved for pump-over \(^{42}\). Discrepancies in the results are likely due to the type of pump-over operation performed and the length of time it is performed as well as the initial grape composition \(^{3}\).

References


CHAPTER 2

Investigating the Effect of Cold Soak Duration on Phenolic Extraction During Cabernet Sauvignon Fermentation

Abstract

The impact of increasing cold soak (CS) duration (0, 1, 4, 7, and 10 days at 10 °C) on the extraction of phenolic compounds during the CS period and primary fermentation as well as the final composition of Cabernet Sauvignon wine was investigated. The results showed that CS duration had no effect on hydroxycinnamate and flavonol extractions. Greater amounts of gallic acid, (+)-catechin, (-)-epicatechin, and total tannins were extracted with increasing CS duration, with differences maintained during bottle aging. Anthocyanin extraction and color density increased with longer periods of CS; however, by the end of primary fermentation, as well as three months’ bottle aging, there were no significant differences due to CS duration. The wines made with seven and 10 days of CS had higher seed tannin contributions and total tannin compared to the non-CS wine, which could potentially result in increased astringency.

Introduction

Phenolic compounds are important to red wine quality, as they are responsible for the color, mouthfeel, and ageability of wine. The phenolic composition of wine depends on the grapes used and also on the winemaking processes, as these will influence phenolic extraction into the must as well as subsequent reactions. The main phenolic compounds from a wine quality perspective are the anthocyanins, flavanols (including the oligomeric proanthocyanidins also referred to as condensed tannins),
hydroxycinnamic acids, and flavonols. Phenolics are distributed throughout the grape berry, being found in the pulp, juice, skin, and seeds. Each component of the grape berry contains different classes of phenolic compounds, with each class contributing differently to the sensory properties of the wine. Proanthocyanidins and monomeric flavanols are found primarily in the grape skins and seeds and contribute to the bitterness and astringency of the wine. Anthocyanins are red pigments and the principal source of pigmentation in red wine. Anthocyanins are found in the skin of the grape berry for most Vitis vinifera varieties including Cabernet Sauvignon, as well as in the pulp of teinturier cultivars (e.g. Alicante Bouschet). Hydroxycinnamates are present throughout the grape berry, and react with anthocyanins as co-pigments, thereby stabilizing the color. Hydroxycinnamates are also strong antioxidants, and when oxidized can form brown pigments. The brown form has an effect on the color of white wine but only has a minor effect on the color of red wine. The flavonols are yellow pigments found in the cells of the grape skin. While less abundant than the other phenolics, the flavonols contribute to wine color as they are co-factors/pigments similar to hydroxycinnamates contributing to the color-enhancing phenomenon known as copigmentation.

Due to the significant influence of phenolic compounds on red wine quality, many winemaking processes have been developed to enhance the extraction of these compounds. One such process is cold soak (CS), a period of prefermentative maceration lasting for several days (typically one to 10 days) in which the temperature of the must is kept low enough to prevent spontaneous fermentation (10–15 °C). It is claimed that CS favors the extraction of the more hydrophilic phenolic compounds, such as the anthocyanins, in the aqueous environment of the must as well as favoring skin tannin extraction over seed extraction. Several studies examined the effect of CS
on anthocyanin concentration in red wine with varying results. Gómez-Míguez, et al. showed that prefermentative cold maceration of Syrah grapes at 15 °C for seven days was successful at increasing the extraction of anthocyanins and other phenolic compounds, producing wines that were darker in color and less brown. De Santis and Frangipane showed that a Merlot wine produced with CS at 8 °C for four days had a higher concentration of anthocyanins and volatile compounds than wines produced with traditional maceration. The CS technique promoted a high level of anthocyanin extraction in Cabernet Sauvignon wines when kept at 10 °C for seven days according to Gil-Muñoz, et al. Busse-Valverde, et al. reported that CS (10 °C for 10 days) increased the seed proanthocyanidin concentration in Monastrell and Cabernet Sauvignon wines but had no effect on Syrah wines. Subsequently, they found that although CS also increased the extraction of anthocyanins in Monastrell wine it was not significant by the end of fermentation.

A study with Pinotage also found that CS at 10 °C improved the quality of the wine but did not produce a large difference in the phenolic compounds of the finished wine. CS decreased the concentration of acetate and ethyl esters with an increased skin contact time at 15 °C for two or four days prior to fermentation. Reynolds, et al. found that Shiraz wine displayed an increase in anthocyanin extraction when CS (2 °C for 10 days) was combined with lower fermentation temperatures (15 and 20 °C), but not when combined with a high fermentation temperature (30 °C). Moreover, the effect of the time of CS on the evolution of phenolic compounds and color of Syrah wine was studied and it was found that 12 days’ cold maceration time resulted in wines with higher phenolic content in addition to more stable color with more red-bluish tonalities than shorter cold maceration time (8 days) and traditional maceration wines. Peyrot
des Gachons and Kennedy\textsuperscript{14} showed that a CS of 4 and 10 days had no significant effect on the concentration of proanthocyanins in the final Pinot noir wine.

Studies investigating CS have produced variable results, depending mainly on the duration of CS and grape variety. Previous studies have mostly focused on how CS affects the color and phenolic composition of the finished wine and did not evaluate phenolic evolution during the CS period or fermentation. This study investigated how CS duration affects phenolic extraction during the CS period and active fermentation as well as the final composition of Cabernet Sauvignon wine.

**Materials and Methods**

**Harvest and Winemaking**

Approximately 3000 kg of Cabernet Sauvignon grapes were harvested in 2013 from Lodi, California and received by the UC Davis Teaching and Research Winery (Davis, CA, USA) and immediately processed. The chemical characteristics of the grapes at harvest were 26 °Brix, 4.4 g/L tartaric acid (TA), and pH of 3.7. Clusters (approximately 300 berries) were randomly selected and stored at \(-20\,^\circ\text{C}\) for further analysis prior to crushing. The grapes were destemmed and crushed using a Bucher Vaslin Delta E2 (Santa Rosa, CA, USA) directly into a Bucher Vaslin PMV must pump. The must was pumped into UC Davis/Cypress Semiconductor research fermentors. An addition of 15% potassium metabisulfite solution was performed for all fermenters, giving a final concentration of 60 mg/L sulfur dioxide (SO2).

To evaluate the effect of CS duration on grape skin and seed phenol extraction, 15 research-scale (75 L) fermentations were performed in jacketed, cylindrical, variable-capacity, stainless steel fermentor tanks with the fermentation conditions controlled by the Integrated Fermentation Control Systems (IFCS) units. All
fermentations were cooled to 10 °C (overnight using jacket temperature and mixing) following addition of SO2 to prevent spontaneous fermentation. Experimental treatments were performed in triplicate with five CS durations investigated (0, 1, 4, 7, and 10 days). During CS pump-overs were performed twice per day with each pump-over volume being half of the must volume. At the end of the CS duration fermentations were heated to 25 °C prior to inoculation with Saccharomyces cerevisiae strain D254 (Lallemand Lalvin®). During fermentation two fermentor volumes were pumped over twice daily. All fermentations were performed at 25 °C with temperature maintained by means of the water jacket. Prior to inoculation, diammonium phosphate was added to increase the yeast assimilable nitrogen to 300 mg/L and tartaric acid was adjusted to 6 g/L. All treatments were sampled twice a day following pump-overs during the CS period and fermentation. The first sampling point was when CS maceration temperature was reached (10 °C) and fermentation started (conversion of sugar) in the no CS treatment, approximately 15 h after yeast inoculation. The total skin contact time of treatments after the end of alcoholic fermentation were 7.6, 9.6, 12.6, 15.6, and 18.6 days for 0, 1, 4, 7, and 10 days of CS treatment, respectively. Samples were centrifuged (Eppendorf Centrifuge 5810R, Eppendorf AG, Hamburg, Germany) at 3220 rpm for 15 min at 4 °C and subsequently stored at −20 °C until analysis.

°Brix measurements were measured manually with an Anton Parr DMA35 density meter (Anton Parr, Ashland, VA, USA). Fermentations were pressed at 0 °Brix using a prototype Cypress Semiconductor Corporation hydraulic basket press. Finished wines were inoculated with Oenococcus oeni (Chr. Hansen, Inc., Milwaukee, USA) and all wines completed malolactic fermentation within four weeks.
After completion of malolactic fermentation, the free sulfur dioxide concentration was adjusted to 30 mg/L with the addition of a 15% potassium metabisulfite solution. All treatments were sterile filtered and bottled in 750 mL screw top bottles (Bordeaux style, green glass), purged with nitrogen gas prior to filling. The finished wines were stored at 14.4 °C in the Teaching and Research Winery at UC Davis until analysis three months after bottling.

The wine chemical compositions were determined at time of bottling for all treatments. The ethanol content was measured with an Alcolyzer (Anton Parr, Ashland, VA, USA). The pH was measured using an Orion 5-star pH meter (Thermo Scientific, MA, USA). The total acidity was measured automatically with the Mettler-Toledo DL50 titrator (Mettler-Toledo Inc., OH, USA). The measurements of acetate, malate, and residual sugar were made using the Thermo Scientific Gallery automated analyzer (Thermo Scientific, MA, USA).

Grape Skin and Seed Tannin Extraction

Four sets of 20 berries were chosen at random from frozen clusters. Skins and seeds were separated from the berry mesocarp with a scalpel and were washed with deionized water, patted dry with paper towels, and weighed. The skins and seeds were extracted separately with 0.1 g of samples per 1 mL of 1:1 ethanol:water containing 0.1% v/v HCl and 0.1% w/v ascorbic acid. All samples were homogenized using an IKA ULTRA-TURRAX®T18 basic (IKA® Works, Inc., NC, USA) and allowed to extract at 4 °C overnight. The samples were centrifuged at 3220 rpm for 15 min, after which the supernatant was collected. The homogenized samples were subsequently extracted with a 70:30 acetone:water solution containing 0.1% w/v ascorbic acid, which was added in the same ratio of sample to solvent as that of the ethanol solution and allowed to extract at 4 °C overnight. Samples were then centrifuged and the
supernatants combined prior to concentration under reduced pressure at 35 °C followed by lyophilization.

**Determination of Color and Adams–Harbertson Assay Correlation**

Absorbance measurements were made using a Hewlett-Packard 8453 UV-vis spectrophotometer (Hewlett-Packard, Palo Alto, CA, USA) with 0.1 mm path length flow cell (Starna Cells, Atascadero, CA, USA). The frozen samples were thawed at room temperature, centrifuged, and absorption spectra were collected from 230–900 nm. Color density was calculated as the sum of absorbance at 420, 520, and 620 nm, and hue was calculated as the ratio between absorbance at 420 and 520 nm. The predicted Adams–Harbertson values for tannins with the coefficient of determination of ($r^2$) 0.86 were generated for all samples using the Skogerson–Boulton model.15

**Reagents**

Gallic acid monohydrate, (+)-catechin, (-)-epicatechin, caffeic acid, quercetin, quercetin-rhamnoside, trans-ferulic acid, and p-coumaric acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Malvidin-3-O-glucoside chloride was purchased from Extrasynthese (Genay, France). Acetonitrile (Sigma Chemical Co., St. Louis, MO, USA) and formic acid (Fisher Scientific, Bridgewater, NJ, USA) were HPLC grade. HPLC-grade water was prepared in house to a final resistance of 18 MΩ and filtered through a 0.22 µm filter prior to use.

**Reversed Phase HPLC (RP-HPLC) Analysis of Monomeric Phenols**

Frozen wine samples were thawed, centrifuged, and filtered through 0.45 µm PTFE syringe-tip filters (Econo filter 25 mm, Agilent, Wilmington, DE) prior to analysis. All wine samples were analyzed by RP-HPLC using an Agilent (Santa Clara, CA, USA) 1260 Infinity HPLC equipped with a binary pump, column compartment, and diode array detector. The column used was an Agilent Poroshell 120 SB-C18 (4.6
× 150 mm. 2.7 µm particle) maintained at 35 °C. The mobile phases used for the separation were solvent A (water with 5% v/v formic acid) and solvent B (10% v/v solvent A in acetonitrile). The mobile phase flow rate was set at 1.25 mL/min and 20 µL injection volumes were used for all samples. The gradient for the separation was 0–23 min, 5–27% B; 23–24 min, 27–95% B; 24–26 min, 95% B; 26–26.5 min, 95–5% B; 26.5–32 min, 5% B. Eluting peaks were monitored at 280 (gallic acid, (+)-catechin, (-)-epicatechin, polymeric phenols), 320 (caftaric acid, caffeic acid, coutaric acid, p-coumaric acid), 370 (quercetin-3-galactoside, quercetin-3-glucuronide, quercetin-3-glucoside, quercetin), and 520 nm (anthocyanins, polymeric pigment). Compounds eluting from the HPLC were identified and quantified based on spectral and retention time comparisons to authentic standards. Phenolics were quantitated by external calibration, with calibration curves generated for gallic acid, (+)-catechin, (-)-epicatechin, caffeic acid, quercetin, quercetin-rhamnoside, p-coumaric acid, and malvidin-3-O-glucoside chloride. Chromatograms were integrated using Agilent® CDS ChemStation software. Compounds were quantified as themselves if an authentic standard was available; otherwise they were quantified as follows: polymer phenols as (+)-catechin equivalents; caftaric acid as caffeic acid equivalents; coutaric acid, as p-coumaric acid equivalents; quercetin-3-galactoside, quercetin-3-glucuronide, quercetin-3-glucoside as quercetin-3-rhamnoside equivalents; and anthocyanins and polymeric pigments as malvidin-3-O-glucoside chloride equivalents.

**Isolation and Characterization of Proanthocyanidins**

Solid phase extraction (SPE) was performed to isolate tannins in triplicate for each sample using the method of Oberholster, et al. The proanthocyanidins were purified using Toyopearl® HW-40F size exclusion media. The columns were packed to a bed volume of 10 mL and equilibrated with 20 mL of 55:45 ethanol/water.
containing 0.05% v/v trifluoroacetic acid (TFA). Lyophilized seed and skin extracts were dissolved in 15% methanol solution at concentrations of 5 mg/mL and 10 mg/mL respectively. Reconstituted extracts were centrifuged at 3220 rpm for 15 min and loaded onto columns, with 1 mL for seed and 2 mL for skin extract solutions. Wine samples were thawed and centrifuged, and 2 mL were loaded onto the conditioned column. Sugars, protein, low molecular weight flavan-3-ols (monomers and dimers), and all other monomeric phenols were eluted with 40 mL of 55:45 ethanol:water containing 0.05% TFA. The proanthocyanidins were then eluted with 30 mL of 60:40 acetone:water containing 0.05% TFA. The proanthocyanidin fraction was concentrated under reduced pressure at 35 °C to remove all solvents and then dissolved in 0.5 mL of methanol. The concentrated proanthocyanidin samples were stored at −20 °C for a maximum of one month prior to analysis by phloroglucinolysis.

The isolated proanthocyanidins were analyzed using the phloroglucinolysis method optimized by Kennedy and Jones. A phloroglucinol solution of 0.2 N HCl in MeOH containing 100 g/L phloroglucinol and 20 g/L ascorbic acid was prepared. The phloroglucinolysis reactions were performed in duplicate. Equal volume aliquots of the proanthocyanidin fraction and phloroglucinol solution were mixed and heated at 50 °C for 20 min. The reaction was quenched by the addition of five reaction volumes of 40 mM aqueous sodium acetate. Quenched digests were centrifuged for 5 min at 13000 rpm (Eppendorf Centrifuge 5415D) and transferred into an HPLC vials. Due to the instability of the cleavage products, sample vials were held for a maximum of 12 h. An Agilent® Infinity series 1260 HPLC was used for all phloroglucinolysis analyses. Phloroglucinolysis reaction products were analyzed using RP-HPLC with an Agilent Poroshell 120 SB-C18 (4.6 × 150 mm, 2.7 µm particle) HPLC column utilizing a binary gradient system of water with 0.1% formic
acid (mobile phase A) and acetonitrile with 0.1% formic acid (mobile phase B). The mobile phase flow rate was set at 2.0 mL/min, 10 µL injection volumes were used for seed samples, and 20 µL injection volumes were used for skin and wine samples. The gradient for the separation was 0–2.96 min, 3% B; 2.96–10.30 min, 3%–16% B; 10.30–10.40 min, 16%–20% B; 10.40–12.10 min, 20% B; 12.10–13.0 min, 20%–80% B; 13.0–14.34 min, 80% B; 14.34–15.34 min, 80%–3% B; 15.34–20.0 min, 3% B. The column temperature was maintained at 35 °C and the eluting peaks were monitored at 280 nm. Quantitation of reaction products was performed using an external calibration generated with (+)-catechin using their response factor relative to catechin and with molar extinction coefficients corrected using values determined by Kennedy and Jones. The chromatographs were integrated using Agilent® CDS ChemStation software. Tannin concentration, mean degree of polymerization (mDP), percentage galloylation, and percentage gallo units were determined for each sample. The percentage of seed and skin tannin contribution in wine samples was also calculated by comparing the proportional extension subunit composition in wine relative to the proportional extension subunit in the corresponding grape.

Statistical Analysis

Statistical analysis was performed using R (ver. 2.15.1). Fisher’s least significant differences (LSD) were used to discriminate the means between all fermentation treatments using the function found in the Agricolae package, which was built under R version 2.15.1.
Results and Discussion

Chemical Composition of the Finished Wine

The chemical composition of the wines made with different CS durations was determined at the time of bottling (Table 1). The results indicate that increasing the duration of CS had no effect on the basic chemical composition of the resulting wines (the percentage ethanol, pH, total acidity, acetic acid, malic acid, and residual sugar).

Table 1. Chemical composition of finished wines made with different CS durations.

<table>
<thead>
<tr>
<th>Cold Soak Duration</th>
<th>% Ethanol</th>
<th>pH</th>
<th>Total Acidity (g/L)</th>
<th>Acetic Acid (g/L)</th>
<th>Malic Acid (mg/L)</th>
<th>Residual Sugar (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day</td>
<td>15.06 ± 0.10a</td>
<td>3.81 ± 0.02a</td>
<td>5.34 ± 0.03a</td>
<td>0.38 ± 0.01a</td>
<td>45 ± 4.0a</td>
<td>0.36 ± 0.01a</td>
</tr>
<tr>
<td>1 day</td>
<td>14.96 ± 0.08a</td>
<td>3.82 ± 0.03a</td>
<td>5.39 ± 0.03a</td>
<td>0.38 ± 0.01a</td>
<td>48 ± 1.0a</td>
<td>0.36 ± 0.02a</td>
</tr>
<tr>
<td>4 days</td>
<td>14.92 ± 0.10a</td>
<td>3.81 ± 0.05a</td>
<td>5.41 ± 0.14a</td>
<td>0.37 ± 0.02a</td>
<td>47 ± 2.0a</td>
<td>0.37 ± 0.01a</td>
</tr>
<tr>
<td>7 days</td>
<td>14.92 ± 0.02a</td>
<td>3.82 ± 0.03a</td>
<td>5.48 ± 0.03a</td>
<td>0.38 ± 0.02a</td>
<td>49 ± 3.5a</td>
<td>0.36 ± 0.02a</td>
</tr>
<tr>
<td>10 days</td>
<td>14.82 ± 0.22a</td>
<td>3.80 ± 0.04a</td>
<td>5.49 ± 0.17a</td>
<td>0.38 ± 0.01a</td>
<td>52 ± 0.6a</td>
<td>0.34 ± 0.01a</td>
</tr>
</tbody>
</table>

Notes: Means ± SD followed by same letter within the same column indicates no significant difference (p < 0.05, n = 3).

Chromatic Composition of the Must and Wines

The evolution of color density during CS and active fermentation at different CS durations as determined by UV-vis are shown in Figure 1 (end of CS marked by a dashed line). The significant difference in color density between CS and non-CS (control) treatments is due to the fact that the first sampling point was at the start of fermentation for the non-CS treatment (15 h after inoculation) with simultaneous sampling of the different CS duration treatments as they reached CS maceration temperature (10 °C) at the same time point. This difference represents the impact of temperature on extraction as the control non-CS treatment was maintained at 25 °C whereas CS treatments (1 to 10 days) were cooled to reach CS maceration temperature.
(10 °C) at this point. Thus the end of CS for 1 day is shown at 39 h (time from start of cooling of different CS duration treatments in addition to actual CS maceration time at 10 °C) on the time line and subsequently 111, 183, and 255 h for 4, 7, and 10 days of CS. From the results shown in Figure 1, it can be seen that the color density increased with a longer cold maceration, although it did not persist beyond the CS period. The greatest color density at the end of the CS period was observed for the 10 days’ CS treatment at 4.14 AU (Table 2). These differences persisted for the first two days of active fermentation, but by day 4 few differences existed in color density for the treatments. By the end of fermentation there were no significant differences in color density values among the different CS duration wines.

![Figure 1](image_url)

**Figure 1.** Color density evolution at different CS durations during CS and active fermentation as determined by UV-vis (n = 3). The end of CS for 1, 4, 7, and 10 days of CS treatment are marked as a dashed line at 39, 111, 183, and 255 h, respectively.

All finished wines were also analyzed five months after the end of treatment (three months’ bottle aging), and their color density and hue value are shown in Table 2. No significant differences were seen in either the color density or the hue among the different CS treatments in the bottled wine. The color density of the wines decreased...
by approximately 11% during the five-month post-treatment period, with a simultaneous increase of approximately 30% in hue. The decrease in color density and increase in hue value during aging are due to the loss of free anthocyanins as a result of polymerization and other modification reactions with other compounds in red wine to form polymeric pigments as well as degradation reactions \(^{18-20}\). The concentration of anthocyanins in red wines changes significantly during the first year of storage. Potential degradation reactions include glycoside hydrolysis or breakdown of the carbon chain of the chalcone molecule as a result of a shift in the equilibrium towards the colorless chalcone form \(^{21}\).
Table 2. Color density and hue of wines made with different CS durations at the end of CS, the end of alcoholic fermentation (AF), and in bottled wines.

<table>
<thead>
<tr>
<th>Absorbance Unit</th>
<th>Cold Soak Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Day</td>
</tr>
<tr>
<td><strong>Color density</strong></td>
<td></td>
</tr>
<tr>
<td>(420 + 520 + 620 nm)</td>
<td></td>
</tr>
<tr>
<td>End of CS</td>
<td>−</td>
</tr>
<tr>
<td>End of AF</td>
<td>7.68 ± 0.09a</td>
</tr>
<tr>
<td>Bottle</td>
<td>6.96 ± 0.12a</td>
</tr>
<tr>
<td><strong>Hue (420/520 nm)</strong></td>
<td></td>
</tr>
<tr>
<td>End of CS</td>
<td>−</td>
</tr>
<tr>
<td>End of AF</td>
<td>0.47 ± 0.01b</td>
</tr>
<tr>
<td>Bottle</td>
<td>0.73 ± 0.01a</td>
</tr>
</tbody>
</table>

Notes: Means ± SD followed by same letter within the same row indicates no significant difference (p < 0.05, n =3). (−): no analysis as there is no end of CS for non-CS treatment.
Figure 2. Total anthocyanin concentration in treatments with different CS durations during CS period and active fermentation, as determined by RP-HPLC (n = 3). The end of CS for 1, 4, 7, and 10 days of CS treatment are marked by a dashed line at 39, 111, 183, and 255 h, respectively.

Monomeric phenol concentrations were determined by RP-HPLC and tannin concentration was estimated by UV-vis using the Skogerson–Boulton model. Phenolic extraction profiles in the must and wine for different CS durations during CS, active fermentation, and in the finished wine after three months of bottle aging were determined. The extraction profiles of both hydroxycinnamates (caffeic acid, caftaric acid, coutaric acid, p-coumaric acid) and flavonols (quercetin, quercetin-glycosides) were similar to the extraction profile shown for total anthocyanins in Figure 2. Hydroxycinnamate extraction increased with CS duration but by the end of primary fermentation there were no significant differences between the different CS treatments. Although the extraction profile of the flavonols (quercetin and quercetin-glycosides) were similar to the hydroxycinnamates, a lower percentage was extracted during CS durations compared to during active fermentation due to lower solubility of the flavonols in the aqueous must. Higher temperature and increasing ethanol content
during fermentation increased extraction and solubility \(^1\), resulting in similar concentrations in the final wines made with different CS durations. Thus CS duration had no significant impact on the hydroxycinnamate and flavonol content of the finished wine (Table 3).

The extraction profiles of total anthocyanins during different CS durations and primary fermentation are shown in Figure 2. The evolution of extracted anthocyanins showed an almost constant increase during the CS period for all treatments. However, for the 7- and 10-day CS, treatments a maximum was reached after five days of CS, followed by a decrease during the remainder of the CS period. According to Singleton and Trousdale \(^{22}\), this decrease is due to the fact that during the maceration period, parallel to the extraction, the anthocyanins are slowly reacting with other compounds. Another possibility is readsorption of the extracted anthocyanins on to the grape cell walls, similar to their adsorption on to yeast cell walls during fermentation \(^{23}\). The significantly higher anthocyanin concentration for the non-CS treatment compared to the CS treatments at the first sampling point is due to faster extraction of anthocyanins at higher temperatures (25 °C vs 10 °C), as discussed previously in Section 2.2. The 1-, 4-, 7-, and 10-day treatments showed significantly higher anthocyanin concentrations at the start of fermentation (sample point after dashed lines) when compared to the control (first sample point). Extraction of anthocyanins increased during fermentation due to increased fermentation temperature and ethanol generation \(^1\). The musts with longer CS duration, especially the 10-day CS treatment, also showed greater anthocyanin extraction during active fermentation, which is in agreement with a study that compared no CS with eight and 12 days of cold maceration \(^{13}\). The 10-day CS treatment showed greater rates of extraction during the first four days of fermentation (Figure 2). Faster extraction rates with longer CS periods could be due to increased
permeability of the cell membranes as a result of longer contact time, which only becomes apparent during fermentation due to solubility limitations in the 10 °C aqueous must. However, at the end of fermentation all the treatments had similar concentrations of extracted anthocyanins, which persisted with bottle aging (Table 3). There were, however, significant differences in acylated anthocyanins. Both peonidin-3-glucoside-acetate and malvidin-3-glucoside-acetate concentrations were significantly lower in the finished wines made with longer CS duration (seven and 10 days) compared with the control. It has been shown that the profile of anthocyanin derivatives can be influenced by adsorption of anthocyanins onto yeast cell walls. The acylated anthocyanins are more strongly adsorbed onto yeast cell walls than non-acylated anthocyanins and the same may potentially be true for grape cell walls.

The amount of tannin extracted during CS and primary fermentation for different CS durations is shown in Figure 3. Increased amounts of tannin were extracted in the treatments with increasing CS duration, although there were no significant differences in total tannin concentration among the 1- to 10-day CS treatments at the end of CS. This is due to slow extraction into the aqueous medium, potentially reaching a temporary saturation point at 10 °C. Other related research in our laboratory supports this claim. With the onset of fermentation and the simultaneous increase in both fermentation temperature and ethanol content, extraction of tannins from both the grape skins and seeds continues. We hypothesize that the extended maceration time in the longer CS duration treatments resulted in the increased permeability of the cell walls, resulting in increased extraction of phenolics in these treatments when solubility of the compounds improved due to increased temperature and ethanol content. The extraction profiles shown for tannin were also true for gallic acid (benzoic acid mainly present in the seeds and released as hydrolysis
product) and the monomeric flavan-3-ols ((+)-catechin and (-)-epicatechin) monitored, with greater amounts extracted with increasing CS duration during CS and active fermentation. Differences in phenol concentrations (gallic acid, (+)-catechin, (-)-epicatechin, and tannin) at the end of fermentation persisted among the different CS duration wines (0 to 10 days) and were still present five months post-treatment (Table 3). The 4-, 7-, and 10-day CS treatments resulted in wines with significantly higher concentrations of gallic acid and monomeric flavan-3-ols when compared to the control wine, although there were no significant differences between the 4- to 10-day CS wines for monomeric flavan-3-ols.

Figure 3. Tannin concentrations in treatments with different CS durations during CS period and active fermentation, as determined by the Skogerson–Boulton model (n = 3). The end of CS for 1, 4, 7, and 10 days of CS treatments are marked as a dash line at 39, 111, 183, and 255 h, respectively.
Table 3. Concentration of phenolic compounds (mg/L ± SD) in finished wines made with different CS durations.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cold Soak Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Day</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>27.40 ± 0.28d</td>
</tr>
<tr>
<td>(+)-Catechin</td>
<td>38.52 ± 1.05c</td>
</tr>
<tr>
<td>(-)-Epicatechin</td>
<td>17.65 ± 1.12c</td>
</tr>
<tr>
<td>Caftaric acid</td>
<td>3.10 ± 0.31a</td>
</tr>
<tr>
<td>Coutaric acid</td>
<td>1.47 ± 0.14a</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>33.17 ± 1.11c</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>10.77 ± 0.41a</td>
</tr>
<tr>
<td>Quer-glycoside</td>
<td>12.11 ± 0.70a</td>
</tr>
<tr>
<td>Quercetin</td>
<td>2.27 ± 0.55a</td>
</tr>
<tr>
<td>Delph-3-glu</td>
<td>4.49 ± 0.13a</td>
</tr>
<tr>
<td>Pet-3-glu</td>
<td>5.43 ± 0.26a</td>
</tr>
<tr>
<td>Peo-3-glu</td>
<td>3.71 ± 0.37a</td>
</tr>
<tr>
<td>Mlv-3-glu</td>
<td>135.65 ± 8.40a</td>
</tr>
<tr>
<td>Delph-3-glu-ac</td>
<td>1.63 ± 0.09a</td>
</tr>
<tr>
<td>Pet-3-glu-ac</td>
<td>2.33 ± 0.15a</td>
</tr>
<tr>
<td>Peo-3-glu-ac</td>
<td>1.56 ± 0.09a</td>
</tr>
<tr>
<td>Mlv-3-glu-ac</td>
<td>48.53 ± 4.40a</td>
</tr>
<tr>
<td>Pet-3-glu-cou</td>
<td>1.42 ± 0.05ab</td>
</tr>
<tr>
<td>Peo-3-glu-cou</td>
<td>1.56 ± 0.09a</td>
</tr>
<tr>
<td>Mlv-3-glu-cou</td>
<td>11.22 ± 1.06a</td>
</tr>
<tr>
<td>Total Anthocyanin</td>
<td>218.83 ± 14.70a</td>
</tr>
<tr>
<td>Total tannin</td>
<td>436.31 ± 2.69c</td>
</tr>
<tr>
<td>Poly-pigment</td>
<td>17.21 ± 0.59a</td>
</tr>
</tbody>
</table>

Notes: Means ± SD followed by same letter within the same row indicates no significant difference (p < 0.05, n = 3). Quer-glycoside, quercetin glycosides; Poly-
pigment, Polymeric pigment; Delph-, Pet-, Peo-, and Mlv-3-glu; delphinidin-, petunidin-, peonidin-, and malvidin-3-glucoside, respectively. Delph-, Pet-, Peo-, and Mlv-3-glu-ac; delphinidin-, petunidin-, peonidin-, and malvidin-3-glucoside-acetate, respectively. Pet-, Peo-, and Mlv-3-glu-cou; petunidin-, peonidin-, and malvidin-3-glucoside-p-coumarate, respectively.

Proanthocyanidin Composition of Wines

Proanthocyanidin composition of musts and wines were determined at the end of CS, the end of fermentation, and five months post-treatment by phloroglucinolysis (Table 4). There were no significant differences in the mean degree of polymerization (mDP), tannin concentration, and average molecular weight among the different CS treatments. However, there were some significant differences in the percentage galloylation of the different CS samples. In general, the percentage galloylation increased with increasing CS duration and increased from the end of CS to the end of fermentation for all treatments, followed by a decrease post-fermentation. There were also small but significant differences in percentage gallo units with generally a decrease with CS duration and an increase from the end of CS to the end of fermentation. The percentage gallo units and galloylation have been shown to be estimates of the skin (epigallocatechin subunit) and seed (epicatechin gallate subunit) tannin extracted into the wine, respectively. We can thus conclude from the results that seed tannin contribution increased in wines with longer CS duration. Tannins are extracted from skins and seeds during maceration, and it has been reported that skin tannins extract more readily, whereas extraction from seeds requires longer maceration and is accelerated by the presence of ethanol 25-29. The longer CS contact time (seven to 10 days) also slightly increased tannin concentration at the end of fermentation and
five months post-treatment, although it was not statistically significant. The differences in tannin concentration determined by the Skogerson–Boulton model and phloroglucinolysis are due to the fact that the Skogerson–Boulton model estimates protein precipitable tannin, which includes quantification of indirect polymerization products such as polymeric pigments, whereas phloroglucinolysis only quantifies grape skin and seed proanthocyanidins.

The percentage of seed and skin tannin contribution in wine was also calculated using the method of Peyrot des Gachons and Kennedy. The results are presented as the percentage skin tannin contribution in Figure 4. At the end of CS there was a slight decrease in skin tannin with longer CS durations, although the differences between treatments were not significant. At the end of fermentation, there were significant differences in the proportion of skin tannin between treatments of 0 and 1 day CS versus 7 and 10 days’ CS, confirming that the skin tannin proportion declined in relation to seed tannin with the longer CS durations. The percentage of seed tannin increased from 34.6% in the control to 39.3% in the 10-day CS treatment. Increases in the proportion of seed tannin may potentially affect the sensory profile of the wine. The differences in seed tannin contribution among treatments mostly persisted in the finished wine. It can be noted that CS duration had no significant effect on the total skin and seed tannin concentration but had a significant effect on the skin and seed tannin proportions. Similarly, Peyrot des Gachons and Kennedy found increased seed tannin proportions at the beginning of fermentation when comparing four and 10 days of CS. However, they found no differences in skin and seed tannin proportions or final concentrations by the end of fermentation. This study showed that the seed tannin proportion and total protein precipitable tannin increased with the longer CS duration,
and the 7- and 10-day CS treatments may potentially have increased bitterness and astringency (mouthfeel)\textsuperscript{30, 31}.
Table 4. Proanthocyanidin composition of wines made with different CS durations at the end of CS, the end of alcoholic fermentation (AF), and in finished wine, including the mean degree of polymerization (mDP), average tannin concentration, average molecular weight (MW), percentage galloylation, and percentage gallo units.

<table>
<thead>
<tr>
<th></th>
<th>0 Day</th>
<th>1 Day</th>
<th>4 Days</th>
<th>7 Days</th>
<th>10 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cold Soak Duration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>mDP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of CS</td>
<td>-</td>
<td>6.86 ± 0.47a</td>
<td>6.62 ± 0.15a</td>
<td>6.88 ± 0.99a</td>
<td>5.95 ± 0.09a</td>
</tr>
<tr>
<td>End of AF</td>
<td>10.97 ± 0.47a</td>
<td>11.36 ± 0.49a</td>
<td>10.92 ± 0.09a</td>
<td>10.82 ± 0.13a</td>
<td>11.00 ± 0.23a</td>
</tr>
<tr>
<td>Bottle</td>
<td>10.86 ± 0.24a</td>
<td>10.94 ± 0.43a</td>
<td>10.37 ± 0.64a</td>
<td>10.17 ± 0.37a</td>
<td>10.60 ± 0.42a</td>
</tr>
<tr>
<td><strong>Tannin (mg/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of CS</td>
<td>-</td>
<td>43.98 ± 7.79a</td>
<td>47.95 ± 5.03a</td>
<td>41.49 ± 4.52a</td>
<td>43.19 ± 5.57a</td>
</tr>
<tr>
<td>End of AF</td>
<td>623.66 ± 50.25a</td>
<td>546.71 ± 36.80a</td>
<td>569.09 ± 29.49a</td>
<td>628.07 ± 12.34a</td>
<td>675.76 ± 34.16a</td>
</tr>
<tr>
<td>Bottle</td>
<td>518.17 ± 23.44a</td>
<td>499.15 ± 52.24a</td>
<td>513.54 ± 53.83a</td>
<td>543.35 ± 30.63a</td>
<td>612.25 ± 10.61a</td>
</tr>
<tr>
<td><strong>Average MW</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of CS</td>
<td>-</td>
<td>3298.05 ± 143.90a</td>
<td>3414.46 ± 150.42a</td>
<td>3285.60 ± 23.19a</td>
<td>3261.52 ± 38.90a</td>
</tr>
<tr>
<td>End of AF</td>
<td>3235.54 ± 71.48a</td>
<td>3257.42 ± 128.91a</td>
<td>3085.02 ± 190.25a</td>
<td>3030.39 ± 111.92a</td>
<td>3161.16 ± 126.05a</td>
</tr>
<tr>
<td>Bottle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>% Galloylation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of CS</td>
<td>4.86 ± 0.09b</td>
<td>4.73 ± 0.13b</td>
<td>5.05 ± 0.34ab</td>
<td>5.46 ± 0.19a</td>
<td>5.59 ± 0.06a</td>
</tr>
<tr>
<td>End of AF</td>
<td>2.98 ± 0.07ab</td>
<td>2.84 ± 0.13b</td>
<td>2.78 ± 0.05b</td>
<td>3.18 ± 0.02ab</td>
<td>3.42 ± 0.10a</td>
</tr>
<tr>
<td>Bottle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>% Gallo units</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of CS</td>
<td>-</td>
<td>20.56 ± 2.27ab</td>
<td>21.73 ± 1.68a</td>
<td>19.06 ± 1.47ab</td>
<td>15.91 ± 2.34b</td>
</tr>
<tr>
<td>End of AF</td>
<td>30.88 ± 0.57ab</td>
<td>31.27 ± 0.43a</td>
<td>30.08 ± 0.96abc</td>
<td>29.08 ± 0.73bc</td>
<td>28.70 ± 0.33c</td>
</tr>
<tr>
<td>Bottle</td>
<td>31.15 ± 0.80ab</td>
<td>32.14 ± 0.18a</td>
<td>31.11 ± 0.71ab</td>
<td>26.62 ± 1.27b</td>
<td>29.62 ± 0.35b</td>
</tr>
</tbody>
</table>

Notes: Means ± SD followed by same letter within the same row indicates no significant difference (p < 0.05, n = 3). (-) means no analysis as there is no end of CS for non-CS treatment.
Figure 4. The percentage of skin tannin contribution with different CS durations at the end of CS, the end of fermentation and in finished wines. Means with different letters are significantly different (p < 0.05, n = 3).

Conclusions

It can be concluded that Cabernet Sauvignon wine made with a CS duration of 4 to 10 days at 10 °C had greater extraction of phenolic compounds (gallic acid, (+)-catechin, (−)-epicatechin, and tannin) when compared to the control that did not experience CS. Not all phenolic compounds increased with longer CS duration as there was no significant effect on hydroxycinamate and flavonol extractions during fermentation and these results also persisted in the final wine. Although anthocyanin extraction and color density increased with longer periods of CS, there were no significant differences due to CS duration by the end of primary fermentation. Furthermore, 7 and 10 days of CS resulted in wines with higher seed tannin proportions and total tannin when compared to the non-CS wines and potentially enhanced the astringency in the wines.
References


10. Busse-Valverde, N.; Gómez-Plaza, E.; López-Roca, J. M.; Gil-Munoz, R.; Bautista-Ortín, A. B., The extraction of anthocyanins and proanthocyanidins from grapes to wine


CHAPTER 3

Impact of Different Cold Soak Durations on Cabernet Sauvignon Fermentation and Phenolic Composition

Abstract

The impact of different cold soak duration (0, 1, 2, 4, 7, and 10 days at 10 °C) on the extraction profiles of phenolic compounds was investigated for Cabernet Sauvignon wines. The results showed that cold soak duration had no effect on the extraction of the hydroxycinnamates, tannins, and total anthocyanins. Wines produced with four to ten days cold soak showed increased concentrations of gallic acid, (+)-catechin, and (-)-epicatechin compared to non-cold soak wines at the end of alcoholic fermentation. Analysis of wines after three months of bottle aging showed differences in concentration for only (-)-epicatechin. Tannin analysis of the bottled wines showed that wines made with ten days cold soak had increased seed tannin contributions although this was only observed at the end of cold soak.

Introduction

During the red winemaking process, phenolic compounds are extracted from mostly the grape skins and seeds into the must. The most important phenolic classes extracted are the anthocyanins, flavanols (including proanthocyanidins), hydroxycinnamates, and flavonols, all of which contribute to red wine quality. These phenolics contribute to sensory characteristics of the wine, particularly color, astringency, and bitterness. The phenolic composition of a red wine depends on several factors such as grape variety, phenolic content of the berry, and vinification techniques. For that reason, many winemaking techniques have been developed to enhance the extraction of these compounds. One such technique is the use of
cold soak. Cold soak (CS) is defined as prefermentative maceration in which the must is kept at low temperature (8-15 °C) for several days prior to alcoholic fermentation. It is widely believed that the use of CS increases the extraction of anthocyanins, skin tannins, and aroma compounds from grape skin to the wine.

The results obtained in previous studies have shown that CS has positive, negative, and no effect on the phenolic composition of the resulting wines. The Mencia wines made with CS had lower level of anthocyanins when compared with non-CS wines were reported by Pérez-Lamela, et al. González-Neves, et al. found that the level of anthocyanins in Tannat red wines produced with CS (10-15 °C for five days) showed no significant differences with non-CS wine. Busse-Valverde, et al. reported that CS (10 °C for 10 days) had no effect on the seed proanthocyanidin concentration in Syrah wines compared to non-CS wines. Conversely, a lot of studies note the positive influence on the final composition and sensory quality. For instance, Gómez-Miguez, et al. showed that Syrah wines produced with CS (15 °C for seven days) contained a greater amount of anthocyanins. Gil-Muñoz, et al., Gordillo, et al., De Santis and Frangipane, and Damijanić, et al. also found an increase in anthocyanin extraction with CS in Cabernet Sauvignon, Teran, Merlot, and Tempranillo wines, respectively. Additionally, the CS technique increased the extraction of skin proanthocyanidins in Cabernet Sauvignon according to Koyama, et al. Moreno-Pérez, et al. reported that the CS (10 °C for 10 days) led to higher proanthocyanidin content in Monastrell wines from Montealegre area when compared with non-CS wines. Moreover, the effect of the length of CS on phenolic composition was studied by Ortega-Heras, et al. The results showed that Mencia red wine made with CS at 5 ± 2 °C for six days had higher level of phenolic compounds than three days CS wine. Cejudo - Bastante, et al. also reported that longer CS period (12 days) resulted in higher phenolic content in Syrah wines than 8-day CS and traditional maceration wines. Similar results were reported by Panprivech, et al. in
which higher levels of gallic acid, (+)-catechin, and (-)-epicatechin were extracted with increasing duration of the CS (10 °C for four to 10 days). In addition, they also found that Cabernet Sauvignon wines made with seven and 10 days of CS had increased amount of tannin compared to non-CS wine, with the increase being due to seed tannin rather than skin tannin. The differences in results obtained by the CS studies discussed are potentially due to different grape varieties used, CS periods and temperatures investigated as well as the potential impact of winemaking processes and method of analysis. The effect of CS at different temperature on the sensory properties of the final wines was studied by Damijanić, et al. The results showed that the prefermentative cryomaceration (5 °C for five days with dry ice) increased red color, fruity flavor and good body/finish in Teran wines compared to non-cryomaceration wines. Parenti, et al. also reported that Sangiovese wines obtained with cold maceration at 5 °C with solid CO₂ and at 0°C and 5 °C with liquid nitrogen showed greater intensity of all the descriptors (color, flavor, and balance) than traditional wine.

Previous studies have mostly focus on how the CS affects levels of different phenolics in the final wines and did not investigate the impact on phenolic extraction during CS and fermentation. Additionally there is little data on the impact of CS duration on sensory properties of the resulting wines. Therefore, the aim of this study was to investigate the effects of increasing CS duration on phenolic extraction during both CS and fermentation, and to evaluate the sensory properties of different CS duration wines. In addition, a sub-selection of the research-scale experiments was repeated on a commercial scale, to confirm the impact of CS on Cabernet Sauvignon in large-scale fermentations.
Materials and methods

Research-Scale Fermentation

Cabernet Sauvignon grapes (approximately 4000 kg) were harvested in 2014 from Paso Robles, CA, USA. Initial juice analysis showed the following: sugar content 24.4 °Brix, 5.37 g/L tartaric acid (TA), 178.9 mg/L yeast assimilable nitrogen and pH of 3.58. Grapes were received by the Teaching and Research Winery at UC Davis and immediately processed. Prior to crushing, clusters (about 300 berries) were randomly collected from each harvest bin and stored at -20°C for further analysis. Grapes were destemmed and crushed using a Bucher-Vaslin Delta E2 (Sata Rosa, CA, USA) into a Bucher-Vaslin PMV must pump. Must was pumped directly into fermentors to a final volume of 90 L. To reduce vineyard variations in the fermentation, fermentors were filled sequentially in 22.5 L lots to the final volume.

To more fully investigate the effect of CS on red wine production, 18 research-scale Cabernet Sauvignon fermentations were performed using UC Davis/Cypress Semiconductor fermenters equipped with an Integrated Fermentation Control Systems (IFCS) unit as described in more detail in Lerno, et al. All fermentations were initially cooled to 10 °C overnight following an addition of potassium metabisulfite solution (final concentration of 50 mg/L sulfur dioxide). Six different CS durations (0, 1, 2, 4, 7, and 10 days) were investigated, with all treatments performed in triplicate. At the end of CS the fermentation temperature was increased to 25 °C, diammonium phosphate was added to adjust YAN to 300 mg/L and TA was adjusted to 6 g/L prior to inoculation with *Saccharomyces cerevisiae* strain D254 (Lallemand Lalvin, Santa Rosa, CA, USA). Automated pump-overs were controlled by the IFCS, which were programed at half fermentor volumes twice daily during both CS and fermentation. All treatments were sampled twice daily (8 am and 8 pm) following pump-over during both CS and fermentation with the first sampling point occurring when CS temperature was reached (10 °C) and fermentation had started in the no CS treatment.
(approximately 9 h after yeast inoculation). The total skin contact time of treatments after the end of alcoholic fermentation were 7.4, 8.7, 9.7, 12.4, 15.4, and 18.4 days for 0, 1, 2, 4, 7, and 10 days of CS treatment, respectively. Samples were centrifuged (Eppendorf Centrifuge 5810R, Eppendorf AG, Hamburg, Germany) at 3220 rpm for 15 min at 4 °C and subsequently frozen at −20 °C until analysis.

Brix measurements were collected manually with an Anton Parr DMA35 density meter (Anton Parr, Ashland, VA, USA). Fermentations were pressed after 8 days of alcoholic fermentation (approximately 0°Brix) using a prototype Cypress Semiconductor Corporation hydraulic basket press. After pressing wines were inoculated with Oenococcus oeni (Chr. Hansen, Inc., Milwaukee, WI, USA) for malolactic fermentation. After completion of malolactic fermentation potassium metabisulfite was added to adjust the concentration of free sulfur dioxide to 30 mg/L prior to bottling. All wines were filtered through a 0.5-1.0 μm nominal membrane pre-filter followed by 0.45 μm absolute membrane filter. Wines were bottled in 750 ml screw top bottles (Bordeaux style, green glass) purged with nitrogen gas prior to filling. The finished wines were stored at 14.4°C in the Teaching and Research Winery at UC Davis until analysis three and six months after bottling.

The chemical composition of the finished wines was determined at time of bottling for all treatments. The ethanol content was measured with an Alcolyzer (Anton Parr, Ashland, VA, USA). The pH was measured using an Orion 5-star pH meter (Thermo Scientific, MA, USA). The total acidity was measured automatically with the Mettler-Toledo DL50 titrator (Mettler-Toledo Inc., OH, USA). Acetate, malate, and residual sugar were measured using the Thermo Scientific Gallery automated analyzer (Thermo Scientific, MA, USA).

**Commercial-Scale Fermentation**

Commercial-scale Cabernet Sauvignon fermentations were performed and analyzed at E. & J. Gallo Winery (Courtside Cellars, San Miguel, CA, USA). Approximately 18000 kg of
grapes were harvested from the same vineyard as those used in the research-scale fermentations. Experimental treatments for the commercial-scale fermentations were 0, 1, 2, and 7 days of CS at 10 °C. Due to the volume of the fermentations and the limited facilities available the commercial-scale fermentations could not be replicated. All fermentations were pumped-over once a day during CS (one fermentor volume) and twice daily during alcoholic fermentation (two fermentor volumes). Samples were collected daily following pump-over during both CS and fermentation. The first sampling point occurring when CS at 10°C and fermentation had started approximately 24 h in the CS treatments and non-CS treatment, respectively. Fermentations were pressed after the end of alcoholic fermentation (7-9 days), which the total skin contact time of treatments were 9, 10, 11, and 14 days for 0, 1, 2, and 7 days of CS treatment, respectively.

Reagents

All reagents were of the highest purity possible. Phloroglucinol, ascorbic acid, gallic acid monohydrate, (+)-catechin, (−)-epicatechin, caffeic acid, quercetin, quercetin-rhamnoside, trans-ferulic acid, p-coumaric acid, trifluoroacetic acid (TFA) and acetonitrile were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA). Malvidin-3-O-glucoside chloride was purchased from Extrasynthese (Genay, France). Sodium acetate, potassium hydrogen tartrate, phosphoric acid and formic acid were purchased from Fisher Scientific (Bridgewater, NJ, USA). HPLC-grade water was prepared in house to a final resistance of 18 MΩ and filtered through a 0.22 µm filter prior to use.

Grape skin and seed tannin extraction

Grape skin and seed tannin were extracted from frozen grapes collected at the time of harvest using a previously described method \(^9\). All tannin extracts were lyophilized and stored at -20 °C until analysis.
**Determination of Color measurement and Adams-Harbertson Assay Correlation**

The frozen must or wine samples were thawed at room temperature and centrifuged prior to analysis. UV-visible spectra were collected from 230-900 nm with a Hewlett-Packard 8453 UV-vis spectrophotometer (Hewlett-Packard, Palo Alto, CA, USA) using 0.1 mm path length flow cell (Starna Cells, Atascadero, CA, USA). Color density was quantified as the sum of absorbance at 420, 520, and 620 nm. Hue was calculated as the ratio between the absorbance at 420 and 520 nm. The predicted Adam-Harbertson values for tannins with the coefficient of determination ($r^2$) of 0.86 were generated for all samples using the Skogerson-Boulton model.

**Determination of phenolic profiles using reversed phase HPLC (RP-HPLC)**

Frozen wine samples were thawed, centrifuged and filtered through 0.45 µm PTFE syringe-tip filters (Econo filter 25 mm, Agilent, Wilmington, DE) prior to analysis. Samples were analyzed on an Agilent (Santa Clara, CA, USA) 1260 Infinity HPLC equipped with a binary pump, column compartment, and diode array detector. Monomeric phenols were separated on an Agilent Poroshell 120 SB-C18 (4.6 x 150 mm, 2.7 µm particle) column maintained at 35°C. The solvents used were water acidified with 1.5% (v/v) phosphoric acid (solvent A) and acetonitrile containing 20% (v/v) solvent A (solvent B). The elution conditions were as follows: 0-23 min, 8-27% B; 23-25 min, 27% B; 25-28 min, 27-85% B; 28-30 min, 85% B; 30-32 min, 85-8% B; 32-40 min, 8% B. The flow rate was at 1.5 mL/min and the injection volume was 20 µL. Eluting peaks were monitored at 280 nm (gallic acid, (+)-catechin, and (-)-epicatechin), 320 nm (caftaric acid, caffeic acid, coutaric acid, and p-coumaric acid), 360 nm (quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-glucoronide, quercetin-rhamnoside, and quercetin), and 520 nm (anthocyanins). Phenolics of interest were identified using authentic standards when possible as well as literature. Phenolics were quantified by external standard with calibration curves generated for gallic
acid, (+)-catechin, caffeic acid, p-coumaric acid, quercetin-3-glucoside, quercetin, and malvidin-3-O-glucoside chloride. Compounds were quantified as themselves with the following exceptions: (-)-epicatechin as (+)-catechin equivalents; caftaric acid as caffeic acid equivalents; coutaric acid as p-coumaric acid equivalents; quercetin-3-galactoside, quercetin-3-glucuronide, and quercetin-rhamnoside as quercetin-3-glucoside equivalents; anthocyanins and polymeric pigments as malvidin-3-O-glucoside chloride equivalents. Instrument control and data analysis (peak identification, integration, and calibration) was done using Agilent® CDS ChemStation (Rev. B.04.03) software.

Isolation and Characterization of Proanthocyanidins

Solid phase extraction (SPE) was performed in triplicate to isolate proanthocyanidins as described previously. Following isolation, proanthocyanidin fractions were analyzed in duplicate by phloroglucinolysis using the method of Kennedy and Jones. Briefly, a phloroglucinol solution of 0.2 N HCl in MeOH containing 100 g/L phloroglucinol and 20 g/L ascorbic acid was added to aliquots of the isolated proanthocyanidins at a ratio of 1:1 (v/v), and heated at 50°C for 20 min. After 20 minutes the reaction was quenched by the addition of five volumes of 40 mM sodium acetate and then centrifuged for 5 min at 13,200 rpm (Eppendorf Centrifuge 5415D). Supernatants were transferred to an HPLC vial and all cleavage products were analyzed within 12 h (kept at 8°C).

Following acid-catalyzed cleavage of proanthocyanidins, all cleavage products were analyzed by RP-HPLC using a previously published method. The average tannin composition of each sample as well as the mean degree of polymerization (mDP), tannin concentration, average molecular weight (MW), percentage galloylation, and percentage gallo units were determined. Additionally, the percentage of skin and seed tannin contributions during wine production were calculated using the method of Peyrot des Gachons and Kennedy.
Sensory evaluation

Descriptive analysis of the different CS duration wines were performed after six months of bottle aging by the E. & J. Gallo Consumer & Product Insights and Development division. Eight experienced panelists evaluated the six wine treatments in duplicate. Prior to descriptive analysis the fermentation replicates for each treatment were evaluated and deemed similar. Subsequently the three fermentation replicates were blended prior to descriptive analysis. Sixty mL of wine was served in black glasses at room temperature and presented blind to panelists in a balance rotation one at time. Intensity scores were assigned by panelists to attributes of each sample based on the universal wine ballot using a 15-point spectrum scale. A list of known red wine attributes were provided in Table 1.
<table>
<thead>
<tr>
<th>Table 1. A list of red wine attributes.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aroma</strong></td>
</tr>
<tr>
<td><strong>Flavor</strong></td>
</tr>
<tr>
<td><strong>Taste</strong></td>
</tr>
<tr>
<td><strong>Mouthfeel / Aftertaste</strong></td>
</tr>
</tbody>
</table>
**Statistical Analysis**

The Fisher’s least significant differences (LSD) \((p < 0.05)\) were used to determine the statistical differences among treatment means using the function found in the Agricolae package, which was built under R version 2.15.1. For the sensory evaluation, the Analysis of Variance (ANOVA) with Dunnett’s test was used to identify significant differences among samples. Significances were determined at 90% confidence level.

**Results and discussion**

**Influence of CS duration on chemical composition of finished wine**

General chemical composition of finished wines made with different CS durations at time of bottling is given in Table 2. Wine compositions (ethanol, pH, total acidity, acetic acid, malic acid, and residual sugar) were not significantly different among treatments, indicating that the different CS durations had no effect on the general chemical composition of wines.

**Table 2.** The chemical composition of finished wines made with different cold soak durations at the time of bottling.\(^a\)

<table>
<thead>
<tr>
<th>Cold soak duration</th>
<th>% Ethanol (g/L)</th>
<th>pH</th>
<th>Total acidity (g/L)</th>
<th>Acetic acid (g/L)</th>
<th>Malic acid (mg/L)</th>
<th>Residual sugar (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day</td>
<td>14.60 ± 0.19(^a)</td>
<td>3.73 ± 0.05(^a)</td>
<td>5.58 ± 0.08(^a)</td>
<td>0.04 ± 0.01(^a)</td>
<td>0 ± 0(^a)</td>
<td>0.18 ± 0.01(^a)</td>
</tr>
<tr>
<td>1 day</td>
<td>14.63 ± 0.12(^a)</td>
<td>3.71 ± 0.01(^a)</td>
<td>5.57 ± 0.03(^a)</td>
<td>0.03 ± 0.01(^a)</td>
<td>0 ± 0(^a)</td>
<td>0.16 ± 0.01(^a)</td>
</tr>
<tr>
<td>2 days</td>
<td>14.48 ± 0.11(^a)</td>
<td>3.68 ± 0.02(^a)</td>
<td>5.59 ± 0.06(^a)</td>
<td>0.05 ± 0.02(^a)</td>
<td>0 ± 0(^a)</td>
<td>0.18 ± 0.01(^a)</td>
</tr>
<tr>
<td>4 days</td>
<td>14.73 ± 0.20(^a)</td>
<td>3.68 ± 0.04(^a)</td>
<td>5.55 ± 0.11(^a)</td>
<td>0.04 ± 0.01(^a)</td>
<td>0 ± 0(^a)</td>
<td>0.16 ± 0.02(^a)</td>
</tr>
<tr>
<td>7 days</td>
<td>14.92 ± 0.42(^a)</td>
<td>3.71 ± 0.04(^a)</td>
<td>5.67 ± 0.12(^a)</td>
<td>0.05 ± 0.01(^a)</td>
<td>0 ± 0(^a)</td>
<td>0.15 ± 0.02(^a)</td>
</tr>
<tr>
<td>10 days</td>
<td>14.40 ± 0.07(^a)</td>
<td>3.66 ± 0.02(^a)</td>
<td>5.58 ± 0.26(^a)</td>
<td>0.07 ± 0.01(^a)</td>
<td>0 ± 0(^a)</td>
<td>0.14 ± 0.01(^a)</td>
</tr>
</tbody>
</table>

\(^a\) Results are expressed as means ± standard deviation \((n = 3)\). Values followed by the same letter within the same column indicate no significant difference \((p < 0.05)\).
Color evolution of the must and wines

The evolution of color during CS and fermentation for each treatment was evaluated (Figure 1). The color density increased fairly constant during the CS period for all treatments, which agrees with the results obtained by Gordillo, et al. The low temperature of the must during CS period might promote the degradation of grape skin cells, which increase in color density. The low temperature also inhibit the activity of some enzymes such as polyphenol oxidase and microorganism development, which avoid or retard anthocyanin degradation favoring the color stability. At the end of CS the 10-day treatment had significantly more color than all the other treatments. Additionally, the 7- and 4-day CS treatments respectively had significantly higher color densities compared to the 1- and 2-day CS treatments (Table 3). The color density increases following the start of fermentation, with the 4-, 7-, and 10-day CS treatments (11.5 ± 0.3, 12.9 ± 1.2, and 10.5 ± 0.7, respectively) had significantly higher color density values compared to 0-, 1-, and 2-day CS treatments (6.4 ± 0.4, 9.2 ± 0.2, and 9.5 ± 0.5, respectively). These results are in agreement with those observed by Gómez-Miguez, et al., who found that CS wines had much darker with a more bluish color than the non-CS wines at the beginning of the alcoholic fermentation by using UV-vis. The increases in color density of the longer CS treatments may be due to the greater degradation or disruption of cell structure of grape skin than the shorter CS treatments during the CS period, resulting in the release of more phenolics from the skins. These differences in color density did not persist beyond the start of fermentation. The 4-day CS treatment had a significantly higher color density compared to all other treatments during the first five days of fermentation. Around day four of fermentation the evolution of color density slowed and began to decrease from day six throughout the remainder of the fermentation period in all treatments. From day six to the end of fermentation, no significant differences in color density were observed among the treatments. The decrease in color density during the latter
stages of fermentation are potentially due to the fact that the anthocyanins are slowly reacting with other compounds, and being re-adsorbed on to solid such as yeast cell walls \textsuperscript{31, 32}. Ribéreau - Gayon, et al. \textsuperscript{33} suggested that the decrease in color could be a result of a shift in the equilibrium towards colorless chalcone form and subsequent breakdown of the carbon molecule.

![Figure 1](image)

**Figure 1.** Evolution of color density of wines made with different CS duration during CS and alcoholic fermentation as determined by analyzing the morning samples of each treatment using UV-vis (n=3). The end of CS for 1, 2, 4, 7, and 10 days of CS treatment are marked as a dashed line at 33, 57, 105, 177, and 249 h, respectively.

*Note:* The hours on y-axis correspond with total skin contact time as it took the must 9 h to reach the CS temperature of 10 ± 1°C and 1 day CS correspond to 33 h.

Changes in the hue were also observed, although the trends were opposite to those for color density. The increases in color density during CS reduced in hue (420 nm to 520 nm ratio) values due to larger contributions to 520 nm (red color range) (Table 3). The longer CS durations (7- and 10-day CS) had significantly lower hue compared to 1- and 2-day CS treatments at the end of CS. The differences in hue did not persist during alcoholic
fermentation as all the treatments finished with similar hues (Table 3). Findings on color density and hue from this study are in agreement with those of Panprivech, et al. 19, who observed that longer CS treatments had significant higher color density and lower hue value than non-CS treatment at the end of CS. However, these differences disappeared during alcoholic fermentation. This is in agreement with the results found by Gómez-Míguez, et al. 8.

Color density and hue were also evaluated after three-month bottle aging (five months post-treatment). No significant differences among treatments were found for either color density or hue (Table 3). The color density decreased approximately 32 % from the values measured at the end of alcoholic fermentation while the hue increased approximately 45 % for all treatments after three months in bottle. The decrease in color density and increase in hue during aging most probably result from the degradation of anthocyanins and the reaction between anthocyanins and tannins, forming pigmented polymeric phenolics 31, 34.
Table 3. Color density and hue of wines made with different CS durations at the end of CS, the end of alcoholic fermentation, and after three months of bottle aging. *

<table>
<thead>
<tr>
<th>Absorbance unit</th>
<th>Cold soak duration</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
<td>1 day</td>
</tr>
<tr>
<td></td>
<td>end of CS</td>
<td></td>
</tr>
<tr>
<td>Color density (420 + 520 + 620 nm)</td>
<td>-</td>
<td>4.23±0.46 a</td>
</tr>
<tr>
<td>end of AF</td>
<td>15.83±1.55 a</td>
<td>14.99±0.30 a</td>
</tr>
<tr>
<td>bottle</td>
<td>9.68±1.42 a</td>
<td>11.37±0.16 a</td>
</tr>
<tr>
<td>Hue (420/520 nm)</td>
<td>-</td>
<td>1.06±0.08 a</td>
</tr>
<tr>
<td>end of AF</td>
<td>0.48±0.02 a</td>
<td>0.45±0.01 a</td>
</tr>
<tr>
<td>bottle</td>
<td>0.71±0.01 a</td>
<td>0.69±0.01 a</td>
</tr>
</tbody>
</table>

* Results are expressed as means ± standard deviation (n = 3) and followed by same letter within the same row indicates no significant difference (p < 0.05). (−) means no analysis as there is no end of CS for non-CS treatment.
Evolution of phenolic profiles during CS and active fermentation

The primary monomeric phenolics in the musts and wines were analyzed by RP-HPLC. The extraction profiles of (+)-catechin during CS and alcoholic fermentation are shown in Figure 2. (+)-Catechin extraction slowly increased from the beginning to the end of CS period (from 6.2 to 9.1 mg/L, 5.9 to 9.2 mg/L, 7.2 to 12.8 mg/L, 6.9 to 13.0 mg/L, and 5.8 to 13.9 mg/L for 1, 2, 4, 7, and 10 days CS treatment, respectively), followed by greater extraction and solubility when temperature and alcohol level increased during fermentation.

Similar extraction trends of over the whole winemaking period were previously reported for CS wines by Ševcech, et al. By the end of both CS and fermentation, significantly higher concentrations of (+)-catechin were found in the treatments with increasing CS duration. Thus the 4-, 7-, and 10-day CS treatments had significantly higher concentrations of (+)-catechin at the end of fermentation compared to the control. The extraction profiles of (-)-epicatechin and gallic acid showed similar trends to that of (+)-catechin. Significantly higher concentration of (-)-epicatechin and gallic acid were seen in the 4-, 7-, and 10-day CS treatments compared to the rest of treatments (0-, 1-, and 2-day CS) throughout the CS period and fermentation, although there were no significant differences between 4- to 10-day CS treatments. The increase in some key phenolic compounds (gallic acid, (+)-catechin, and (-)-epicatechin with increasing CS duration during CS period and fermentation was also reported by Panprivech, et al. However, after three months in bottle only (-)-epicatechin was still significantly different with the 10-day CS treatment having significantly higher concentrations than the 0-, 1-, and 2-day CS treatments (Table 4). During bottle aging the concentration of (+)-catechin and (-)-epicatechin decreased by 15 ± 2 % and 26 ± 2 %, respectively. The decrease in the concentration of both (+)-catechin and (-)-epicatechin may be due to condensation and polymerization reactions with other flavonols (flavanol-flavanol) and anthocyanins as well as oxidation reactions. In addition, increases in polymeric
phenols and polymeric pigments during aging were seen by 42.4 % and 102.7%, respectively. However, the polymeric phenols and pigments showed no significant differences among treatments after three months bottling (Table 4). Monagas, et al.\textsuperscript{39} reported that (+)-catechin and (-)-epicatechin progressively decreased in Cabernet Sauvignon wine during aging in the bottle. Gómez-Plaza, et al.\textsuperscript{40}, Revilla and González-SanJosé\textsuperscript{38} also found losses of monomeric flavanol during aging in the bottle. The concentration of gallic acid in the bottled wines increased by 59 ± 4 % from the concentration measured at end of alcoholic fermentation. These results are in agreement with results reported by Monagas, et al.\textsuperscript{39}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{graph.png}
\caption{Evolution of (+)-catechin concentration of wines made with different CS duration during CS and alcoholic fermentation as determined by analyzing the morning samples of each treatment using RP-HPLC (n=3). The end of CS for 1, 2, 4, 7, and 10 days of CS treatment are marked as a dashed line at 33, 57, 105, 177, and 249 h, respectively. \textbf{Note}: The hours on y-axis correspond with total skin contact time as it took the must 9 h to reach the CS temperature of 10 \pm 1°C and 1 day CS correspond to 33 h.}
\end{figure}
The extraction profiles of the hydroxycinnamates (caftaric acid, caffeic acid, coutaric acid, and p-coumaric acid) are shown in Figure 3. Hydroxycinnamate extractions slowly increased during the CS period with the majority of the extraction took place at the beginning of alcoholic fermentation. There were no significant differences among treatments throughout the CS period and fermentation. These results are in agreement with results presented in a previous work, in which CS duration had no effect on hydroxycinnamate extractions. In contrast, these results contradict the results reported by Koyama, et al., the concentration of hydroxycinnamates of CS wines (13 °C for two days) reached a maximum on the forth day fermentation, followed by a decrease during the remainder of fermentation compared to the control wines. It is possible that the extraction of hydroxycinnamates can be influenced by differences in CS conditions (temperature and duration). The primary hydroxycinnamates that extracted during CS and alcoholic fermentation were caftaric and coutaric acid. After bottle aging the concentration of caffeic and p-coumaric acid increased, reflecting the release of free hydroxycinnamic acid forms due to hydrolysis of the tartaric esters. Even though no significant differences in total hydroxycinnamate extraction were seen at the end of alcoholic fermentation, there were significant differences among treatments in the bottled wines (Table 4). The significant differences in p-coumaric acid levels are potentially due to an additional source of p-coumaric acid (hydrolysis of p-coumaroylated anthocyanin) during aging. In addition, the amount of total hydroxycinnamates in the bottled wine had a small decrease (approximately 2.3 %) from the concentration measured at the end of fermentation for all treatments.
Figure 3. Evolution of total hydroxycinnamate concentration of wines made with different CS duration during CS and alcoholic fermentation as determined by analyzing the morning samples of each treatment using RP-HPLC (n=3). The end of CS for 1, 2, 4, 7, and 10 days of CS treatment are marked as a dashed line at 33, 57, 105, 177, and 249 h, respectively.

Note: The hours on y-axis correspond with total skin contact time as it took the must 9 h to reach the CS temperature of 10 ± 1°C and 1 day CS correspond to 33 h.

The evolution of total flavonols (sum of quercetin-galactoside, quercetin-glucoside, quercetin-glucoronide, quercetin, and quercetin-rhamnoside) for each CS treatment is presented in Figure 4. The main flavonols found in all wine treatments was glycoside of quercetin with quercetin-glucoside being the main quercetin-glycoside present. During CS the total flavonols slowly increased with increasing CS duration. At the end of CS the 4-, 7-, and 10-day CS treatments (42.0 ± 3.9, 41.9 ± 3.2, and 43.2 ± 4.9 mg/L, respectively) contained significantly higher concentrations of flavonols compared to 1- and 2-day CS treatments (28.4 ± 0.3 and 27.7 ± 1.2 mg/L, respectively). The low extraction of flavonols during CS period might be due to flavonols being less soluble in water medium and at low temperature. An increase in the extraction rate of flavonols was seen during fermentation.
followed mostly by a decrease at the end of fermentation. This behavior was also observed by Koyama, et al. in wines from the same grape variety. The increase of flavonols extracted with the start of fermentation is mostly due to increase their solubility as a result of an increase in temperature. Although the 7- and 10-day CS wines had lower total flavonol levels compared to the rest of the CS and non-CS wines at the end fermentation, it was not significant. These results remained after three months of bottle aging (Table 4). Quercetin-glycoside concentrations decreased (approximately 15.37 %) with aging for all treatments. Zafrilla, et al. and Monagas, et al. also noticed a decrease in the concentration of glycosylated forms during aging. These losses of flavonol glycosides cannot be explained only by the hydrolysis of glycosidic linkages because no parallel increase in the amounts of the corresponding aglycones (quercetin) was observed. The losses are probably due to other kinds of reactions (condensation, oxidation reactions) involving flavonol glycosides or even their corresponding aglycones after hydrolysis as suggested by Gutiérrez, et al.  

Figure 4. Evolution of total flavonols concentration of wines made with different CS duration during CS and alcoholic fermentation as determined by analyzing the morning
samples of each treatment using RP-HPLC (n=3). The end of CS for 1, 2, 4, 7, and 10 days of CS treatment are marked as a dashed line at 33, 57, 105, 177, and 249 h, respectively.

**Note:** The hours on y-axis correspond with total skin contact time as it took the must 9 h to reach the CS temperature of 10 ± 1°C and 1 day CS correspond to 33 h.

The extraction of anthocyanins during the CS as well as alcoholic fermentation was monitored by RP-HPLC for all treatments. The extraction profiles for total anthocyanins (calculated as the sum of all anthocyanins quantified by RP-HPLC data) are shown in Figure 5. The extraction of anthocyanins almost increased in all treatments during the CS period, however the 7- and 10-day treatments reaching a maximum respectively on day sixth and seventh of CS period after which a slight decrease occurred. This decrease may possibly be due to the adsorption of anthocyanin extracted on to the grape solids (similar to their adsorption on to yeast cell walls during fermentation)\(^{19, 31}\). Additionally, the anthocyanins can be involved in secondary reactions in addition to potential degradation reactions\(^{31, 32, 34}\) although this will probably be a slow process at 10 °C. By the end of CS there were no significant differences in anthocyanin concentration among treatments. During the early stages of fermentation, the CS treatments showed higher anthocyanin extracted (approximately 194 mg/L) than the non-CS treatment (98 mg/L). This difference may be due to degradation of the skin cell structure during CS, allowing for easier extraction at the start of fermentation. Extraction of anthocyanins increased during fermentation due to increased fermentation temperature and ethanol production\(^{6}\). As can be seen in Figure 5, the concentration of total anthocyanins increased to a maximum around day six–seven of alcoholic fermentation and decreased thereafter throughout the remainder of fermentation, which is consistent with other studies\(^{9, 14, 15, 19, 45, 46}\). As previously discussed the reason for this decrease in concentration after a maximum was reached was due to the re-adsorption of anthocyanin extracted onto yeast cell walls anthocyanins and polymerization with other
compounds to form polymeric pigments as well as degradation reactions \(^{31, 32}\). However, no significant differences in total anthocyanins were seen among treatments by the end of fermentation. Thus in this study, CS duration had no effect on total anthocyanins, which is in agreement with several study studies \(^{5, 11, 19}\). In contrast, an increase in anthocyanin extraction and final concentration was observed in CS wine when compared to non-CS wine in several other studies \(^{8, 11, 13, 47}\). The different results might be depended on temperature and duration of CS, grape variety, or vinification process.

![Figure 5](image)

**Figure 5.** Evolution of total anthocyanin concentration of wines made with different CS duration during CS and alcoholic fermentation as determined by analyzing the morning samples of each treatment using RP-HPLC (n=3). The end of CS for 1, 2, 4, 7, and 10 days of CS treatment are marked as a dashed line at 33, 57, 105, 177, and 249 h, respectively.

**Note:** The hours on y-axis correspond with total skin contact time as it took the must 9 h to reach the CS temperature of 10 ± 1°C and 1 day CS correspond to 33 h.

The extraction profiles of different types of anthocyanins (non-acylated, acetylated, and \(p\)-coumaroylated) were also investigated. The non-acylated anthocyanins predominated in the must and wines for all treatments (61.4%), followed by acetylated (27.8%) and \(p\)-
coumaroylated (10.8%) anthocyanins, respectively. The extraction profiles for non-acylated and acetylated anthocyanins (Figure 6a and 6b) showed similar trends to the total anthocyanin extraction profile. The p-coumaroylated anthocyanins had a different extraction behavior during CS (Figure 6c). Relatively little of the p-coumaroylated anthocyanins extracted during CS, possibly due to their increased hydrophobic character compared to the rest of the anthocyanins and limited solubility at 10 °C. As temperature and ethanol content increased during fermentation the p-coumaroylated anthocyanin showed greater extraction similar to the non-acylated and acetylated forms. The concentration of anthocyanins in the different wine treatments after three months of bottle aging are given in Table 5. There were no significant differences in total anthocyanins among treatments, although significant differences were seen in acetylated anthocyanins. The total acetylated anthocyanins were significantly lower in the finished wines made with longer CS duration (7- and 10-day CS) compared to the non-CS wine, which is in agreement with our previous work. The acylated anthocyanins (including acetylated form) might be more strongly adsorbed onto yeast cell walls than non-acylated anthocyanins. Compared to the end of fermentation the concentration of anthocyanins three months after bottling decreased, the greatest decrease being in the p-coumaroylated (37 %), followed by non-acylated (17 %) and acetylated (14 %) forms. These results are in agreement with He, et al. and Puertas, et al., which reported that during maceration and aging the concentration of anthocyanins in red wine declines, especially the acylated anthocyanins.
Figure 6. Evolution of non-acylated (a), acetylated (b), and \( \mu \)-coumaroylated (c) anthocyanin concentration of wines made with different CS duration during CS and alcoholic fermentation as determined by analyzing the morning samples of each treatment using RP-HPLC (n=3). The end of CS for 1, 2, 4, 7, and 10 days of CS treatment are marked as a dashed line at 33, 57, 105, 177, and 249 h, respectively.

Note: The hours on y-axis correspond with total skin contact time as it took the must 9 h to reach the CS temperature of 10 ± 1°C and 1 day CS correspond to 33 h.
The extraction profiles of tannins during CS and alcoholic fermentation were determined by UV-vis spectroscopy using the Skogerson-Boulton correlation model (Figure 7). There were no significant differences in total tannin concentration among treatments at the end of CS and fermentation. These results agree with those reported by Marais in Pinotage wines and Ortega-Heras, et al. in Mencia wines. Considering the extraction profile of tannins, slow extraction was observed during CS period, with faster extraction occurring during fermentation. Slow extraction during CS period can be related to the fact that tannins have low solubility in water and at low temperature. The increase in fermentation temperature and ethanol content during fermentation can contribute to the extraction by increasing tannin solubility and breaking down cellular structures.

Figure 7. Evolution of tannin concentration of wines made with different CS duration during CS and alcoholic fermentation as determined by analyzing the morning samples of each treatment using the Skogerson-Boulton model (n=3). The end of CS for 1, 2, 4, 7, and 10 days of CS treatment are marked as a dashed line at 33, 57, 105, 177, and 249 h, respectively. Note: The hours on y-axis correspond with total skin contact time as it took the must 9 h to reach the CS temperature of 10 ± 1°C and 1 day CS correspond to 33 h.
Table 4. Concentration of phenolic compounds (mg/L) in finished wines made with different CS durations after three months of bottle aging. a

<table>
<thead>
<tr>
<th>Compound</th>
<th>0 day</th>
<th>1 day</th>
<th>2 days</th>
<th>4 days</th>
<th>7 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>22.47±1.13 a</td>
<td>22.83±0.34 a</td>
<td>21.50±0.69 a</td>
<td>22.52±0.69 a</td>
<td>23.83±0.75 a</td>
<td>23.63±0.80 a</td>
</tr>
<tr>
<td>(+)-Catechin</td>
<td>34.01±1.78 a</td>
<td>33.76±0.53 a</td>
<td>32.57±2.12 a</td>
<td>33.92±0.75 a</td>
<td>34.75±1.66 a</td>
<td>35.54±1.10 a</td>
</tr>
<tr>
<td>(-)-Epicatechin</td>
<td>16.22±1.05 b</td>
<td>16.46±0.36 b</td>
<td>16.41±1.15 b</td>
<td>17.59±1.2 ab</td>
<td>18.11±0.96 ab</td>
<td>19.61±0.80 a</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>35.17±1.24 a</td>
<td>33.07±0.68 a</td>
<td>33.04±0.65 a</td>
<td>32.23±1.48 a</td>
<td>32.10±1.54 a</td>
<td>30.81±4.18 a</td>
</tr>
<tr>
<td>Caftaric acid</td>
<td>9.91±0.31 a</td>
<td>9.21±0.20 ab</td>
<td>8.96±0.71 ab</td>
<td>8.45±0.35 ab</td>
<td>8.37±0.68 ab</td>
<td>8.00±1.34 b</td>
</tr>
<tr>
<td>Coutaric acid</td>
<td>2.00±0.18 a</td>
<td>1.74±0.23 a</td>
<td>2.07±0.31 a</td>
<td>1.79±0.48 a</td>
<td>1.71±0.72 a</td>
<td>2.00±1.05 a</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>35.17±1.24 a</td>
<td>33.07±0.68 a</td>
<td>33.04±0.65 a</td>
<td>32.23±1.48 a</td>
<td>32.10±1.54 a</td>
<td>30.81±4.18 a</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>5.88±0.28 a</td>
<td>5.65±0.19 ab</td>
<td>5.46±0.58 ab</td>
<td>5.32±0.17 ab</td>
<td>5.39±0.34 ab</td>
<td>4.89±0.23 b</td>
</tr>
<tr>
<td>Total hydroxycinnamate</td>
<td>51.43±0.89 a</td>
<td>49.00±0.28 ab</td>
<td>49.77±0.61 ab</td>
<td>47.79±1.78 b</td>
<td>47.41±1.72 b</td>
<td>46.72±1.71 b</td>
</tr>
<tr>
<td>Quercetin-galactoside</td>
<td>53.36±2.07 a</td>
<td>51.02±2.06 ab</td>
<td>50.12±4.18 ab</td>
<td>48.14±0.94 ab</td>
<td>50.00±3.01 ab</td>
<td>45.33±2.33 b</td>
</tr>
<tr>
<td>Quercetin-glucoside</td>
<td>8.15±0.17 a</td>
<td>7.83±0.31 a</td>
<td>7.81±0.58 a</td>
<td>7.90±0.14 a</td>
<td>8.27±0.64 a</td>
<td>7.17±0.44 a</td>
</tr>
<tr>
<td>Quercetin-rhamnoside</td>
<td>0.89±0.07 a</td>
<td>0.77±0.08 ab</td>
<td>0.75±0.07 a</td>
<td>0.77±0.01 ab</td>
<td>0.76±0.08 ab</td>
<td>0.66±0.04 b</td>
</tr>
<tr>
<td>Quercetin-glycoside</td>
<td>68.28±2.48 a</td>
<td>65.28±2.63 ab</td>
<td>64.15±5.39ab</td>
<td>62.13±1.00 ab</td>
<td>64.43±4.02 ab</td>
<td>58.05±3.03 b</td>
</tr>
<tr>
<td>Quercetin</td>
<td>9.34±3.31 a</td>
<td>9.65±3.03 a</td>
<td>11.87±0.1 a</td>
<td>8.88±2.48 a</td>
<td>12.22±0.04 a</td>
<td>10.33±1.09 a</td>
</tr>
<tr>
<td>Total flavonol</td>
<td>77.62±5.46 a</td>
<td>74.93±4.89 a</td>
<td>76.01±5.24 a</td>
<td>71.01±3.00 a</td>
<td>76.65±4.05 a</td>
<td>68.38±4.13 a</td>
</tr>
<tr>
<td>Polymeric phenol</td>
<td>145.67±32.76 a</td>
<td>124.38±5.83 a</td>
<td>122.45±10.76 a</td>
<td>126.15±5.67 a</td>
<td>178.46±19.28 a</td>
<td>129.77±18.64 a</td>
</tr>
<tr>
<td>Polymeric pigment</td>
<td>12.07±2.81 a</td>
<td>9.96±0.56 a</td>
<td>10.43±0.88 a</td>
<td>11.07±0.87 a</td>
<td>15.26±1.42 a</td>
<td>10.42±1.52 a</td>
</tr>
</tbody>
</table>

Results are expressed as means ± standard deviation (n = 3). The significant differences are indicated as Table 3.
Table 5. Anthocyanin composition (mg/L) in finished wines made with different CS durations after three months of bottle aging. 

<table>
<thead>
<tr>
<th>Compound</th>
<th>0 day</th>
<th>1 day</th>
<th>2 days</th>
<th>4 days</th>
<th>7 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delph-3-glu</td>
<td>4.99±0.51 a</td>
<td>4.98±0.25 a</td>
<td>4.77±0.30 ab</td>
<td>4.37±0.24 ab</td>
<td>4.21±0.60 ab</td>
<td>3.90±0.24 b</td>
</tr>
<tr>
<td>Cya-3-glu</td>
<td>0.27±0.02 a</td>
<td>0.29±0.03 a</td>
<td>0.25±0.02 a</td>
<td>0.19±0.05 a</td>
<td>0.24±0.06 a</td>
<td>0.20±0.03 a</td>
</tr>
<tr>
<td>Pet-3-glu</td>
<td>8.62±0.60 a</td>
<td>8.27±0.28 ab</td>
<td>8.35±0.53 ab</td>
<td>8.05±0.33 ab</td>
<td>7.61±0.92 ab</td>
<td>7.14±0.55 b</td>
</tr>
<tr>
<td>Peo-3-glu</td>
<td>3.98±0.31 a</td>
<td>3.73±0.10 ab</td>
<td>3.66±0.22 ab</td>
<td>3.49±0.06 ab</td>
<td>3.30±0.27 b</td>
<td>3.24±0.25 b</td>
</tr>
<tr>
<td>Mlv-3-glu</td>
<td>129.48±6.64 a</td>
<td>122.85±3.14 a</td>
<td>127.79±4.18 a</td>
<td>128.70±5.10 a</td>
<td>123.24±9.04 a</td>
<td>119.60±8.17 a</td>
</tr>
<tr>
<td>Total nonacylated</td>
<td>142.35±7.53 a</td>
<td>135.13±3.49 a</td>
<td>140.05±4.93 a</td>
<td>140.43±5.43 a</td>
<td>134.40±10.27 a</td>
<td>130.18±8.93 a</td>
</tr>
<tr>
<td>Delph-3-glu-ac</td>
<td>1.60±0.166 a</td>
<td>1.33±0.21 a</td>
<td>1.32±0.23 a</td>
<td>1.17±0.09 a</td>
<td>2.40±0.94 a</td>
<td>2.12±0.42 a</td>
</tr>
<tr>
<td>Cya-3-glu-ac</td>
<td>1.20±0.08 a</td>
<td>1.02±0.02 ab</td>
<td>1.08±0.06 ab</td>
<td>1.04±0.07 ab</td>
<td>0.95±0.10 b</td>
<td>0.91±0.06 b</td>
</tr>
<tr>
<td>Pet-3-glu-ac</td>
<td>2.92±0.20 a</td>
<td>2.73±0.11 ab</td>
<td>2.70±0.21 ab</td>
<td>2.56±0.11 ab</td>
<td>2.48±0.29 ab</td>
<td>2.30±0.10 b</td>
</tr>
<tr>
<td>Peo-3-glu-ac</td>
<td>2.81±0.18 a</td>
<td>2.58±0.08 ab</td>
<td>2.67±0.13 a</td>
<td>2.48±0.09 ab</td>
<td>2.24±0.19 b</td>
<td>2.25±0.14 b</td>
</tr>
<tr>
<td>Mlv-3-glu-ac</td>
<td>64.30±2.68 a</td>
<td>58.53±1.26 ab</td>
<td>61.24±2.65 ab</td>
<td>58.65±1.83 ab</td>
<td>52.94±4.26 b</td>
<td>54.52±2.86 b</td>
</tr>
<tr>
<td>Total acetylated</td>
<td>71.23±0.10 a</td>
<td>64.86±1.42 ab</td>
<td>67.70±3.04 ab</td>
<td>64.73±2.01 a</td>
<td>58.61±4.82 b</td>
<td>59.98±3.10 b</td>
</tr>
<tr>
<td>Delph-3-glu-cou</td>
<td>0.69±0.12 a</td>
<td>0.59±0.04 a</td>
<td>0.67±0.03 a</td>
<td>0.66±0.02 a</td>
<td>0.71±0.07 a</td>
<td>0.68±0.08 a</td>
</tr>
<tr>
<td>Cya-3-glu-cou</td>
<td>0.72±0.07 a</td>
<td>0.66±0.03 a</td>
<td>0.69±0.07 a</td>
<td>0.70±0.02 a</td>
<td>0.70±0.08 a</td>
<td>0.62±0.07 a</td>
</tr>
<tr>
<td>Pet-3-glu-cou</td>
<td>0.93±0.07 a</td>
<td>0.84±0.04 a</td>
<td>0.93±0.04 a</td>
<td>0.84±0.08 a</td>
<td>0.79±0.01 a</td>
<td>0.88±0.05 a</td>
</tr>
<tr>
<td>Peo-3-glu-cou</td>
<td>1.32±0.10 a</td>
<td>1.19±0.06 ab</td>
<td>1.26±0.06 ab</td>
<td>1.21±0.05 ab</td>
<td>1.16±0.09 ab</td>
<td>1.10±0.06 b</td>
</tr>
<tr>
<td>Mlv-3-glu-cou</td>
<td>16.71±1.39 a</td>
<td>14.81±0.53 a</td>
<td>16.17±0.74 a</td>
<td>15.72±0.79 a</td>
<td>14.73±1.53 a</td>
<td>14.71±1.02 a</td>
</tr>
<tr>
<td>Total p-coumaroylated</td>
<td>19.67±1.62 a</td>
<td>17.50±0.62 a</td>
<td>19.06±0.91 a</td>
<td>18.46±0.92 a</td>
<td>17.39±1.71 a</td>
<td>17.30±1.20 a</td>
</tr>
<tr>
<td>Total Anthocyanin</td>
<td>240.54±12.83 a</td>
<td>224.40±5.97 a</td>
<td>233.57±9.34 a</td>
<td>229.83±7.98 a</td>
<td>217.72±16.45 a</td>
<td>214.17±13.53 a</td>
</tr>
</tbody>
</table>

a Results are expressed as means ± standard deviation (n = 3). The significant differences are indicated as Table 3.

b Delph-, cya-, pet-, peo-, and mlv-3-glu; delphinidin-, cyanidin-, petunidin-, peonidin-, and malvidin-3-glucoside, respectively. Glu-ac, and glu-cou; glucoside-acetate, and glucoside-p-coumarate, respectively.
Proanthocyanidin composition

The proanthocyanidin composition of the CS treatments was determined by phloroglucinolysis at the end of CS, the end of fermentation, and after three months of bottle aging (Table 6). Significant differences were seen in the mean degree of polymerization (mDP), average molecular weight (MW), and the percentage of gallo units among treatments during the CS period only. The 10- and 7-day CS had respectively significantly lower mDP, average MW, and percentage gallo units compare to the rest of CS and non-CS treatments at the end of CS. The percentage of gallo units (epigallocatechin subunit in skins) was lowest in 10-day CS treatment (22.36 %) while 1-, 2-, and 4-day CS treatments had similar levels of 27.51 %, 27.14 %, and 26.46 %, respectively. Due to tannins having a higher hydrophobicity, a higher mDP and percentage of gallo units might have been trapped in skin cells during CS and released into the must later when ethanol concentration increased during alcoholic fermentation. At the end of fermentation with increasing CS duration, resulted in a decreased in percentage of gallo units and increases in percentage galloylation. Moreover, there were no significant differences observed among treatments. These results indicate that the longer CS duration could potentially favor seed tannin extraction. These results also agree with those of Busse-Valverde, et al., who found that seed tannin content increased in Monastrell and Cabernet Sauvignon wines when CS was applied. To more fully investigate skin and seed tannin extraction, the percentage skin tannin contribution was determined (Table 6). At the end of CS there were significant decreases in skin tannin content with increasing CS duration. The wines made with 10-day CS had significantly higher seed tannin contribution (56.88 %) than the control wine (48.16 %) at the end of CS. At the end of fermentation, a similar trend to the end of CS could be discerned but no significant differences among treatments were observed. These results are similar to those reported by
Panprivech, et al. \textsuperscript{19} and Peyrot des Gachons and Kennedy \textsuperscript{28}, who found that longer CS duration had higher seed tannin contributions.
Table 6. Proanthocyanidin composition of wines made with different CS durations at the end of CS, the end of alcoholic fermentation, and after three months of bottle aging.\(^a\)

<table>
<thead>
<tr>
<th>Tannin (mg/L)</th>
<th>0 day</th>
<th>1 day</th>
<th>2 days</th>
<th>4 days</th>
<th>7 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>end of CS</td>
<td>-</td>
<td>9.96±0.35 a</td>
<td>9.60±0.28 a</td>
<td>9.23±0.18 ab</td>
<td>8.52±0.33 bc</td>
<td>8.26±0.28 c</td>
</tr>
<tr>
<td>end of AF</td>
<td>12.67±0.44 a</td>
<td>12.88±0.21 a</td>
<td>12.78±0.39 a</td>
<td>12.58±0.11 a</td>
<td>13.21±0.38 a</td>
<td>11.67±0.85 a</td>
</tr>
<tr>
<td>bottle</td>
<td>12.77±0.15 a</td>
<td>13.40±0.77 a</td>
<td>13.66±0.50 a</td>
<td>12.69±0.09 a</td>
<td>13.38±0.15 a</td>
<td>13.22±0.37 a</td>
</tr>
<tr>
<td>end of CS</td>
<td>-</td>
<td>120.76±4.31 a</td>
<td>107.87±8.80 a</td>
<td>119.43±7.42 a</td>
<td>117.55±8.87 a</td>
<td>120.09±13.42 a</td>
</tr>
<tr>
<td>end of AF</td>
<td>770.92±92.96 a</td>
<td>935.27±15.92 a</td>
<td>928.67±67.24 a</td>
<td>852.05±6.53 a</td>
<td>984.54±71.23 a</td>
<td>733.47±37.81 a</td>
</tr>
<tr>
<td>bottle</td>
<td>896.72±36.98 a</td>
<td>938.22±13.14 a</td>
<td>893.68±86.11 a</td>
<td>914.24±16.53 a</td>
<td>820.74±60.18 a</td>
<td>866.39±35.40 a</td>
</tr>
<tr>
<td>end of CS</td>
<td>-</td>
<td>2957.84±102.45 a</td>
<td>2848.61±84.67 a</td>
<td>2736.48±54.22 ab</td>
<td>2525.02±99.93 bc</td>
<td>2445.17±83.49 c</td>
</tr>
<tr>
<td>end of AF</td>
<td>3506.36±130.68 a</td>
<td>3869.07±61.02 a</td>
<td>3837.37±118.48 a</td>
<td>3779.54±31.83 a</td>
<td>3969.84±113.53 a</td>
<td>3504.94±254.70 a</td>
</tr>
<tr>
<td>Bottle</td>
<td>3815.67±49.29 a</td>
<td>4003.87±234.54 a</td>
<td>4083.95±149.79 a</td>
<td>3792.68±24.09 a</td>
<td>4004.70±46.80 a</td>
<td>3956.10±111.27 a</td>
</tr>
<tr>
<td>Average MW c</td>
<td>-</td>
<td>2.91±0.10 a</td>
<td>2.62±0.07 a</td>
<td>2.73±0.22 a</td>
<td>2.77±0.15 a</td>
<td>2.83±0.14 a</td>
</tr>
<tr>
<td>% Galloyl d</td>
<td>-</td>
<td>2.91±0.10 a</td>
<td>2.62±0.07 a</td>
<td>2.73±0.22 a</td>
<td>2.77±0.15 a</td>
<td>2.83±0.14 a</td>
</tr>
<tr>
<td>Skin tannin</td>
<td>-</td>
<td>2.89±0.37 a</td>
<td>2.86±0.06 a</td>
<td>2.82±0.04 a</td>
<td>3.07±0.16 a</td>
<td>3.11±0.09 a</td>
</tr>
<tr>
<td>contribution</td>
<td>-</td>
<td>27.51±0.39 a</td>
<td>27.14±0.53 a</td>
<td>26.46±0.44 a</td>
<td>24.71±1.63 ab</td>
<td>22.36±1.04 b</td>
</tr>
<tr>
<td>% Gallo units e</td>
<td>-</td>
<td>36.07±0.86 a</td>
<td>37.56±0.36 a</td>
<td>37.04±1.30 a</td>
<td>36.98±0.13 a</td>
<td>36.73±1.07 a</td>
</tr>
<tr>
<td>% Skin tannin contribution</td>
<td>-</td>
<td>37.81±1.61 a</td>
<td>38.27±1.11 a</td>
<td>39.84±0.47 a</td>
<td>37.96±1.60 a</td>
<td>38.74±0.31 a</td>
</tr>
<tr>
<td>% Galloyl d</td>
<td>-</td>
<td>36.07±0.86 a</td>
<td>37.56±0.36 a</td>
<td>37.04±1.30 a</td>
<td>36.98±0.13 a</td>
<td>36.73±1.07 a</td>
</tr>
<tr>
<td>% Gallo units e</td>
<td>-</td>
<td>37.81±1.61 a</td>
<td>38.27±1.11 a</td>
<td>39.84±0.47 a</td>
<td>37.96±1.60 a</td>
<td>38.74±0.31 a</td>
</tr>
<tr>
<td>Skin tannin</td>
<td>-</td>
<td>66.87±1.40 a</td>
<td>69.03±0.57 a</td>
<td>68.11±2.26 a</td>
<td>68.10±0.21 a</td>
<td>67.37±1.94 a</td>
</tr>
<tr>
<td>contribution</td>
<td>-</td>
<td>69.53±3.04 a</td>
<td>69.95±1.62 a</td>
<td>72.86±0.76 a</td>
<td>69.86±2.99 a</td>
<td>70.98±0.55 a</td>
</tr>
</tbody>
</table>

\(^a\) Results are expressed as means ± standard deviation (n = 3). (-) means no analysis as there is no end of CS for non-CS treatment. The significant differences are indicated as Table 3.

\(^b\) mDP; mean degree polymerization.

\(^c\) Average MW; average molecular weight.

\(^d\) % Galloyl; percentage galloylation (epicatechingallate and epicatechingallate-phloroglucinol) of the total.

\(^e\) % Gallo units; percentage gallo units (epigallocatechin and epigallocatechin-phloroglucinol) of the total.
Comparison of research-scale and commercial-scale CS

The effect of CS has mostly been investigated using small, research-scale fermentations. As commercial winemaking occurs on a much larger scale the trends and effects of CS at the research-scale may not be representative of what occurs in commercial fermentations. Given that the extraction data for the two scales were analyzed at different laboratories and using different RP-HPLC methods, so a direct comparison for all phenolics was not possible although extraction trends can be compared. Both research and commercial scale fermentations had similar trends when comparing extraction profiles for catechin, caftaric acid, and polymeric tannin. In short, extraction increased with longer CS durations. However, no differences among treatments were seen at the end of fermentation for each scale. Malvidin-3-O-glucoside extraction increased with a longer CS for both scales. During fermentation malvidin-3-O-glucoside extraction of each treatment in both scales increased and reached a maximum then decreased during the latter of fermentation. However, there were no significant differences among treatments at the end of fermentation for both scales. For quercetin-glycoside extraction, their extraction increased with longer CS for all research-scale treatments. But for the commercial-scale, quercetin-glycoside extraction reached a maximum after four days of fermentation, followed by a decrease during the remainder of the fermentation period. Both winemaking scales showed no significant differences in quercetin-glycoside extraction by the end of fermentation although the different extraction trends were seen.
Figure 8. Evolution of anthocyanin (a) and tannin (b) concentrations of wines made with different CS duration during CS and alcoholic fermentation in research- and commercial-scale fermentation as determined by analyzing the morning samples of each treatment using the Skogerson-Boulton model. For commercial-scale the end of CS for 1, 2, and 7 days of CS treatments are marked as a drop line (solid line) at 24, 48 and 168 h, respectively. For research-scale the end of CS for 1, 2, and 7 days of CS treatments are marked as a drop line (dashed line) at 33, 57 and 177 h, respectively.

Additionally, UV-vis data was collected on both the research scale and commercial scale samples. Thus the Adams-Harbertson values for anthocyanins and tannins could be calculated using the correlation model and be compared directly (Figure 8a and 8b). Anthocyanins and tannins extraction trends were similar in both
commercial and research-scale wine treatments. The 7-day CS treatment showed greater extraction of anthocyanins and tannins than other treatments but no significant differences among treatments were seen. Furthermore, the results from both scales showed a large difference in the actual amount of anthocyanins and tannins extracted. The commercial-scale fermentations exhibited lower amounts of extraction than the research-scale for all treatments. This may partly be due to the fact that efficient cap management for the removal of phenolic and temperature gradients in commercial-scale fermentations. In the same way, the correlation between the chemical composition of small (microscale) versus production scale wines (CS at 7 °C for four days) were studied by Sampaio, et al. They also reported that both scales showed the similar trends in chemical extraction. However, Sampaio, et al. observed higher amounts of phenolic extraction in the commercial-scale than the research-scale fermentations. The differences in results might be due to the different in winemaking practice (cap management techniques).

**Sensory evaluation**

The wines made with different CS duration were evaluated by descriptive analysis after six months of bottle aging to determine any potential sensory and thus quality impact. Results from descriptive analysis indicated that there were no significant differences among treatments made with 1-, 2-, and 10-day CS at 10 °C for any of the attributes. However, the 4- and 7-day CS wines were significantly higher in caramelized/vanilla/browned flavor compared to the 1-day CS wine (Table 7, Figure 9). Bitterness decrease with up to 2 days of CS, but there is no added benefit for this attribute with additional CS duration. According to sensory analysis there was no impact of CS duration on the mouthfeel of the finished wine. This is in agreement with the phenolic compositional analyses of the wines. Similarly, Casassa, et al. 56
found that the application of CS with solid SO₂ (9 ± 2.5 °C for four days) had no effect on perceived aroma, taste (bitterness), and mouthfeel sensations for any six wine cultivars (Barbera D’Asti, Cabernet Sauvignon, Malbec, Merlot, Pinot noir, and Syrah). In Cabernet Sauvignon wines with or without CS (7 °C for one day), no sensory differences were also detected in the study of Gardner, et al. 57

**Table 7.** Significant descriptive analysis attributes for difference CS duration wines after six months of bottle aging.

<table>
<thead>
<tr>
<th>Significant attribute</th>
<th>Cold soak duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>Caramelized / Vanilla / Browned</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.6 b</td>
</tr>
<tr>
<td>Bitter taste</td>
<td>4.4 a</td>
</tr>
</tbody>
</table>

* Different letters within the same row indicates significance at $p < 0.1$ level (n = 2).

**Figure 9.** Significant attribute intensities for the different CS duration wines after six months of bottle aging.
Conclusions

In conclusion, the impact of different CS durations on phenolic extraction during CS and fermentation in Cabernet Sauvignon wines were investigated. The results obtained show that the CS duration had no significant effect on the extraction of hydroxycinnamates, tannins, and total anthocyanins at the end of CS and fermentation as well as in the bottled wines. Wines produced with four to ten days CS showed increased concentrations of gallic acid, (+)-catechin, and (-)-epicatechin than non-CS wines at the end of alcoholic fermentation but this did not persisted in 3-month bottled wine except for (-)-epicatechin. The wines made with ten days CS showed higher seed tannin contributions than other treatments although this was only observed at the end of CS. In general, there was very little difference in phenol composition when comparing the bottled wines made with different CS durations. Similarly, sensory analysis indicated very little difference among the wines made with different CS duration with only two attributes showing some significant impact. Furthermore, similar trends in phenolic extraction between research-scale and commercial-scale wines were observed for different CS durations. Our research indicate that in the case of Cabernet Sauvignon and the CS and winemaking conditions used in this study, CS had very little impact on the final wines.

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CHAPTER 4
The Effect of Pump-over Conditions on the Extraction Rates of Phenolic Compounds and Their Compositions during Cabernet Sauvignon Fermentation

Abstract

The formation of the cap during red wine fermentation necessitates some mode of cap management be employed so that proper control of fermentation temperatures can be maintained and phenolic extraction can be promoted during the course of alcoholic fermentation. One often-employed cap management technique is the pump-over, in which fermenting must is pumped from below the cap and sprayed over the top of it. The efficacy of the pump-over at maintaining temperature and promoting phenolic extraction will be affected by both the volume and frequency of the pump-over, parameters that have not been thoroughly investigated. Cabernet Sauvignon fermentations were performed in which the volume and frequency of the pump-over were varied according to standard winemaking practices and phenolic extraction was monitored. In addition the effects of extremely low pump-over volumes and frequencies were also investigated. Pump-over volume and frequency were found to have no significant effects on phenolic extraction although trends in extraction were observed, with increasing volume and frequency leading to greater extraction. The trends at the end of fermentation persisted during bottle aging. Tannin analysis of the bottled wines showed no differences in tannin concentration, mead degree of polymerization, percent galloylation, or percent gallo units.
Introduction

During red wine production juice from crushed grapes is fermented in the presence of grape skins and seeds so that the phenolic content in these solids may extract into the fermenting wine. The major classes of phenolics extracted during fermentation are the anthocyanins, flavanols (including flavanol polymers, called proanthocyanidins or tannins), flavonols, and hydroxycinnamates. The two most abundant classes of phenolics in wine are the anthocyanins and the flavanols, which contribute greatly to the quality of the wine, as they are responsible for the color, mouthfeel, and taste. Anthocyanins (red pigments found in grape skins) are the phenolics responsible for the color of red wines. The extraction of anthocyanins during the early part of fermentation has been documented and accredited to the more hydrophilic nature of anthocyanins. The primary contributors to mouthfeel and taste are the flavanols, especially the proanthocyanidins, which are located in the skins and seeds. Interactions between the flavonols in the wine and proline-rich salivary proteins produce the sensation of the astringency, which is often an identifying sensory characteristic of red wines. While proanthocyanidins are found in both grape skin and seed, the chemical and sensory properties of proanthocyanidins from the two tissues are distinctly different. Seed proanthocyanidins are less polymerized (an approximate mean degree of polymerization of 10) than those found in skins (an approximate mean degree of polymerization of 30). From a sensory standpoint the astringency of proanthocyanidins increases with size, while bitterness decreases. These trends impact the sensory properties of a wine, as increased skin tannin extraction produces wines that are perceived as being less harsh and a more favorable mouthfeel. The phenolics found in lesser concentrations (primarily the
hydroxycinnamates and flavonols) serve to primarily stabilize wine color through interactions with the anthocyanins, such as in co-pigmentation \textsuperscript{10,11}.

As the bulk of grape phenolics are located in the skins and seeds contact between the juice and these grape solids is necessary for extraction. The presence of skins and seeds during fermentation results in the formation of a cap at the surface of the fermentor, as carbon dioxide produced during fermentation causes the solids to rise to the surface \textsuperscript{12}. This dense cap has two potentially negative effects on the fermenting wine: the trapping of the heat of fermentation and reduction in the contact between the solids and the fermenting juice \textsuperscript{1,3}. The trapping of the heat of fermentation in the cap has been shown to lead to temperature gradients as large as 12 °C between the liquid and the center of the cap \textsuperscript{13}. These elevated cap temperatures may lead to difficult to control fermentations with the yeast populations in the must and cap fermenting at uneven rates as well as deactivation, or even death, of parts of the yeast population \textsuperscript{14}. Reduction in contact time between the bulk of the juice and the grape solids can lead to decreased phenolic extraction, resulting in wines with less color and unexpected mouthfeel. During fermentation phenolic content increases until equilibrium is reached between the phenolic content in the solids and the liquid in contact with the solids \textsuperscript{15}. Once this equilibrium is reached further increases in phenolic extraction are not possible. Minimal juice contact will most likely lead to this equilibrium being reached relatively early during fermentation and most likely only in the lowest portions of the cap (where relatively constant juice contact occurs). Reduction in contact between the cap and bulk juice may also lead to decreases in the phenolic content of the finished wine as extracted phenolics are lost to adsorption to solids, such as yeast hulls \textsuperscript{16}.
To minimize the effects of cap formation on red wine production, winemakers employ various cap management techniques to mix the fermentor, homogenizing the temperature as well as the phenolic content. Even though many cap management techniques exist, the pump-over has become one of the most widespread and oft used techniques owing largely to the simplicity and ease with which it can be performed. During the pump-over fermenting must is pumped from the bottom portion of the fermentor and sprayed over the top of the cap. Previous studies investigating the effect of pump-overs on phenolic extraction have focused largely on the comparison of pump-overs to other cap management techniques, such as punch-down and submerged cap. Early investigations comparing pump-over and punch-down during Cabernet Sauvignon fermentations found that pump-overs produced wines with slight increases of color and tannin. A more recent investigation comparing pump-over to punch-down (both manual and mechanical) during the fermentation of Pinot noir, Dornfelder, and Portugieser found that the effects of the cap management technique were dependent on the variety. In this study it was found that the only phenolic increased by pump-over in all varieties was quercetin and Dornfelder being the only variety in which pump-over increased extraction. Pinotage fermentations in which phenolic extraction was compared using manual punch-down, pump-over, and rotary mixing found that the pump-over fermentations extracted the least total flavonols, anthocyanins, and tannins. The authors of this study also found that the rate of extraction for the pump-over fermentations was slower than the other fermentations in the study.

Previous studies have focused their analyses on finished wines, comparing cap management treatments after fermentation was finished. While these investigations have provided valuable insight on the effect of cap management on finished wines,
relatively little information on how phenolic extraction processes and red wine fermentation as a whole are available as extraction was not followed during the course of fermentation. Recently, the effects of two other often used cap management techniques (submerged cap and plunging) during red wine fermentations have been thoroughly investigated. For Barbera wines produced using the traditional floating cap and submerged cap techniques it was found that the submerged cap fermentations extracted less phenolics than the floating cap. The decreased phenolic content in the submerged cap fermentations was accredited primarily to limited mechanical intervention, such as mixing, and to slower rates of alcoholic fermentation. A different study investigated phenolic extraction during Merlot production using different plunging regimes. In this study it was found that the extraction of phenolics (anthocyanins, tannins, and total phenolics) was significantly greater in the fermentations with no plunging than in the fermentations plunged twice daily. Analysis of the phenolics content in the bottled wines showed that over the course of 11 months the non-plunged wines had significantly greater phenolic content than the plunged wines. The authors accredit the decreased extraction in the plunged wines to oxidation from increased oxygen exposure during mechanical disruption of the cap as well as increased adsorption of phenolics to berry solids.

The efficacy of the pump-over at mixing the fermentor, maintaining proper cap temperature, and promoting phenolic extraction will most likely be affected by the volume and frequency of the pump-over. Surprisingly, these parameters of the pump-over have yet to be fully investigated. To investigate the effects of pump-over volume and frequency on red wine fermentation research-scale Cabernet Sauvignon fermentations were performed in which phenolic extraction and cap and must temperatures were monitored. Finished wines were also analyzed for phenolic content
five months post-treatment to determine if effects at the end of fermentation were maintained following bottling.

**Materials and Methods**

**Reagents**

Acetone (reagent grade), acetonitrile (HPLC grade), L-ascorbic acid (molecular biology grade), caffeic acid (98%), (+)-catechin hydrate (98%), p-coumaric acid (98%), (-)-epicatechin (95%), gallic acid monohydrate (99%), hydrochloric acid (37%, reagent grade), methanol (HPLC grade), phloroglucinol (99% purity), quercetin (95%), trifluoroacetic acid (HPLC grade), and Toyopearl HW-40F size exclusion media were all purchased from Sigma Aldrich (St. Louis, MO). Quercitrin (98.5%) was purchased from Indofine Chemical Company (Hillsborough, NJ). Malvidin-3-O-glucoside (95%) was purchased from Extrasynthese (Genay Cedex, France). Phosphoric acid (88%) and sodium acetate trihydrate (HPLC grade) were purchased from Fisher Scientific (Pittsburgh, PA). Koptec brand ethanol (95%) was purchased from Decon Laboratories, Inc. (King of Prussia, PA). Deionized water was prepared in-house to a final purity of 18 MΩ. All solvents were filtered through a 0.22 µm filter prior to use.

**Winemaking**

All winemaking occurred at the Teaching and Research Winery of the University of California, Davis (Davis, CA) during the 2012, 2013, and 2014 harvests. All winemaking practices were identical with the exception of cap management and skin contact time. The experimental parameters for the three sets of fermentations are summarized in Table 1. The pump-over parameters investigated were volume (2012 harvest), frequency (2013 harvest), and low volume and
frequency extremes (2014 harvest). Fermentations were performed in triplicate for all three harvests. Cabernet Sauvignon grapes were sourced from the same vineyard in Lodi, CA for all three harvests. Grapes were destemmed and crushed directly into a must pump, from which the research fermentors were filled. Cypress/UC Davis research fermentors were filled with must to a final volume of 90 L and brought to 25 °C prior to inoculation with Saccharomyces cerevisiae strain D254 (Lallemand Lalvin, Scott Laboratories, Petaluma, CA). All fermentations were performed at 25°C and were sampled twice daily (morning and evening). All fermentations were pressed after the specified skin contact time (Table 1). Following pressing all wines were inoculated with Oenococcus oeni (Chr. Hansen, Inc., Milwaukee, WI, USA) and allowed to proceed through malolactic fermentation, after which they were racked, sterile filtered, and bottled.

Table 1. Summary of experimental conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total pump-over volume</th>
<th>Frequency</th>
<th>Total skin contact (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2012 Harvest</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HV2X</td>
<td>1</td>
<td>2</td>
<td>163.5</td>
</tr>
<tr>
<td>1V2X</td>
<td>2</td>
<td>2</td>
<td>163.5</td>
</tr>
<tr>
<td>2V2X</td>
<td>4</td>
<td>2</td>
<td>163.5</td>
</tr>
<tr>
<td><strong>2013 Harvest</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4V1X</td>
<td>4</td>
<td>1</td>
<td>184</td>
</tr>
<tr>
<td>2V2X</td>
<td>4</td>
<td>2</td>
<td>184</td>
</tr>
<tr>
<td>1V4X</td>
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<tr>
<td>HV8X</td>
<td>4</td>
<td>8</td>
<td>184</td>
</tr>
<tr>
<td><strong>2013 Harvest</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HV2X</td>
<td>1</td>
<td>2</td>
<td>177</td>
</tr>
<tr>
<td>HV1X</td>
<td>0.5</td>
<td>1</td>
<td>177</td>
</tr>
<tr>
<td>QV2X</td>
<td>0.5</td>
<td>2</td>
<td>177</td>
</tr>
<tr>
<td>NOPO</td>
<td>0</td>
<td>0</td>
<td>177</td>
</tr>
</tbody>
</table>

*a* Pump-over volume is given as multiples of fermentor volume.

*b* Number of times per day pump-over was performed

Pump-over events were controlled by the integrated fermentation control system (IFCS) of the Cypress/UC Davis research fermentors and performed by the integrated pump. The exception was the once per day pump-over fermentations
during the 2013 harvest, which were controlled manually. Cap and must temperatures, as well as Brix measurements, were monitored by the IFCS during fermentation. All acquired fermentation data was transmitted wirelessly to a central dashboard computer for storage and monitoring.

**Reverse-phase HPLC**

Monomeric phenolics were monitored during extraction using a high-throughput reverse-phase HPLC (RP-HPLC) method described previously. The phenolics monitored were gallic acid, (+)-catechin, (-)-epicatechin, caftaric acid, quercetin-glycosides (the summed peak areas for quercetin-3-O-glucoside, quercetin-3-O-galactoside, and quercetin-3-O-glucuronide), and malvidin-3-O-glucoside. Analyses were performed on an Agilent 1260 Infinity HPLC (Agilent Technologies, Santa Clara, CA) equipped with a binary pump, autosampler, column compartment, and diode array detector. Instrument control and data analysis was performed in Agilent CDS ChemStation software (Rev. B.04.03). Identification of phenolics was based on comparison with authentic standards. Phenolics were quantified by external calibration, with curves generated for gallic acid, (+)-catechin, (-)-epicatechin, caffeic acid (used for caftaric acid), quercetin-3-O-rhamnoside (used for quercetin-glycosides), and malvidin-3-O-glucoside. The phenolic content of the bottled wines was analyzed for the above phenolics with the addition of quercetin (quantitated by external calibration using a quercetin curve).

**Determination of proanthocyanidins**

The concentration of proanthocyanidins in each fermentation sample was determined by UV-VIS spectroscopy and the Skogerson-Boulton correlation method for determination of the Adams-Harbertson values. UV-Vis absorption spectra were collected from 230-900nm using a 0.1 mm quartz flow cell (Starna Cells, Atascadero,
CA) on an Agilent 8453 UV-VIS spectrophotometer (Agilent Technologies, Santa Clara, CA). Samples were clarified by centrifugation prior to spectral acquisition. Absorption spectra were analyzed using the publicly available Microsoft Excel correlation worksheet (http://boulton.ucdavis.edu).

**Isolation and analysis of proanthocyanidins**

Proanthocyanidins were isolated from grape samples collected at the time of crushing as well as the finished wines. Proanthocyanidins were extracted from grape skins and seeds by solvent extraction and then isolated and purified by solid phase extraction (SPE) using Toyopearl HW-40F size exclusion media ²⁴. Wine proanthocyanidins were isolated and purified from wine samples using the same SPE method employed for skin and seed extracts. All proanthocyanidin fractions from SPE purification were dried under vacuum at 35 °C and reconstituted in 0.5 mL of methanol. Purified proanthocyanidins were stored at -20 °C and analyzed within three months of purification. Proanthocyanidin fractions were analyzed by phloroglucinolysis and RP-HPLC using previously described methods ²⁵. Proanthocyanidin fractions were characterized based on the mean degree of polymerization (mDP), percent galloylation, percent gallo units, and average molecular weight.

**Data analysis**

Fermentation data was plotted in Microsoft Excel 2011 (Microsoft, Redmond, WA). The Fisher’s least significant differences (LSD) ($p < 0.05$) were used to determine the statistical differences among treatment means using the function found in the Agricolae package, which was built under R version 2.15.1.
Results and Discussion

Pump-over volume

The effects of pump-over volume on phenolic extraction were investigated during the 2012 harvest. Pump-over volumes investigated were half a fermentor volume, one fermentor volume, and two fermentor volumes. All pump-overs were performed at a frequency of twice per day and the fermentor volume was 75 L. The extraction profiles for malvidin-3-O-glucoside (mlv-3-glu), quercetin-glycosides, (+)-catechin, and tannins are shown in Figure 1. These phenolics were chosen as being representative of skin extraction (malvidin-3-O-glucoside and quercetin-glycosides), seed extraction ((+)-catechin), and extraction from both skins and seeds (tannins). While (+)-catechin is found in both the skin and seeds of grapes, it has been shown that the concentration is much greater in the seeds than the skins and can be used as an indicator of seed extraction. The extraction data for the skin phenolics (Figure 1a and 1b) showed minor differences in extraction pump-over volume was increased. For mlv-3-glu (Figure 1a) the two-volume fermentations reached maximum extraction first (99.5 h), followed by the half-volume pump-over (115.5 h), and the one-volume fermentations taking the longest to reach maximum extraction (139.5). The maximum concentration of mlv-3-glu reached in all treatments was approximately 148 mg/L. Regardless of when maximum extraction was reached the concentration of mlv-3-glu became relatively uniform in all treatments by about 163.5 h. Caftaric acid extraction was similar to that of mlv-3-glu, increasing during the first 72 h of fermentation and then remaining fairly constant during the rest of the fermentation (Figure 2a). The extraction of the quercetin-glycosides (Figure 1b) was relatively uniform among treatments, with a slight difference in extraction occurring around 91.5 h, although the
differences among treatments at this point were not significant and were lost by the next pump-over (115.5 h).

Figure 1. The extraction profile of malvidin-3-O-glucoside (a), quercetin-glycoside (b), (+)-catechin (c), and tannins (d) of wine made with different pump-over volume during fermentation as determined by RP-HPLC (n=3).
Figure 2. The extraction profile of caftaric acid wine made with different pump-over volume during fermentation as determined by RP-HPLC (n=3).

The extraction of (+)-catechin was not affected by increases in pump-over volume, with all three treatments having similar extraction (Figure 1c). Little extraction occurred during the first 24 hours of fermentation, after which extraction occurred in two fairly linear portions, the first being between 48-96 hours and the second being 96-168 hours. The combined effect of these two portions is an overall extraction that is non-linear during the first several days of fermentation. This non-linearity in extraction has been accredited to increased extraction rates from increasing ethanol concentrations and is an extraction trend that has previously been described by Lerno, et al. 22, Canals, et al. 27, Hernández-Jiménez, et al. 28, Oszmianski, et al. 29. Similar extraction trends were observed for both (-)-epicatechin and gallic acid (Figure 2b and 2c), two other phenolics that have been shown to be much more abundant in grape seeds 26. Tannin extraction was linear in all treatments, with increasing pump-over volume having no significant effect on extraction (Figure 1d).

The extraction data for the monitored phenolics suggests that increases in pump-over volume led to faster extraction, as the phenolic content in the two-volume treatments generally increased faster than the one-volume treatments. This was
especially true for mlv-3-glu. The two-volume treatments reached maximum extraction 40 h before the one-volume fermentations. The faster extraction may be due to increased mixing and homogenization of the phenolic content in the fermentor with greater pump-over volumes. It is known that phenolic extraction requires a concentration gradient exist between the phenolic content in the solids and the must. During pump-over the distribution of the extracted phenolics throughout the fermentor is homogenized, with the localized concentration of phenolics in the cap decreasing, restoring concentration gradients, and driving further extraction. The extraction behavior of the half-volume ferments (being similar to the two-volume) may be a result of the cap temperatures in these fermentations remaining elevated following pump-over. Temperature data recorded by the IFCS showed that in the half-volume fermentations the cap temperature following pump-over was 2-3 °C greater than the must temperature. The one- and two-volume fermentations had complete homogenization of temperature, with the cap cooling to the temperature of the must during the pump-over. Investigations into the effects of cap and fermentation temperatures on phenolic extraction during Cabernet Sauvignon fermentation showed that increased cap temperatures led to an earlier extraction of skin phenolics but not greater overall extraction.

**Pump-over frequency**

Pump-over frequency treatments of one-, two-, four-, and eight-times per day were investigated during the 2013 harvest (total pump-over volume was constant at four fermentor volumes, 90 L). The overall extraction behavior for the monitored phenolics was similar to those in the volume treatments with the exception of the skin phenolics (Figure 3a and 3b). The extraction data for mlv-3-glu did not show the characteristic profile for anthocyanins as no maximum was reached during the period
of skin contact. The extraction profile observed is not likely an effect of pump-over frequency as the two times per day treatment is the same cap management regime as the two-volume treatment (2012 harvest, Figure 1), which showed a more characteristic extraction profile. If skin-contact time had been increased a more characteristic extraction profile may have been obtained. The extraction profile of the quercetin-glycosides was also different than those obtained in the pump-over volume experiments (Figure 3b). The quercetin-glycosides concentration reached a maximum after 84 hours for the four times per day treatments and just after 108 hours for the other treatments. Following these maxima the concentration of quercetin-glycosides decreased. Increased loss of quercetin-glycosides during fermentations using pump-overs have been reported and were accredited to increased hydrolysis of the glycosides or increased rates of oxidation \(^{18}\). Neither conclusion is readily evident from the extraction data as increases in quercetin were linear during fermentation and do not correlate well with the loss of the glycosides and the oxidized forms of the flavonols were not monitored (data not shown). The extraction of (+)-catechin (Figure 3c) and tannins (Figure 3d) were similar to the extractions in the pump-over volume treatments.
Figure 3. The extraction profile of malvidin-3-O-glucoside (a), quercetin-glycoside (b), (+)-catechin (c), and tannins (d) of wine made with different pump-over frequency during fermentation as determined by RP-HPLC (n=3).

While no significant differences were observed among treatments with different pump-over frequencies a trend towards increased phenolic extraction with increasing pump-over frequency was observed. This trend is clearly evident in the extraction of mlv-3-glu (Figure 3b) and to lesser extents in the extraction of (+)-catechin and tannins (Figure 3c and 3d). The cap and must temperature recorded by the IFCS show cap and must temperatures were well homogenized in all the treatments. Minor increases (27 °C rather than 25 °C) occurred in the must temperature of the more frequent pump-over treatments during the most active periods of fermentation. The must temperature of the lower frequency treatments (one and two times-per-day) remained at 25°C during the fermentation. Must
temperature has been shown to be more of a driver of phenolic extraction than cap temperature, which may explain the trend and the slight increases in phenolic extraction that were observed \(^{22}\). The increased phenolic extraction in the more frequent pump-over treatments may also be an effect of longer periods of concentration gradients within the cap. Increased pump-over frequencies would minimize the buildup of extracted phenolics in the cap, increasing the total amount of time with a maximum concentration gradient.

**Low extremes in pump-over volume and frequency**

The pump-over volume and frequency experiments performed during the 2012 and 2013 harvests investigated pump-over parameters that are more common to standard winemaking practices. As these experiments showed no significant differences among treatments a third set of fermentations were performed during 2014 in which low extremes of pump-over volume (half- and quarter-volume) as well as a no pump-over fermentation were investigated (Table 1). For these experiments the pump-over frequencies was either once or twice per day. The extraction profiles for mlv-3-glu, quercetin-glycosides, (+)-catechin, and tannins are shown in Figure 4. The skin phenolics (mlv-3-glu and quercetin glycosides) had very similar extraction profiles (Figure 4a and 4b, respectively) and will be discussed as one. For all treatments the greatest rates of extraction occurred in the first 69 hours of fermentation, with the rate of extraction being the greatest in the half-volume twice-per-day treatment and the other treatments having a slower rate. After 69 hours the rate of extraction decreased in all treatments. Maximum extraction was reached at 129 hours for both half-volume treatments, while the quarter-volume and no pump-over treatments reached maximum concentrations at 141 hours. No significant differences occurred in extraction although a general trend of increased phenolic extraction with increased
pump-over volume was observed. While not significant the extraction profile of the half-volume twice per day treatment was different than the other treatments. The half-volume twice per day treatment extracted more phenolics than the other treatments during early fermentation and the concentration of skin phenolics remained greater in this treatment for the duration of fermentation. Extraction in the other treatments was similar during fermentation with clear differences occurring at 141 h, when extraction in the no pump-over treatments became less than the half-volume once per day and quarter-volume treatments (although this difference was not significant).

![Graph](image)

**Figure 4.** The extraction profile of malvidin-3-O-glucoside (a), quercetin-glycoside (b), (+)-catechin (c), and tannins (d) of wine made with different low extremes in pump-over volume and frequency as determined by RP-HPLC (n=3).

The extraction of (+)-catechin and tannins showed similar trends (Figure 4c and 4d, respectively). Extraction was linear during fermentation in both half-volume as well as the quarter-volume treatments. For these treatments the half-volume once-
per-day and quarter-volume fermentations were nearly identical in extraction and extracted less than the half-volume twice-per-day fermentations, although the differences were not significant. Extraction in the no pump-over treatments was similar to the other treatments until 141 h, after which the increase in concentration slowed, indicating that extraction was reaching equilibrium or the rate of extraction and adsorption were becoming similar. The similarity in extraction profiles between the no pump-over treatment and the treatments receiving pump-over may be due to some degree of natural mixing in the no pump-over treatment as carbon dioxide produced during active fermentation bubbled up through the fermentor. This mixing may have been enough to maintain the necessary concentration gradients for extraction to occur and as fermentation slowed (and carbon dioxide production slowed) any mixing effects would become less, allowing equilibrium to be reached sooner than the other treatments.

The extraction of caftaric acid (Figure 5a) was similar to both mlv-3-glu and the quercetin-glycosides (Figure 4a and 4b, respectively), with the greatest extraction occurring early during the fermentation (within 69 h) followed by gradual extraction during the remainder of fermentation. All four treatments extracted the same, with no differences in rate or amount being observed. This is most likely due to caftaric acid being located primarily within the pulp of the grape. The extraction of (-)-epicatechin and gallic acid (Figures 5b and 5c, respectively) were similar to those of (+)-catechin and tannins.
Figure 5. The extraction profile of caftaric acid (a), (-)-epicatechin (b), and gallic acid (c) of wine made with different low extremes in pump-over volume and frequency as determined by RP-HPLC (n=3).

**Phenolic content in bottled wines**

The phenolic content of the bottled wines was analyzed five months post-treatment. As the fermentations in the three harvests had different total skin contact time, as well as different time in the bottle, comparison of the phenolic content of the bottled wines will only be made between the treatments in a given harvest. No
significant differences were found in the phenolic content of the treatments in the 2012, 2013, or 2014 harvests (Table 2). For the pump-over volume and frequency treatments (2012 and 2013 respectively) the lack of significant differences in the phenolic content of the bottled wines continues the trends observed at the end of fermentation. The low volume and low frequency fermentations (2014 harvest) maintained the general extraction trends observed among treatments at the end of fermentation although the differences were not significant. The wines produced with no pump-over had the least skin- and seed-derived phenolics than the other treatments, followed by the quarter volume and half volume once-per-day treatments. The greatest phenolic content was in the half volume twice-per-day treatments. While not monitored in the fermentation samples, polymeric pigments were measured in the bottled wines. No significant differences existed in the concentration of polymeric pigments among the treatments in 2012, 2013, or 2014 harvests.

The tannin content of the bottled wines was characterized by phloroglucinolysis and no significant differences were found among the treatments in 2012, 2013, and 2014 (Table 3). No trends in the mDP, average molecular weight, percentage of gallo units, and percent galloylation existed between the treatments in the pump-over volume and frequency treatments (2012 and 2013 harvests respectively). While significant differences did not exist among the 2014 treatments, trends in tannin extraction and content did. Mean degree of polymerization increased slightly with less pump-overs, with the half volume twice per day treatments having the lowest mDP and the no pump-over treatments having the largest (12.77 ± 0.15 and 13.79 ± 0.36 respectively). Increased values for average molecular weight and percentage of gallo units also occurred with decreasing pump-over. The average molecular weight of the half volume twice per day treatment was 3815.67 ± 49.29
g/mol while the no pump-over treatment was 4120.23 ± 105.98 g/mol. The percentage of gallo units in the tannins increased from 37.81 ± 1.61% in the half volume twice per day treatment to 39.36 ± 1.37% in the no pump-over treatments. The percentage of galloylation showed an opposite trend, decreasing with decreasing pump-overs. The percentage of galloylation in the half volume twice per day treatments was 2.89 ± 0.37% while in the no pump-over treatments it was 2.68 ± 0.11%. These differences in tannin characteristics among the treatments imply that more skin tannin was extracted with decreasing pump-overs, which is indicated by the increases in mDP and average molecular weight as well as the decrease in percentage of galloylated tannins. The trend towards increased skin extraction with less pump-overs may be due to equilibrium in tannin extraction being reached with the more accessible skin tannins. The location of the skin tannins in the vacuoles of the hypodermal cells makes their extraction easier while seed tannins require increased amounts of liquid contact to begin breaking down seed cell structures. As these differences are minor (and not significant) the increase in skin tannin contribution is most probably negligible and would not likely have any impact on the mouthfeel of the final wines.
Table 2. Concentration of phenolic compound (mg/L) in finished wines (five months post-treatment) made with different pump-over conditions in the 2012, 2013, and 2014 harvest.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gallic acid</th>
<th>(+)-Catechin</th>
<th>(-)-Epicatechin</th>
<th>Caffeic acid</th>
<th>Quercetin-glycoside</th>
<th>Quercetin</th>
<th>Malvidin</th>
<th>Tannin</th>
<th>Polymeric pigments</th>
</tr>
</thead>
<tbody>
<tr>
<td>HV2X</td>
<td>27.12 ± 0.66 a</td>
<td>38.97 ± 1.49 a</td>
<td>27.55 ± 0.84 a</td>
<td>6.44 ± 0.89 a</td>
<td>22.17 ± 2.70 a</td>
<td>14.30 ± 2.35 a</td>
<td>94.46 ± 6.37 a</td>
<td>499.84 ± 73.08 a</td>
<td>4.77 ± 0.35 a</td>
</tr>
<tr>
<td>2012 1V2X</td>
<td>29.14 ± 2.85 a</td>
<td>40.71 ± 5.72 a</td>
<td>27.21 ± 3.51 a</td>
<td>4.04 ± 1.47 a</td>
<td>24.64 ± 1.73 a</td>
<td>15.85 ± 1.43 a</td>
<td>94.79 ± 8.45 a</td>
<td>566.20 ± 20.56 a</td>
<td>4.55 ± 0.31 a</td>
</tr>
<tr>
<td>Harvest</td>
<td>2V2X</td>
<td>28.63 ± 0.65 a</td>
<td>37.93 ± 2.33 a</td>
<td>24.98 ± 0.75 a</td>
<td>3.89 ± 0.49 a</td>
<td>25.44 ± 0.58 a</td>
<td>15.13 ± 0.89 a</td>
<td>92.13 ± 6.57 a</td>
<td>447.19 ± 18.75 a</td>
</tr>
<tr>
<td></td>
<td>4V1X</td>
<td>26.16 ± 0.55 a</td>
<td>36.61 ± 3.75 a</td>
<td>16.89 ± 0.64 a</td>
<td>3.03 ± 0.20 a</td>
<td>10.81 ± 1.06 a</td>
<td>1.68 ± 0.57 a</td>
<td>140.92 ± 18.82 a</td>
<td>941.74 ± 61.54 a</td>
</tr>
<tr>
<td>2013 2V2X</td>
<td>27.38 ± 0.28 a</td>
<td>38.48 ± 1.05 a</td>
<td>17.60 ± 1.12 a</td>
<td>2.92 ± 0.30 a</td>
<td>11.82 ± 0.62 a</td>
<td>2.26 ± 0.55 a</td>
<td>135.63 ± 8.40 a</td>
<td>1036.34 ± 46.87 a</td>
<td>17.20 ± 0.61 a</td>
</tr>
<tr>
<td>Harvest</td>
<td>1V4X</td>
<td>25.93 ± 0.72 a</td>
<td>38.24 ± 1.72 a</td>
<td>17.91 ± 1.14 a</td>
<td>2.56 ± 1.17 a</td>
<td>11.85 ± 1.01 a</td>
<td>2.27 ± 0.74 a</td>
<td>135.85 ± 1.77 a</td>
<td>934.44 ± 33.04 a</td>
</tr>
<tr>
<td></td>
<td>HV8X</td>
<td>27.04 ± 1.04 a</td>
<td>36.48 ± 3.32 a</td>
<td>17.41 ± 0.69 a</td>
<td>2.49 ± 0.66 a</td>
<td>11.32 ± 0.19 a</td>
<td>1.74 ± 0.48 a</td>
<td>133.60 ± 4.93 a</td>
<td>972.34 ± 44.18 a</td>
</tr>
<tr>
<td></td>
<td>HV2X</td>
<td>21.08 ± 0.63 a</td>
<td>29.22 ± 0.97 a</td>
<td>22.06 ± 1.05 a</td>
<td>5.04 ± 0.78 a</td>
<td>32.67 ± 1.24 a</td>
<td>10.65 ± 3.61 a</td>
<td>131.07 ± 2.48 a</td>
<td>896.72 ± 36.98 a</td>
</tr>
<tr>
<td>2014 1V1X</td>
<td>20.31 ± 1.22 a</td>
<td>29.31 ± 2.85 a</td>
<td>20.58 ± 0.56 a</td>
<td>5.48 ± 1.02 a</td>
<td>32.10 ± 2.20 a</td>
<td>13.67 ± 0.90 a</td>
<td>124.10 ± 2.65 a</td>
<td>744.16 ± 39.23 a</td>
<td>18.52 ± 2.56 a</td>
</tr>
<tr>
<td>Harvest</td>
<td>QV2X</td>
<td>20.63 ± 0.69 a</td>
<td>29.27 ± 0.78 a</td>
<td>21.79 ± 1.77 a</td>
<td>5.19 ± 0.27 a</td>
<td>31.54 ± 2.91 a</td>
<td>13.10 ± 0.65 a</td>
<td>128.47 ± 11.27 a</td>
<td>799.91 ± 44.53 a</td>
</tr>
<tr>
<td></td>
<td>NOPO</td>
<td>17.72 ± 0.80 a</td>
<td>24.56 ± 0.87 a</td>
<td>19.36 ± 2.95 a</td>
<td>5.50 ± 2.32 a</td>
<td>28.79 ± 4.15 a</td>
<td>10.83 ± 3.05 a</td>
<td>114.08 ± 8.47 a</td>
<td>692.30 ± 79.86 a</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviation (n=3). Values followed by the same letter within the same column indicate no significant difference (p < 0.05).
Table 3. Proanthocyanidins composition in finished wines (five months post-treatment) made with different pump-over conditions in the 2012, 2013, and 2014 harvest.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>mDP b</th>
<th>Average MW c</th>
<th>% Gallo units d</th>
<th>% Galloylation e</th>
<th>Tannin concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012 Harvest</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HV2X</td>
<td>8.45 ± 0.31 a</td>
<td>2518.62 ± 92.78 a</td>
<td>26.89 ± 2.50 a</td>
<td>3.67 ± 0.38 a</td>
<td>499.84 ± 73.08 a</td>
</tr>
<tr>
<td>1V2X</td>
<td>8.71 ± 0.18 a</td>
<td>2592.98 ± 54.27 a</td>
<td>28.08 ± 0.03 a</td>
<td>3.38 ± 0.11 a</td>
<td>566.20 ± 20.56 a</td>
</tr>
<tr>
<td>2V2X</td>
<td>7.89 ± 0.23 a</td>
<td>2348.17 ± 70.50 a</td>
<td>26.63 ± 0.30 a</td>
<td>3.27 ± 0.18 a</td>
<td>447.19 ± 18.75 a</td>
</tr>
<tr>
<td>2013 Harvest</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4V1X</td>
<td>11.53 ± 0.06 a</td>
<td>3435.19 ± 16.79 a</td>
<td>33.50 ± 0.48 a</td>
<td>2.90 ± 0.01 a</td>
<td>941.74 ± 61.54 a</td>
</tr>
<tr>
<td>2V2X</td>
<td>10.86 ± 0.24 a</td>
<td>3235.54 ± 71.48 a</td>
<td>31.51 ± 0.80 a</td>
<td>2.98 ± 0.07 a</td>
<td>1036.34 ± 46.87 a</td>
</tr>
<tr>
<td>1V4X</td>
<td>11.12 ± 0.53 a</td>
<td>3311.47 ± 161.06 a</td>
<td>33.45 ± 0.90 a</td>
<td>2.81 ± 0.05 a</td>
<td>934.44 ± 33.04 a</td>
</tr>
<tr>
<td>HV8X</td>
<td>10.74 ± 0.23 a</td>
<td>3197.02 ± 69.75 a</td>
<td>33.66 ± 0.42 a</td>
<td>2.77 ± 0.08 a</td>
<td>972.34 ± 44.18 a</td>
</tr>
<tr>
<td>2014 Harvest</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HV2X</td>
<td>12.77 ± 0.15 a</td>
<td>3815.67 ± 49.29 a</td>
<td>37.81 ± 1.61 a</td>
<td>2.89 ± 0.37 a</td>
<td>896.72 ± 36.98 a</td>
</tr>
<tr>
<td>HV1X</td>
<td>13.24 ± 0.48 a</td>
<td>3957.26 ± 144.63 a</td>
<td>39.02 ± 0.56 a</td>
<td>2.89 ± 0.08 a</td>
<td>744.16 ± 39.23 a</td>
</tr>
<tr>
<td>QV2X</td>
<td>13.55 ± 0.13 a</td>
<td>4050.64 ± 39.23 a</td>
<td>39.10 ± 0.63 a</td>
<td>2.90 ± 0.13 a</td>
<td>799.91 ± 44.53 a</td>
</tr>
<tr>
<td>NOPO</td>
<td>13.79 ± 0.36 a</td>
<td>4120.23 ± 105.98 a</td>
<td>39.36 ± 1.37 a</td>
<td>2.68 ± 0.11 a</td>
<td>692.30 ± 79.86 a</td>
</tr>
</tbody>
</table>

a Results are expressed as means ± standard deviation (n = 3). The significant differences are indicated as Table 2.
b mDP; mean degree polymerization.
c Average MW; average molecular weight.
d % Gallo units; percentage gallo units (epigallocatechin and epigallocatechin-phloroglucinol) of the total.
e % Galloyl; percentage galloylation (epicatechingallate and epicatechingallate-phloroglucinol) of the total.
Conclusions

Changes in pump-over volume and frequency had no significant effects on the extraction of phenolics during Cabernet Sauvignon fermentation when volumes and frequencies common to modern winemaking practices were employed. While not significant, trends were observed that showed increased pump-over volumes led to more extraction of both grape skin and seed derived phenolics. Similar non-significant trends were observed for pump-over frequency, with more phenolic extraction occurring with increased frequency. Cabernet Sauvignon fermentations performed at extreme lows of volume and frequency as well as no pump-overs more clearly showed trends of increased phenolic extraction with increasing pump-overs, although for the most part these trends were not significant. Analysis of the phenolic content five months post-treatment showed no significant differences among treatments. Analysis of the tannin content in the wines also showed no significant differences among treatments although a slight increase in skin tannin extraction appears to have occurred in the no pump-over fermentations.

References


CHAPTER 5

Chemical Gradients in Pilot Scale Cabernet Sauvignon Fermentations and Their Effect on Phenolic Extraction

Abstract

The phenolic content of red wine is responsible for the color and mouthfeel of the wine. Phenols are extracted from the skins and seeds of the crushed grapes through contact with the juice. Formation of a skin cap at the top of the fermentation decreases this contact, necessitating cap management techniques such as punch downs or pump-overs. While it is clear that these techniques are necessary for extraction, the kinetics of phenolic release, adsorption, and reaction in this heterogeneous system are not well understood. To better understand these kinetics, pilot-scale (2000 L) Cabernet Sauvignon fermentations were performed in two consecutive harvests (2011 and 2012) using a tank modified with a custom sampling grid that allowed the fermentations to be sampled at four depths (two in the cap and two in the liquid) with three replicate sampling points at each depth. Chemical gradients were observed for several phenolics, including gallic acid, (+)-catechin, (-)-epicatechin, caftaric acid, malvidin-3-O-glucoside, total anthocyanins, and condensed tannins. Chemical gradients for skin phenolics, such as the anthocyanins, were observed to develop early during fermentation and became minimal as fermentation progressed. Phenolics found primarily within the seed, such as (+)-catechin, show chemical gradients throughout the fermentation. Seed extraction trends were confirmed using phloroglucinolysis. Interestingly, phenolic gradients were eliminated following a pump-over event, but were re-established during the subsequent few hours and reached a saturation point approximately 8 h post-pump-over with no further extraction.
Introduction

The phenolic component of red wine is responsible for the color, mouth feel, flavor, and ageing potential, all of which are important aspects of the wine’s quality. Anthocyanins and polymeric pigments are responsible for the color of red wine, while the flavan-3-ols and their polymers, also known as condensed tannins, are responsible for the bitter taste as well as astringency through interactions with salivary proteins. It is well known that the phenolic composition of a red wine is dependent on both the grape variety and the winemaking practices employed, as these practices will determine the extent of extraction of phenolics into the wine. However, the mechanisms for the extraction of phenolics and the spatial heterogeneity of phenolics during fermentation are not well understood. This lack of knowledge regarding the extraction mechanisms and spatial heterogeneity of phenolics in the fermentor during fermentation makes informed manipulation of the phenolic content of the finished wine difficult.

Previous work has examined overall phenolic and color extraction as a function of processing conditions (reviewed in Sacchi, et al.), including the effects of temperature. The interaction and binding of free phenolics with isolated cell wall components as well as yeast hulls has been examined, illustrating the potential impact on net extraction. Finally, others have examined reactions of phenolics into polymeric forms not present in the original berry (reviewed in Casassa and Harbertson as well as Fulcrand, et al.). However, generally these studies have used experimental systems simulating a wine environment or have analyzed final wine after extraction has been completed. By examining the kinetics of extraction during a fermentation it would be possible to understand phenomena that vary in time, possibly due to factors such as ethanol concentration, temperature, or mixing.
Much of the knowledge gained thus far on phenolic extraction has assumed a homogeneous fermentation. However, it is clear that red wine fermentations are not spatially homogeneous throughout the bulk of the fermentation with the cap lifted by carbon dioxide evolution. Aside from direct heterogeneity caused by cap formation, Schmid, et al. 17 found that cap formation can also lead to a substantial gradient in temperature, an important factor in extraction and fermentation kinetics 8, 18, 19. This type of heterogeneity is also likely to lead to spatial heterogeneity in phenolic composition, though this has not been quantified to date. Gradients in phenolic concentration, if present, would be important to our understanding of phenolic extraction for multiple reasons. First, one possible scenario is that extraction of components reaches an equilibrium plateau or saturation point in the cap with no further extraction possible until a punch-down or pump-over effectively lowers the concentration in the cap thus allowing further extraction back to the same concentration. If this is the case, understanding the kinetics of this behavior will help determine efficient cap management techniques, and cap phenolic concentrations just prior to mixing may be an early indicator of levels in the final wine. Second, the timing of formation of the gradients throughout the fermentation would give an indication of which component’s solubility is most a function of temperature, ethanol concentration, and mixing, leading to important information that may govern press or other processing decisions leading to tighter control of final wine phenolic profiles.

Therefore, in order to characterize both temporal and spatial changes in phenolic concentration during red wine fermentation, Cabernet Sauvignon fermentations were performed during the 2011 and 2012 harvests at the 2,000 L scale using grapes sourced from the same vineyard. Fermentations in both years were sampled prior to pump-overs throughout the fermentation using a custom built
twelve-position sampling grid that formed a vertical cross-section in the fermentor. Phenolic content in the samples was determined using reverse-phase HPLC and UV-VIS spectroscopy. Extensive sampling between pump-overs also allowed investigation of the presence and kinetics of saturation in the cap for several phenolics.

Materials and methods

Reagents

Acetonitrile, acetone, methanol, trifluoroacetic acid, hydrochloric acid (37%), phloroglucinol, ascorbic acid, gallic acid monohydrate, (+)-catechin, (-)-epicatechin, caffeic acid, quercetin, and Toyopearl HW-40F size exclusion media were purchased from Sigma Aldrich (St. Louis, MO). Phosphoric acid and sodium acetate trihydrate were purchased from Fisher Scientific (Pittsburgh, PA). Malvidin-3-O-glucoside chloride was purchased from Indofine Chemical Company, Inc. (Hillsborough, NJ). Acetonitrile, methanol, acetone, trifluoroacetic acid, phosphoric acid, and sodium acetate trihydrate were all of HPLC grade. Phloroglucinol, ascorbic acid, and hydrochloric acid were all of the highest purity available. Optec brand ethanol (95%) was purchased from Decon Laboratories, Incorporated. (King of Prussia, PA).

Winemaking

All grape processing and winemaking occurred at the University of California, Davis Teaching and Research Winery (Davis, CA), with winemaking practices being identical in the 2011 and 2012 harvest except for the period of extended maceration and altered pump-over schedule in 2012 as described below. The sampling array used in this study (described below) allowed sampling at four levels in the tank (two in the cap and two in liquid) with three replicate sampling points at each level. Seasonal
replicates were performed in consecutive harvests to assess reproducibility of the phenomena using grapes at the same phenological ripeness from the same vineyard. Approximately two tons of Cabernet Sauvignon fruit were sourced from Lodi, California during the 2011 and 2012 harvests and trucked to the winery, where initial composition was determined (Table 1). Grapes were destemmed and crushed into a 2,000 L stainless steel, water-jacketed, cylindrical, closed-top fermentation vessel equipped with a custom sampling array (Figure 1a and 1b). The must was inoculated with hydrated *Saccharomyces cerevisiae* strain D254 (Lallemand Lalvin, Petaluma, CA) at a concentration of 180 mg/L. Musts were initially heated to 25 °C prior to inoculation and maintained at 25 ± 0.5 °C using the tank jacket with chilled water during the duration of the fermentation. Pump-overs were performed twice daily using a rotating, stainless steel irrigator with one tank volume being pumped over each time. Fermentations were sampled twice daily prior to pump-over. On days two and three of the 2012 fermentation, pump-overs were only performed in the morning and samples taken on a regular basis for 24 h to assess longer-term extraction behavior and potential saturation of specific phenolics. Fermentation progress was checked in both seasons at each pump-over using an Anton Paar (Ashland, VA) DMA 35 portable density meter. For the 2011 fermentation, sampling was stopped and the tank was pressed once the fermentation was dry. In 2012 sampling continued for a total of fourteen days, at the end of which the tank was pressed. During this period of extended maceration the tank temperature was maintained at 25 °C and pump-over events were continued twice per day.
Table 1. Initial juice panel analysis.

<table>
<thead>
<tr>
<th>Harvest</th>
<th>pH</th>
<th>°Brix</th>
<th>Total acidity (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>3.41</td>
<td>23.3</td>
<td>4.7</td>
</tr>
<tr>
<td>2012</td>
<td>3.85</td>
<td>24.3</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Figure 1. (a) Diagram showing the approximate locations of the twelve sampling ports. Each layer of sampling ports (1-4) is labeled. The upper layer of sampling ports (those lying above layer 1) was not used in either the 2011 or 2012 experiments. (b) Photo of a single sampling port showing the stainless steel sieve. (c) Photo of the sampling array strung in the 2000 L fermentor.

**Sampling port array**

Fifteen stainless steel sampling point sieves (Figure 1b) were fabricated at the University of California, Davis Engineering machine shop (Davis, CA). Sieves were manufactured from perforated 304 stainless steel mesh and measured 6.35 cm in
length and 1.59 cm in diameter. Sieves were connected to a length of L/S 16 platinum-cured silicone tubing with an internal diameter of 3.05 mm (Masterflex, Vernon Hills, IL). Sampling units were secured to a rigid framework to ensure the units did not move during fermentation. Samples were collected with a Masterflex (Vernon Hills, IL) L/S Digital Standard Drive peristaltic pump equipped with a four-channel pump head. Flow was reversed after sampling for just long enough to assure that sample tubes were emptied between sampling events.

**Reversed-Phase HPLC**

Reversed-phase HPLC was performed using a high-throughput method to generate phenolic extraction profiles for gallic acid, (+)-catechin, (-)-epicatechin, condensed tannins, caftaric acid, quercetin-3-O-glycosides (summed concentrations of quercetin-glucoside, quercetin-galactoside, and quercetin-glucuronide), and malvidin-3-O-glucoside. Chromatographic analyses were performed on an Agilent (Santa Clara, CA) 1260 Infinity HPLC equipped with a binary pump, column compartment, and diode array detector. Separations were performed on an Agilent (Santa Clara, CA) Zorbax Eclipse XDB-C18 column (4.6 x 50 mm, 1.8 µm particle) protected by an Agilent (Santa Clara, CA) Zorbax Eclipse XDB-C18 guard column (4.6 x 12.5 mm, 5 µm). A binary gradient of water with 0.3% phosphoric acid (mobile phase A) and acetonitrile with 0.2% phosphoric acid (mobile phase B) was used at a flow-rate of 1.0 mL/min. with the following time program: 0-10 min., 5-19% B; 10.25-12.5 min., 33% B; 12.5-13.5 min., 33-95% B; and 13.5-14.5 min., 95-5% B. Eluting peaks were monitored at 280 nm (gallic acid, (+)-catechin, (-)-epicatechin, and condensed tannins), 320 nm (caftaric acid), 360 nm (quercetin-glycosides), and 520 nm (malvidin-3-O-glucoside).
Extraction and characterization of grape tannins

Grape clusters were randomly chosen prior to crushing and stored at -20 °C until analysis. Three lots of twenty grapes were randomly chosen from these grapes and the skin and seeds were separated from the pulp using a scalpel, rinsed, dried, and weighed. A volume of ethanol:water:hydrochloric acid (55:45:0.1) was added to the skins and seeds at a ratio of 1 mL of solvent for every 0.1 mg of tissue. Skins and seeds were homogenized using an IKA (Wilmington, NC) T10 Basic Ultra-Turrax disperser and allowed to extract overnight at 4 °C. Supernatants were decanted and stored at -20 °C. A volume of acetone:water (70:30) solution equal in volume to the ethanol solution was added to the pellet and allowed to extract overnight at 4 °C, followed by centrifugation and decanting of the supernatant. Ethanol and acetone fractions were combined, reduced under pressure at 35 °C, reconstituted in 10 mL of deionized water, and lyophilized. Solutions of the lyophilized skin and seed samples were prepared using 15% methanol (aqueous) at concentrations of 10 mg/L for skin extracts and 5 mg/L for seed extracts. Tannins were isolated from skin and seed extracts as well as selected fermentation samples in triplicate by solid phase extraction (SPE) based on a previously described method. Briefly, SPE columns were prepared containing 10 mL (column volume) of Toyopearl HW-40F size exclusion media (Tosoh Biosciences, King of Prussia, PA) which was equilibrated with 20 mL of ethanol:water:trifluoroacetic acid (55:45:0.05 by volume) prior to the application of 2 mL of skin extract or fermentation samples and 1 mL of seed extract. Sugars and monomeric phenols were eluted from the column using 40 mL of ethanol:water:trifluoroacetic acid (55:45:0.05 by volume) while the retained tannins were eluted using 30 mL of acetone: water: trifluoroacetic acid (60:40:0.05 by
Tannin fractions were dried under reduced pressure at 35 °C, reconstituted in 0.5 mL of methanol, and stored at 20 °C until characterization.

Isolated tannins were characterized in duplicate by phloroglucinolysis using a method adapted from Kennedy and Jones. Equal volume aliquots of tannin isolate and phloroglucinol reagent solution (100 g/L phloroglucinol in methanol, 0.2 g ascorbic acid, 0.2 N hydrochloric acid) were incubated at 50°C for twenty minutes, after which the reaction was quenched by the addition of five reaction volumes of 40 mM sodium acetate. All samples were centrifuged at 13,500 rpm for five minutes and supernatants were then transferred to HPLC vials. Phloroglucinolysis cleavage products were analyzed by RP-HPLC. Chromatographic separations were performed on an Agilent (Santa Clara, CA) 1260 Infinity HPLC equipped with a temperature controlled autosampler, column compartment, and diode array detector. An Agilent (Santa Clara, CA) Poroshell 120 SB-C18 (4.6 x 150 mm, 2.7 µm particle) column at a temperature of 35°C was used for all analyses. A gradient separation was performed using water (mobile phase A) and acetonitrile (mobile phase B), both acidified with 0.1% formic acid at a flow-rate of 2.0 mL/min. The time program for the gradient is as follows: 0 min., 3% B; 2.96 min., 3% B; 10.3 min., 16% B; 10.4 min., 20% B; 12.1 min., 20% B; 13 min., 80% B; 14.34 min., 80% B; and 15.34 min., 3% B. Eluting peaks were monitored at 280 nm. Reaction products were quantitated by external calibration using a (+)-catechin calibration curve with concentrations adjusted by multipliers as given in Kennedy and Jones.

**Determination of total anthocyanins**

Total anthocyanins were determined for all samples taken during the 2011 and 2012 experiments by UV-VIS spectroscopy using an Agilent (Santa Clara, CA) 8453 spectrophotometer equipped with a 100 µm path-length quartz flow cell (Starna Cells...
Inc., Atascadero, CA). All samples were thawed at room temperature, centrifuged, and the absorption spectrum from 200-900 nm was collected. All collected spectra were exported to Microsoft Excel (Redmond, WA) where the total anthocyanin content was determined using a partial least squares regression model.

Data analysis

All data were analyzed in Microsoft Excel 2011 (Redmond, WA). Contour plots showing spatial distribution of phenolics in the fermentor were created in SigmaPlot 10 (Systat Software Inc., San Jose, CA).

Results

Formation of phenolic gradients during fermentation

The formation of chemical gradients in phenolics during fermentation was confirmed in pilot scale (2000 L) Cabernet Sauvignon fermentations during the 2011 and 2012 harvests, with seasonal replication performed using grapes with similar phenological ripeness that were sourced from the same vineyard. All trends in extraction that were observed in the 2011 fermentation were supported by similar trends in the 2012 fermentation. The spatial distribution of total anthocyanins prior to a pump-over event at various points during the fermentation (determined by correlation methods using UV-Vis spectroscopy) is shown in Figure 2a for the 2011 fermentation. As expected anthocyanin extraction occurred in the cap with distribution throughout the fermentor occurring during pump-over, which resulted in an increase in anthocyanin concentration in the liquid portion. The extraction profile for total anthocyanins as the average of each horizontal sampling layer is shown in Figure 2b. During the first 24 h of fermentation the anthocyanin concentration throughout the fermentor was relatively homogenous, being 160 ± 13 mg/L.
(malvidin-3-0-glucoside equivalents). Anthocyanin extraction began early in the fermentation, reaching a maximum in the cap (sampling layers one and two) of 631 ±2 mg/L at approximately 64 h, after which the concentration decreased as extraction slowed. During this period the liquid portion (layers three and four) increased in anthocyanin concentration in a relatively linear fashion, reaching a maximum of 429 ± 4 mg/L at 96 h, after which the concentration in the liquid became constant and net extraction stopped. Gradients in anthocyanin concentration developed during the course of fermentation, with the largest gradients occurring between 24 and 72 h of fermentation, with the magnitude of the concentration gradient between layers 1 and layer 4 being fairly constant at 281 ± 11 mg/L. The trends in extraction as measured by the UV-Vis correlation method was confirmed by measurement of malvidin-3-O-glucoside by RP-HPLC, as discussed below.

Figure 2. Spatial distribution of total anthocyanins during the 2011 Cabernet Sauvignon fermentation as measured by UV-VIS spectrophotometry. (a) Contour plots showing anthocyanin concentrations in mg/L malvidin-3-O-glucoside equivalents throughout the fermentor just prior to a pump-over. Fermentation progress
is shown in hours post-inoculation for each contour plot. (b) Anthocyanin concentrations averaged by sampling layers. Layers are color coded to the diagram in Figure 1a.

Concentration gradients also developed for individual monomeric phenols during the course of fermentation, including gallic acid, (+)-catechin, (-)-epicatechin, caftaric acid, and malvidin-3-O-glucoside, with representative layer averaged extraction profiles for the 2011 fermentation shown in Figure 3. The irregular pattern in extraction reflects whether the sample was collected during the morning or evening. Samples collected during the morning had 16 h to extract before a pump-over event while those collected during the evening had only eight hours. Gradients in the concentration of gallic acid (Figure 3a) existed between the cap and liquid portions of the fermentor from the start of the fermentation. During the fermentation the concentration of gallic acid in the cap and the liquid both increased at a similar rate, resulting in a nearly constant gradient between the cap and liquid at the sample points during the fermentation. A small gradient existed for caftaric acid (Figure 3b) at the start of the fermentation, with the cap having a slightly greater concentration than the liquid. Caftaric acid extracted early in the fermentation, with the concentration in the cap rapidly increasing and reaching a maximum around 36 h. After this point the concentration in the cap began to decrease. Liquid levels of caftaric acid increased steadily until 72 h where they became constant. The fermentation gradient in caftaric acid was lost at 96 h. Concentration gradients for (+)-catechin (Figure 3c) developed early in the fermentation (within the first 40 h) and increased throughout the fermentation. The outlier sampling point in the cap (occurring around 68 h) is believed to be due to a non-representative sampling. Malvidin-3-O-glucoside (Figure 3d) behaved in a manner similar to that seen for the
total anthocyanins, with early extraction in the cap reaching a maximum at 64 h and a slower increase in the concentration in the liquid portion.

**Figure 3.** Extraction profiles of representative phenolics averaged by sampling layer for the 2011 fermentation as measured by RP-HPLC. Extraction profiles for (a) gallic acid, (b) caftaric acid, (c) (+)-catechin, and (d) malvidin-3-O-glucoside are shown. Extraction profiles are color coded to the diagram in Figure 1a.

The spatial distribution of tannins during the 2011 experiment is shown in Figure 4, with contour plots showing tannin concentration throughout the fermentor prior to pump-over (Figure 4a). Chemical gradients developed at 40 h as tannin extraction began in the cap. The distribution and concentration of extracted tannins became more homogenous with successive pump-overs as the tannins extracted in the cap were distributed throughout the fermentor, resulting in a decrease in the magnitude of the chemical gradient (Figure 4b). Unlike the anthocyanins, tannin extraction continued throughout fermentation. Tannin analysis by phloroglucinolysis at multiple time points (40, 64, 88, 112, and 136 h) during fermentation demonstrates a spatial variability in tannin size as well as changes in skin and seed tannin
contributions, shown in Figure 5. The mean degree of polymerization (mDP) of the tannins in the cap (layers 1 and 2) decreased during the fermentation from an average of 13.2 ± 0.1 (40 h) to 9.5 ± 0.2 (136 h). The mDP of the tannins in the cap was relatively constant for the first 88 h of the fermentation, after which the mDP began to decrease. The mDP of the tannins in the liquid portion of the fermentor (layers 3 and 4) increased from 10.2 ± 0.5 (40 h) to 13.3 ± 0.3 (64 h) followed by a decrease to 10.6 ± 0.2 (136 h). The percent galloylation (Figure 5b) increased throughout the fermentor during the fermentation with a greater rate of increase occurring in the cap (layers 1 and 2). At the first time point for tannin analysis (40 h) the upper three sampling layers had an average percent galloylation of 2.4 ± 0.1% and the lowest sampling layer had a percent galloylation of 1.7 ± 0.05%. At the last time point (136 h) the cap (layers 1 and 2) had an average percent galloylation of 5.1 ± 0.02% while the liquid (layers 3 and 4) had an average of 3.4 ± 0.2%. Analysis of the seed tannin contributions at these time points (Figure 5c) showed that during the first 64 h of fermentation the extraction of seed tannin was relatively constant, with more seed tannin present in the liquid portion (47%) than in the cap (43%). Seed tannin contribution to total tannins in the cap continued to increase from 64 to 144 h, reaching a contribution of about 75% in the cap. Contribution of seed tannin in the liquid portion increased more slowly from 112 h, reaching 60% of the total tannin concentration at 144 h.
Figure 4. Spatial distribution of tannins during the 2011 Cabernet Sauvignon fermentation as measured by RP-HPLC. (a) Contour plots of tannin concentration in mg/L (+)-catechin equivalents at various points in the fermentation just prior to pump-over. Fermentation progress is given as hours post-inoculation. (b) Tannin concentration averaged by level and color-coded to match the schematic in Figure 1a.
Figure 5. Phloroglucinolysis results from selected time points during the 2011 Cabernet Sauvignon fermentation showing gradients in (a) the mean degree of polymerization, (b) the percent galloylation, and (c) the percent seed tannin in the fermenting wine. Layers are color coded according to the diagram in Figure 1a.

The appearance of saturation concentrations between pump-overs and their effect on phenolic extraction

To determine when saturation levels occur and how these effect phenolic extraction the evening pump-overs were not performed on days two and three in 2012, giving 21.2 h of uninterrupted extraction on day two (hereafter referred to as period 1) and 19.8 h on day three (period 2). During these times the fermentation was
regularly sampled for the first 14 hours during period 1 and the first ten hours during period 2. Pump-overs were performed immediately after sampling at the end of both periods. The extraction profiles for malvidin-3-O-glucoside during periods 1 and 2 are shown in Figures 6a and 6b (periods 1 and 2 respectively). Following pump-over at the start of period 1 (Figure 6a, marked by dashed vertical lines) the concentration of malvidin-3-O-glucoside was fairly homogenous throughout the fermentor. Extraction is evident in the cap (layers 1 and 2) and in the liquid portion closest to the cap (layer 3) within 80 min of the pump-over. Extraction in the cap continued during the remainder of the sampling, increasing from 56 ± 3 mg/L following pump-over to 101 ± 1 mg/L. The liquid portion closest to the cap (layer 3) extracted quickly following pump-over increasing from 51 mg/L to 83 mg/L. The remainder of the bulk liquid showed an increase in concentration following pump-over and then remained relatively constant during the remainder of the period. All four sampling layers showed continued extraction during the remainder of period 1, indicating that saturation did not occur within the fourteen-hour sampling window but was most probably reached prior to the last sampling at 61.6 h. The same general trends were observed during period 2 (Figure 6b), with the exception of all four layers appearing to reach a saturation level at seventy hours of fermentation, when little extraction appeared to occur. In the cap the apparent saturation levels are 150 ± 4 mg/L for layer 1 and 143 ± 8 mg/L for layer 2. A greater spread of concentrations existed in the liquid portion, with layer 3 reaching 112 ± 17 mg/L and layer 4 reaching 93 ± 2 mg/L. Trends similar to those seen for malvidin-3-O-glucoside were observed for the other monitored phenolics located within the grape skin (quercetin-glycosides) and pulp (caftaric acid).
Figure 6. Extraction profiles for malvidin-3-O-glucoside during the 2012 Cabernet Sauvignon fermentation, showing the development of concentration gradients over a twenty-hour period during (a) period 1 and (b) period 2 as measured by RP-HPLC. Pump-overs are marked with a dashed vertical line. Coloring corresponds to the diagram shown in Figure 1a.

Gradient development during extended maceration

The spatial heterogeneity of phenolic concentration during extended maceration (hours 96 - 324 h, 9.5 days) was investigated during the 2012 fermentation, with representative concentration profiles for this period shown in Figure 7. The general trend observed during extended maceration was the loss of concentration gradients as phenolic extraction either slowed or ceased between pump-over events that homogenized the wine. This is clearly visible in the concentration plots for caftaric acid (Figure 7a) and malvidin-3-O-glucoside (Figure 7b). Concentration gradients persisted much longer for (+)-catechin (Figure 7c) and the condensed tannins (Figure 7d), which are representative for late extracting phenolics. The extraction profiles for (+)-catechin and the condensed tannins show concentration gradients for the majority of extended maceration, becoming homogenous at 297 h (12.3 days of total contact). Extraction continued through most of extended maceration with increases in the liquid portion of the fermentor (sampling layers 3
and 4) while the concentration in the cap (layers 1 and 2) remained relatively constant until 204 h, when the concentration at the level of sampling layer 1 decreased below the concentration of layer 2. The contribution of seed tannins to the overall tannin content became more prominent during extended maceration, as the percentage of seed tannins increased from 44 ± 1% to 66 ± 2%, with the distribution of seed tannins throughout the fermentor being fairly homogenous. This is supported by the decrease in mDP values from 14.3 ± 0.2 at the end of active fermentation to 8.5 ± 0.3 at the end of extended maceration, as well as the changes in percent galloylation, which increased from 3.1 ± 0.3% to 4.4 ± 0.3% during extended maceration.

**Figure 7.** Extraction profiles of selected phenolics during the period of extended maceration (105-321 hours) for the 2012 experiment as measured by RP-HPLC. The phenolics shown are (a) caftaric acid, (b) malvidin-3-O-glucoside, (c) (+)-catechin, and (d) tannins. Each averaged sampling layer is color coded to the diagram shown in Figure 1a.
Discussion

Phenolic extraction and spatial heterogeneity of phenolic concentrations were monitored during pilot scale red wine fermentations performed in 2011 and 2012. Extraction trends observed in the 2011 fermentation were validated in the 2012 fermentation. Extraction occurred primarily in the cap for the monitored phenolics (gallic acid, (+)-catechin, (-)-epicatechin, caftaric acid, quercetin-glycosides, malvidin-3-0-glucoside, and condensed tannins), with the concentration in the liquid portion increasing during pump-over as the extracted phenolics were washed from the cap into the bulk liquid. Samples taken prior to and following pump-over events also showed the effectiveness of pumping-over at homogenizing the phenolic content of the fermentor. All extracted phenolics monitored developed concentration gradients during the fermentation (Figure 2 and 3). The kinetics of gradient development were demonstrated between pump-overs on days two and three of the 2012 fermentation. While the extraction data shown in Figure 6 shows that pump-over is the primary mode of homogenizing phenolic content throughout the fermentor, the slow increase in phenolics in the liquid portion of the fermentor indicates that some other form of minimal mixing is also occurring, most likely due to thermal currents arising from temperature gradients and carbon dioxide evolution from yeast growth. As anthocyanins and tannins have been shown to adsorb to yeast hulls as well as grape cell wall material, knowledge of the development of gradients and saturation points would aid winemakers in timing pump-over events, potentially minimizing loss of phenolics to adsorption of solids in the cap.

The monitored skin-derived phenolics (quercetin-glycosides and malvidin-3-O-glucoside) extracted early during fermentation, generally reaching a maximum concentration within the first 72 h for both the 2011 and 2012 fermentations, a trend
that has been shown previously and has been explained by their location within the skin cells as well as their hydrophilic character \(^9, 24\). Following maximum extraction, the concentration of skin phenolics in the cap gradually decreased as these molecules were redistributed throughout the fermentation during pump-overs and secondary processes (such as adsorption to solids, reaction with other compounds, and degradation) began to occur at a higher rate than further extraction, a trend observed in the extended maceration of Merlot wines \(^{26}\). As extraction either slowed or ceased, the magnitude of the concentration gradients became less with successive pump-overs until the fermentor was homogenous, which occurred around 96 h of fermentation (for caftaric acid, quercetin-glycosides, and malvidin-3-O-glucoside). The extraction of \((+)-\)catechin and the condensed tannins continued for a greater time, only ceasing after approximately 250 h (10.5 days) during the 2012 fermentation (Figure 7). The continued extraction of these phenolics during extended maceration is most probably due to increased seed extraction resulting from the breaking down of the lipophilic seed coat during alcoholic fermentation, followed by increased permeability of the seed cells due to hydration \(^{28}\). The continual increase in \((+)-\)catechin and condensed tannin concentration in the liquid portion may be explained by increased extraction from seeds in the bottom of the fermentor as well as in the cap with mixing during pump-over events reducing the concentration gradients and shifting the concentrations towards each other. This latter explanation is supported by a series of fermentations performed in our lab in which the percentage of seeds located in the cap of 24 Cabernet Sauvignon fermentations was estimated to be 93\%. An unexpected observation of these fermentations was that the tannin concentration in the final wine was similar to cap concentrations during the third day of fermentation (81-88 h) for both the 2011 and 2012 fermentations. During the 2011 fermentation,
concentration of tannin in the cap was $217 \pm 5$ mg/L ((+)-catechin equivalents) at 88 h and $209 \pm 14$ mg/L at 136 h, when the tank was pressed. For the 2012 fermentation the tannin concentration was approximately 287 mg/L at 81.4 h and about 265 mg/L at 129.4 hours, when the fermentation was dry. While not conclusive these observations hint at the potential for early extraction markers, which would allow winemakers to better adjust fermentation conditions for final desired phenolic content. The complete mechanism for the extraction of phenolics during fermentation is not fully understood. It can be hypothesized that three distinct processes are occurring: the release of phenolics from cellular structures, adsorption of the released phenolics to solids, and chemical reactions of extracted phenolics with other components of the must. For the phenolics located in the grape skin the process of phenolic release into the fermenting wine is believed to occur by passive diffusion as the cellular structures containing the phenolics break down. As the concentration of phenolics in the cellular structures and the fermenting wine become equal, phenolic extraction will slow, appearing to cease, and a saturation will be observed. To evaluate whether this saturation behavior occurs between pumpovers, the 2012 fermentation was allowed to extract without mixing for periods of roughly 24 hours on day two of fermentation and approximately 20 hours on day three of fermentation (39-81.4 hours of fermentation time). The extraction of phenolics and development of concentration gradients during this time period is shown in Figure 6 for malvidin-3-O-glucoside (similar trends were observed for the other monitored phenolics). No apparent saturation event was reached with the sampling scheme employed during the first sampling period (39-61.6 h). Saturation was observed during the second period (61.6-81.4 h), occurring at 70 h and persisted until tank homogenization by pump-over (an interval of approximately twelve hours). The data collected during period 2 clearly
shows that saturation was reached within seven hours of pump-over and further extraction did not occur until after the subsequent pump-over. However the maximum concentration reached at the end of period one was similar to the maximum concentration reached in the second period (61.6-81.4 h), implying a saturation point may have been reached but not within the time-span that was sampled. The shorter time period leading to saturation during period two may be due to increases in ethanol production, which would increase the solubility of malvidin-3-O-glucoside in the must. An additional implication is that the time needed to reach saturation following further pump-overs will most likely decrease as the overall phenolic content of the fermentation increases. However, as heat and ethanol production both increase the solubility of phenolics further studies into saturation effects during the later stages of fermentation are needed to create a more complete model of saturation kinetics and extraction. Studies during the later stages of extraction may also provide more detail on the saturation kinetics of the later extracting phenolics, such as gallic acid, (+)-catechin, (-)-epicatechin, and condensed tannins.

Conclusions

Evaluation of the spatial heterogeneity of pilot-scale Cabernet Sauvignon fermentations demonstrated the existence of concentration gradients for several phenolics that are important to red wine quality. The trends in extraction and development of chemical gradients of phenolics were similar between the two seasonal replicates performed. The magnitude and appearance of these gradients seems to be dependent on the specific phenolic (or class of phenolics) and the origin within the grape berry. Extraction of compounds located in the skin of the grape, such as the anthocyanins, appears to occur early in fermentation whereas phenolics located
more predominantly in the seed, such as (+)-catechin, extract later. The extraction data clearly show the extraction of phenolics in the cap, with extracted phenolics being washed into the liquid portion of the fermentation during pump-over and demonstrates the importance of cap management in the distribution of extracted phenolics throughout the fermentation. The determination of saturation events in phenolic extraction during early fermentation is of potential importance to winemakers as it could aid in planning cap management events to maximize extraction, as well as in aiding the further development of fundamental mechanisms for phenolic extraction in large-scale fermentations. Furthermore, generated extraction profiles can aid development of studies to investigate the mechanisms of phenolic extraction in large-scale fermentations.

References


29. Bautista-Ortíz, A.; Martínez-Cutillas, A.; Ros-García, J.; López-Roca, J.; Gómez-Plaza, E., Improving colour extraction and stability in red wines: the use
CHAPTER 6

Summary and conclusion

Phenolic compounds are important to red wine and directly effect wine quality. Thus many winemaking techniques have been developed to enhance the extraction of these compounds during the fermentation process. This research study aims to understand how the selected winemaking processes including cold soak duration and pump-over volume/frequency affect the extraction of phenolics during Cabernet Sauvignon fermentation and the impact on the final bottled wine.

Application of CS technique with different CS durations on phenolic extraction was studied during cold soak period and fermentation in Cabernet Sauvignon wines. For 2013 harvest, the impact of increasing CS duration (0, 1, 4, 7, and 10 days at 10 °C) on the extraction of phenolic was investigated. In this investigation, significant differences were found in amounts of gallic acid, (+)-catechin, (-)-epicatechin, and total tannins extracted with increasing CS duration, whereas none were seen for hydroxycinamates and flavonols amounts. Although, anthocyanin extraction and color density increased with the longer periods of CS, it did not persist beyond CS period. Furthermore, 7 and 10 days of CS resulted in wines with higher seed tannin proportions and total tannin when compared to the non-CS wines at the end of fermentation and also persisted in the bottle aging, which could potentially enhanced the astringency in the wines. The experiment was repeated in 2014 with slight variations (added 2-day CS treatment) using fruit from a different source known to be more phenolic rich. For 2014 harvest, wines produced with four to ten days CS showed increased concentrations of gallic acid, (+)-catechin, and (-)-epicatechin than non-CS wines at the end of alcoholic fermentation. The wines made with ten days CS showed higher seed tannin contributions than other treatments
although this was only observed at the end of CS. Analysis of wines after three months of the bottle aging showed differences in concentration only for (-)-epicatechin. Similarly, sensory analysis on 2014 CS wines indicated very little difference among the wines made with different CS duration with only two attributes showing some significant impact. In addition, similar trends in phenolic extraction between research-scale and commercial-scale wines were observed for different CS durations.

In general, the results obtained from 2014 harvest were in agreement with those from 2013, although a significant different source of Cabernet Sauvignon grapes was used. Our research indicate that in the case of Cabernet Sauvignon and the CS and winemaking conditions used in this study, CS had very little impact on the final wines.

The effects of pump-over conditions on phenolic extraction in research-scale fermentations were evaluated. Changes in pump-over volume (two, one, and half volume twice a day) and frequency (one, two, four, and eight times per days) had no significant effect on the extraction of phenolics during Cabernet Sauvignon fermentation. While not significant, trends were observed that showed increased pump-over volumes and frequencies led to more extraction of both grape skin and seed derived phenolics.

As the pump-over volume and frequency showed no significant differences among treatments, a third set of experiments was performed in which low extremes of volume and frequency as well as no pump-overs was investigated. Trends of increased phenolic extraction showed more clearly with increasing pump-overs, although for the most part these trends were not significant during fermentation.
There were no significant differences in phenolic content among wine treatments after three months of bottle aging.

Evaluation of the spatial-heterogeneity of the pilot-scale fermentation demonstrated the existence of chemical gradients for several phenolics that are important to red wine quality. The magnitude and appearance of these gradients seems to be dependent on the class of phenolics and its location within the grape berry. Extraction of phenolics located in the skins appears to occur early in the fermentation while those located in the seeds occurs later. The extraction data generated before and after pumping-over visually reinforced the importance of cap management in the distribution of extracted phenolics throughout the fermentation.

These research studies have shown that neither CS duration or pump-over conditions (volume and frequency) have a major influence on the phenols composition of the final wine in the case of Cabernet Sauvignon. However, a minimal amount of pump-over is needed to ensure homogeneity of the extracted phenolics and thus the removal of gradients, which can result in saturation in the juice close to the cap and ultimately negatively affect phenolic extraction.