

Role of Soluble Cell Wall Carbohydrates in Sensory
Mouthfeel Attributes of Red and White Wines

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Statement of Authorship

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- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate to include the publication in the thesis; and
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Abstract

The overall objective of the work described in this thesis was to define the effects of soluble cell wall carbohydrates (SCWCs) on mouthfeel and other sensory characteristics in wines. The SCWCs and sensory properties were compared in wines made from the white and red grape varieties Chardonnay and Cabernet Sauvignon, which are produced under fundamentally different conditions. Cabernet Sauvignon wines are fermented with grape skins, whereas Chardonnay wines are made from pressed juice, without skins. In addition, SCWC compositions were monitored during ripening of the grapes.

Thus, wine chemical profiles, particularly SCWC compositions, were analyzed in parallel with the sensory evaluation of their effects on mouthfeel and taste attributes. Wines made at different ripening stages exhibited significantly different chemical and sensory profiles. Eight classes of grape-derived and two classes of yeast-derived SCWCs were identified by glycosyl linkage analysis. The SCWCs consisted mainly of oligomeric degradation products of cell wall polysaccharides from grape berries and from yeast. The overall results indicated that no major changes occurred in total grape-derived SCWC *content* during ripening of Chardonnay and Cabernet Sauvignon grape berries and wines, but the large changes in SCWC *composition* suggested possible structural modifications of specific polysaccharides during ripening and the winemaking process. Homogalacturonan (HG) and rhamnogalacturonan (RG I, RG II) were the most abundant parental polysaccharides found in both Chardonnay and Cabernet Sauvignon grapes and in wines. In this study, the amounts of yeast glucan and yeast mannan in the grapes and wine were generally lower than grape-derived SCWCs.

In wines, both phenolic compounds and SCWCs exhibited similar degrees of correlations with mouthfeel attributes, which suggested that these two classes of wine constituent might play comparable roles in affecting mouthfeel. However, the sensory data indicated that wine mouthfeel and taste could be discriminated slightly more through correlations with different types of SCWC. The neutral SCWCs collectively affect the overall sensation in the mouth, irrespective of wine type. In Cabernet Sauvignon wines, arabinan, arabinogalactan (AG I, AG II) and xyloglucan (XG) fragments

correlated strongly with astringency, chalkiness, coating, roughness, viscosity and burn mouthfeel, whereas in Chardonnay wines, AG I and XG were related to burn and viscosity mouthfeel. In contrast, HG correlated strongly with sour and bitter tastes in both types of wine. Yeast glucan and yeast mannan also contributed to the overall sensation of the wine in the mouth. They correlated with burn mouthfeel in Cabernet Sauvignon wines, but demonstrated minimal effect on mouthfeel in Chardonnay wines.

When these polysaccharide and oligosaccharide families that remained in wines were traced back to their origin, it was found that HG, RG I/II, arabinan, AG I/II and XG in the berries could be used as important wine mouthfeel quality markers. Thus, the data generated in this study will enable the development of rapid, predictive assays of specific mouthfeel characteristics in extracts of ripening grapes and early in the winemaking process. Such assays would be invaluable for maximizing desirable mouthfeel contributors and minimizing undesirable mouthfeel components, through the manipulation of viticultural and/or winemaking practices.

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Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Hui Hui Chong

Date

List of Abbreviations

Galactose	Gal
Glucose	Glc
Mannose	Man
Xylose	Xyl
Arabinose	Ara
Rhamnose	Rha
Fucose	Fuc
Glucuronic Acid	GlcA
Galacturonic Acid	GalA
Soluble Cell Wall Carbohydrates	SCWCs
Homogalacturonan	HG
Rhamnogalacturonan I	RG I
Rhamnogalacturonan II	RG II
Arabinogalactan type I	AG I
Arabinogalactan type II	AG II
Xyloglucan	XG
Heteroxylan	HX
Heteromannan	HM

Chapter 1

Introduction

1.1. Background

Grapevines represent one of the most important domesticated crops, with approximately 8 million hectares of globally cultivated area in 2014 (Food and Agriculture Organization of the United Nations, 2014). The increase in the commercial value of the grape is partly attributable to the growing interest in wine consumption. According to the Wine Institute, wine production in the United States and Australia increased substantially from 2011 to 2014 with 12.2% and 8.2% growth by volume, respectively (Wine Institute 2014). It has been reported that the growing number of new US millennial wine consumers, who are high frequency wine drinkers with unique wine style expectations, increases the demand for wine volume (Wine Market Council 2014).

Wine consumption is driven by consumer expectations of positive, impactful sensory attributes. The sensory aspects of wine, particularly mouthfeel, have become integral discriminating quality criteria for consumers (Gawel et al., 2000; Bruwer et al., 2011). Lawless and Heymann (1998) described mouthfeel as “sensations occurring in the oral cavity (such as coating or drying), as opposed to texture, in which the sensations are related to the food itself as in firmness and hardness.” According to Bernadette (2010), mouthfeel is a key attribute that drives the popularity of white wines. The influence of mouthfeel on consumer preferences has also been reported in soft drinks and beers (Langstaff and Lewis, 1993; Redondo et al., 2014). The overall aim of the project described in this thesis was to investigate the role of soluble constituents derived from cell wall polysaccharides as contributors to this important wine sensory characteristic.

Astringency is the most common wine mouthfeel sensory descriptor and is associated with descriptions conveying a wide range of sensations including drying, rough, harsh and chalky (Gawel et al., 2000). Tannins are believed to be the key compounds affecting astringency, and this relationship has been studied extensively over the past three decades (Casassa and Harbertson, 2014). According to Brossaud et al. (2001), wine astringency is mainly a tactile sensation due to the interaction of tannins with salivary glycoproteins, and the molecular size of tannins affect their relative

bitterness and astringency. Robichaud and Noble (1990) reported that gallic acids, in particular flavan-3-ols exhibiting molecular masses less than 500 Da, elicit astringent and bitter tastes in red wines.

However, the complex sensory experience and tannins cannot exclusively explain sensations such as 'body', 'smoothness' or 'fullness' experienced during wine consumption (Bruwer et al., 2011; Runnebaum et al., 2011). Gawel et al. (2000) described 20 different non-astringency related mouthfeel properties of red wines. Moreover, mouthfeel sensory attributes are qualitatively different depending on wine styles (Berger, 2014). The mouthfeel of white wines is often evaluated on the basis of being light-, medium-, or full-bodied (Runnebaum et al., 2011). These observations bring forth the importance of other naturally occurring compounds present in the wine that contribute to these sensations.

Wine soluble cell wall carbohydrates (SCWCs), which are derived from the cell walls of both grapes and yeast, are of great interest because of their organoleptic properties. The stability of cell wall polysaccharides and oligosaccharide products of their partial degradation in the wine matrix and the resistance of these complex cell wall carbohydrates to winemaking processes are two factors that determine the profile of SCWCs in wine (Vicens et al., 2009). Vicens et al. (2009) used the term soluble cell wall polysaccharides to describe wall-derived polymers in wine, but during the course of the current study it became apparent that the soluble fraction contained, in addition to polysaccharides, oligosaccharides of cell wall and yeast origin. Accordingly, the term soluble cell wall carbohydrates (SCWCs) is used throughout this thesis. The impact of SCWCs on wine mouthfeel, such as increasing the wine 'fullness' sensation and modifying wine 'mellowness', has been demonstrated, although individual, contributing carbohydrate components have not been identified (Vidal et al., 2004a; Vidal et al., 2004b). It has been reported that some characteristics of wine SCWCs are similar to those of commercial polysaccharides, such as pectin isolated from citrus, the hydrocolloid properties of which improve the mouthfeel and texture of processed foods and beverages (Holm et al., 2009; Gil et al., 2012). More detailed studies of SCWCs are necessary to reveal important insights into their

contribution to wine mouthfeel and thus to enable the development of differentiated products to meet consumer preferences.

Wine SCWCs comprise grape-derived pectic polysaccharides and hemicelluloses as well as yeast-derived neutral polysaccharides, which have also been shown to influence wine mouthfeel sensory characteristics (Belleville et al., 1993; Pellerin et al., 1996; Doco et al., 2003b; Vidal et al., 2003a; Apolinar-Valiente et al., 2013; Quijada-Morín et al., 2014). These polysaccharides can be classified as non-cellulosic wall polysaccharides and consist of complex and heterogeneous polymers. As noted above, the SCWC fraction described in this thesis may also include oligosaccharide degradation products of the original cell wall polysaccharides.

1.2. General Chemistry of Cell Wall Polysaccharides

1.2.1. Grape-derived Soluble Cell Wall Polysaccharides

The SCWCs that originate from grapes include oligomers and polymers of the common matrix phase cell wall polysaccharides, namely pectic polysaccharides, arabinan, arabinogalactans, xyloglucans, heteromannans and heteroxylans. The major structural features of these complex wall polysaccharides are described below and are summarized diagrammatically in Figure 1.1.

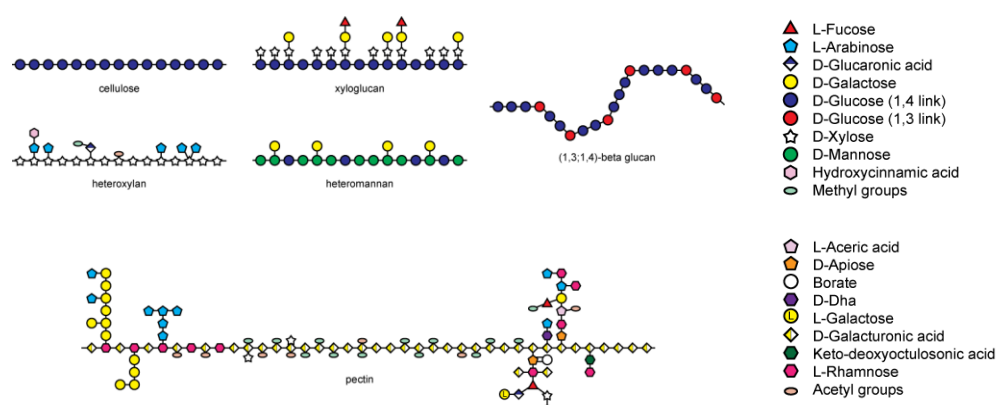


Figure 1.1. Structures of wall polysaccharides in plants. The backbone structures of these polysaccharides are based on (1,4)- β -linked monosaccharides in the case of cellulose, xyloglucan, (1,3;1,4)- β -glucan, heteroxylan and heteromannan, whereas the backbone of the galacturonans that are major constituents of pectic polysaccharides are based on (1,4)- α -linked chains of Gal residues. Extensive chain aggregation of the type found in cellulose is sterically hindered in the non-cellulosic wall polysaccharides mainly through the addition of short oligosaccharides, monosaccharides or acetyl groups. Note that the (1,3;1,4)- β -glucans are not found in grapevines or other eudicots. The structures are redrawn from Mohnen (2008), Burton et al. (2010) and Doblin et al. (2010).

Pectic polysaccharides are a group of polysaccharides containing GalA residues. This group of polysaccharides is the most abundant in wines. There are three structural classes of pectic polysaccharides, namely homogalacturonan (HG), rhamnogalacturonan I (RG I) and rhamnogalacturonan II (RG II).

Homogalacturonan (HG)

This is the major pectic polysaccharide with a linear backbone of (1,4)- α -linked GalA residues. HG is generally negatively charged and can form a stable gel with other pectic polysaccharides in the presence of cations. This phenomenon, which is often described as the “egg-box” model, accounts for approximately 70 % of the pectic gel in the cell walls of plants, where tight packing of HG occurs with ionically cross-linked Ca^{2+} -induced gelling. Nuclear magnetic resonance (NMR) spectroscopy revealed that the HG backbone has a twofold helical structure and, in some cases, threefold helical structures also occur (Caffall and Mohnen, 2009). Depending on the source, HG may be methyl-esterified at the C6 position or acetylated at the O2 position, or both.

Rhamnogalacturonan I (RG I)

RG I is the second most abundant pectic polysaccharide. It contains a backbone comprised of a repeating disaccharide of (- α -GalA-1,2- α -Rha-1,4-) and is branched at the C4 position of Rha residues by arabinan, galactan, and arabinogalactan Type I and Type II side chains (Figure 1.2) (Yapo, 2011). The presence of RG I in cell walls varies greatly. For example, suspension-cultured sycamore walls have approximately 7 % RG I, whereas potato tuber walls have approximately 36 % RG I (Caffall and Mohnen, 2009). RG I serves as the backbone to which other pectic polysaccharides, notably HG and RG II, are covalently linked, forming a macromolecular pectic polysaccharide matrix that contributes to wall strength, mechanical properties and intercellular adhesion (Cosgrove, 2005). RG I side chains are as follows:

- Arabinan is present with a backbone comprising (1,5)- α -linked Araf residues substituted with α -Araf residues at the C2 or C3 positions (Mohnen, 2008). There are implications that

arabinan is required to maintain cell wall flexibility and wall matrix porosity (Verhertbruggen et al., 2009).

- Arabinogalactan Type I (AG I) has a core backbone of (1,4)- α -linked Gal residues with occasional interspersing of (1,5)- α -linked Araf residues and substitutions at the C2 or C3 position with α -Arap residues.
- Arabinogalactan Type II (AG II) is characterized by a backbone of (1,3)- β -linked Gal residues with branch points of one, two, or three (1,6)- β -linked Gal residues. Some AG IIs are substituted with α -Araf residues (Voragen et al., 2009). Both AG I and AG II are considered highly flexible polymers that bind to the surface of cellulose, which helps maintain 'pores' in cell walls and consequently promotes wall flexibility (Cosgrove, 2005; Verhertbruggen et al., 2009).

Rhamnogalacturonan II (RG II)

RG II is a structurally complex pectic polysaccharide that was first identified in 1978 as a quantitatively minor (3 - 4 % of the primary cell wall) component of suspension-cultured sycamore cell walls (Darvill et al., 1978). RG II comprises at least 12 different glycosyl residues, which are linked together by more than 20 different glycosidic linkage types, and its structure is strongly conserved in higher plants, predominantly as a dimer through borate esters (O'Neill et al., 2004). The backbone of RG II has at least eight α -4-GalA residues and can be substituted with rare diagnostic glycosyl residues such as apiose, Kdo (3-deoxy-D-manno-2-octulosonic acid), Dha (3-deoxy-D-lyxo-2-heptulosaric acid), 2-O-methyl fucose, 2-O-methyl xylose, and aceric acid (3-C'-carboxy-5-deoxy-L-xylose) (Figure 1.3). Dimer formation of RG II through borate esters could facilitate the cross-linking of two HG polymer chains and could result in the formation of a three-dimensional pectic polysaccharide network. This network contributes to the mechanical properties of the cell walls and is essential for normal plant growth. It has been reported that structurally complex RG II, which accounts for 1 % - 4 % (w/w) of the pectic polysaccharides of dicotyledonous plants, is one of the major SCWCs in wines and occurs predominantly as the borate cross-linked dimer (O'Neill et al., 2004).

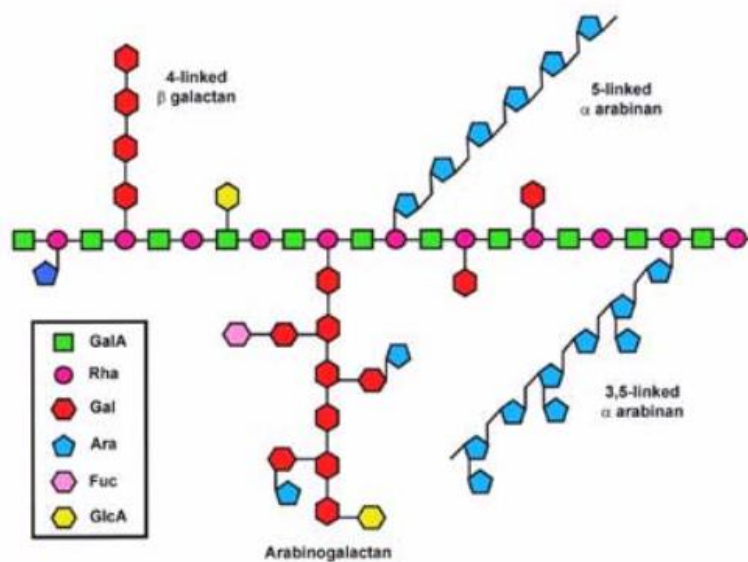


Figure 1.2. The structure of RG I with representative sidechains. This diagram is from <https://www.ccrc.uga.edu/~mao/rg1/rg1.htm>

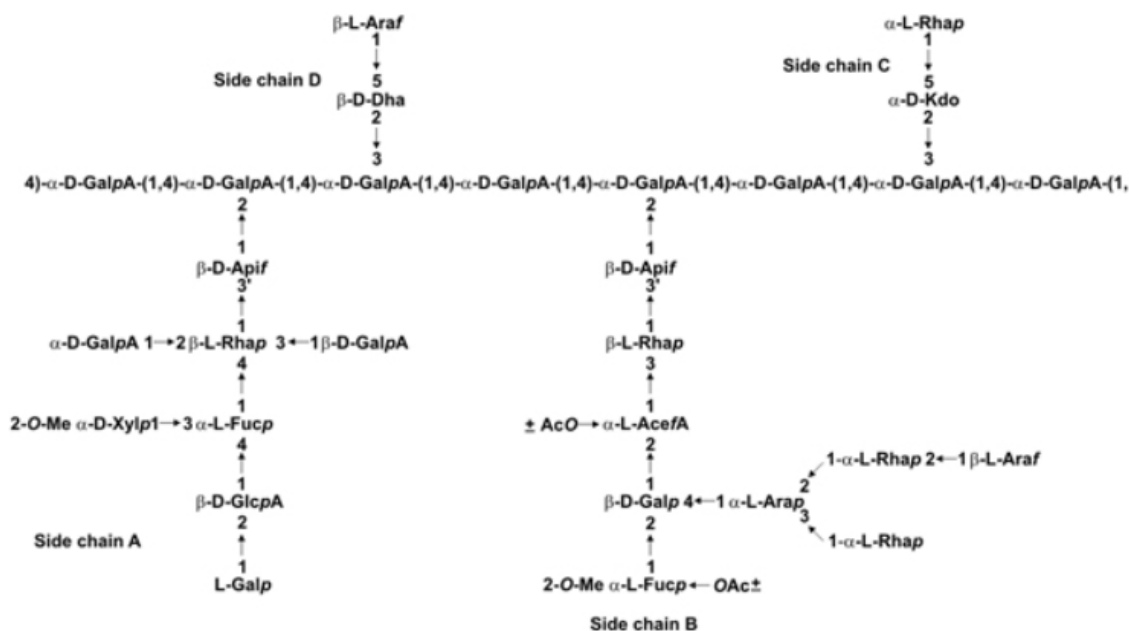


Figure 1.3. The structure of RG II with four well-defined sidechains. This diagram is from <https://www.ccrc.uga.edu/~mao/rg2/intro.htm>

Hemicelluloses include xyloglucans, heteroxylans and heteromannans, which share a common feature of having a (1,4)- β -linked glycosyl residue backbone with an equatorial configuration overall. Their abundance varies greatly between plant species. They are important components of the matrix phase of the wall between cellulose microfibrils and one important role of hemicelluloses is believed to be to strengthen cell walls via their interaction with cellulose (Scheller and Ulvskov, 2010).

Xyloglucan (XG)

XG is the most abundant hemicellulose in the primary cell walls of most dicotyledonous plants and has a core backbone structure of (1,4)- β -linked Glc residues that is heavily substituted at C6 with mono-, di- and triglycosyl side chains. The major components of the side chains are Xyl, Gal and Fuc residues. An important role of XG is believed to be to cross-link cellulose microfibrils, thereby strengthening the cell wall (Burton et al., 2010).

Heteromannan (HM)

HM is found in variable amounts across plant species and its backbone consists of either entirely (1,4)- β -linked Man residues, which can be un-substituted (mannans) or substituted with galactomannans. Alternatively, the main chain can consist of (1,4)- β -linked Man residues and (1,4)- β -linked Glc residues in a non-repeating pattern, which can also be un-substituted (glucomannan) or substituted with galactoglucomannan (Scheller and Ulvskov, 2010). It has been postulated that HM and XG have analogous functions (Schröder et al., 2009). The properties of HM in cell walls depend on its structure and substituted HM and shorter chain HM bind less strongly to the cellulose microfibril network (Whitney et al., 1998).

Heteroxylan (HX)

HX consists of a (1,4)- β -linked xylan core backbone that can be substituted with α -Araf, α -GlcA, or α -4-GlcAOMe, which are commonly linked at the C2 of the xylan backbone of dicotyledonous plants. However, the substituents can also be linked to C3 and/or to both C2 and C3 of the Xyl residues. Nonetheless, it is generally believed that HX and XG have analogous functions and that HX's

interaction with the cellulose microfibril network is weaker (Johnston et al., 2012). The HXs appear to be a major component of walls in monocots, while XGs predominate in eudicots.

1.2.2. Yeast-derived Soluble Cell Wall Polysaccharides

The walls of most yeast species, including the commonly used wine yeast species such as *Saccharomyces cerevisiae*, comprise 85 - 90 % polysaccharide and 10 - 15 % protein (Nguyen et al., 1998). The major polysaccharides of the yeast cell wall are shown diagrammatically in Figures 1.4 and it is important to acknowledge that some polysaccharides and oligosaccharides found in the SCWC fraction of wine will originate from yeast cell walls.

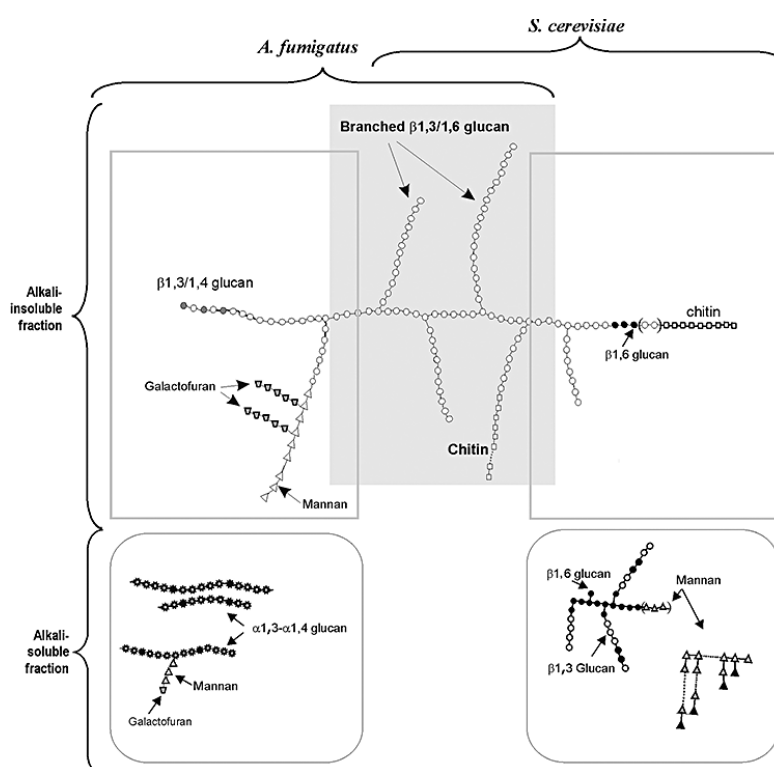


Figure 1.4. Polysaccharides of the fungal cell wall. The core polysaccharide structure that is found in the majority of fungi, including the mould *Aspergillus fumigatus* and the yeast *Saccharomyces cerevisiae*, is shown in grey. The diagram is from Latgé (2007).

Mannan is the main polysaccharide on the surface of yeasts and is involved in yeast cell aggregation. The yeast mannan backbone can consist of up to 150 units of (1,6)- α -linked Man residues with the irregular occurrence of side chains of (1,2;1,3)- α -linked oligo-mannans (Figure 1.5). Thus, the (1,2/1,3;1,6)- α -mannans of the yeast cell wall are structurally quite distinct from the (1,4)- β -

mannans of higher plants. Yeast cell walls also contain mannoproteins (Figure 1.6), some of which could potentially be found in wine.

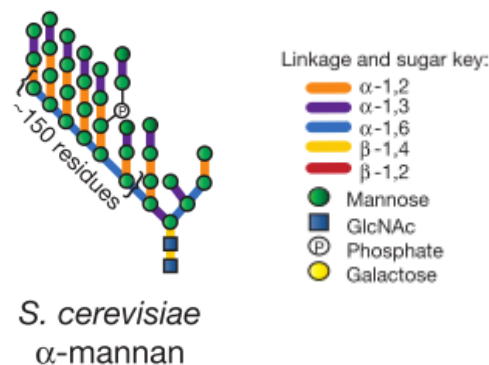


Figure 1.5. Structure of yeast (*Saccharomyces cerevisiae*) mannan. This diagram is from Cuskin et al. (2015).

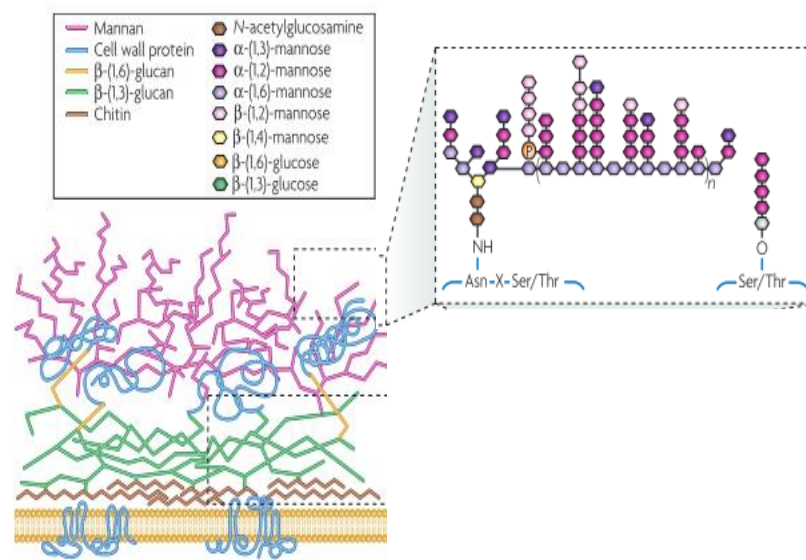


Figure 1.6. Structure of yeast (*Candida albicans*) mannoprotein. This diagram is from Netea et al. (2008).

Glucan plays an important role in the organization of the yeast cell wall and is the central molecule that links to other cell wall components including mannoprotein and chitin. It is well-documented that yeast glucan consists of a complex mixture of polysaccharides with a (1,6)- β -linked Glc backbone, which can be substituted with (1,3)- β -linked Glc residues and other substituents (Kollár et al., 1997). Kwiatkowski et al. (2009) reported that (1,4)- α -glucans covalently bound to (1,6)- β -

glucans are found in *Saccharomyces cerevisiae* cell walls. The α -glucan content varies, depending upon the nutritional status of the cells, the method of isolation and environmental conditions.

1.3. Changes in Grape Cell Wall Polysaccharides During Berry Ripening

As noted above, plant cell walls consist of a network of cellulose microfibrils that are embedded in a gel-like matrix of pectic polysaccharides and hemicelluloses. Hemicelluloses are predominantly represented by XG in eudicots, whereas the highly complex pectic polysaccharides are generally categorized as HG and RG I/II (Caffall and Mohnen, 2009). A typical cell wall of dicotyledonous plants consists of about 50 % cellulose/hemicelluloses, 30 % pectic polysaccharides and 20 % structural proteins (Carpita and Gibeaut, 1993). In accordance with previous research, Zabackis et al. (1995) reported that the cell wall polysaccharides of *Arabidopsis thaliana*, which is a model dicotyledonous plant, consist of 23 % HG, 11 % RG I, 8 % RG II, and 20 % XG, with substituents typical of other higher plants, as well as 4 % glucuronoarabinoxylan, 14 % cellulose and 14 % protein. Levels of HX in these walls were low. The polysaccharides of these cell walls have diverse essential functions in providing both mechanical support and flexibility for the cell, regulating the diffusion of low molecular organic materials, determining the overall shape of cells, modulating the rate of growth and intercellular adhesion, and initiating defense responses against pathogens (Burton et al., 2010). In grape berries, cell wall polysaccharides form a barrier to the diffusion of organoleptic compounds, such as tannins, that are important for wine quality (Downey et al., 2003).

Grape berries, which are non-climacteric fleshy fruits, exhibit a double sigmoidal growth curve with three characteristic developmental phases. The first phase is a period of rapid berry growth through cell division and cell enlargement in parallel with the accumulation of organic acids, tannins, hydroxycinnamates, and the synthesis of phenolic compound precursors (Coombe, 1976; Kuhn et al., 2014). The second is a lag phase with minimal or no berry growth, while rapid berry growth resumes in the third phase. At the beginning of the third phase, berry ripening coincides with berry softening, which is also termed “veraison”, and the growth is limited to cell enlargement (Coombe, 1976; Coombe

and McCarthy, 2000). Veraison includes a group of complex physiochemical and developmental changes that occur during the berry ripening process (Coombe, 1980; Coombe and McCarthy, 2000). After veraison, the berries begin to accumulate sugar, flavor, aroma compounds and anthocyanin in red grape varieties, while organic acids begin to decline (Dokoozian and Kliewer, 1996).

Berry softening is the most obvious biophysical characteristic of grape berry ripening and results from a series of biochemical events that cause changes in the structural properties of the cell walls (Yakushiji et al., 2001; Huang et al., 2005; Thomas et al., 2008). It is generally believed that softening is a consequence of the degradation of berry cell walls, during which the breakdown of wall components occurs continuously during the ripening period (Coombe, 1992). Fruit softening typically coincides with dissolution of the middle lamella, which results in a reduction in intercellular adhesion, together with the depolymerization of hemicelluloses and pectic polysaccharides (Saladié et al., 2007; Paniagua et al., 2014). In the mesocarp tissues of Kyoho grape berries, it was reported that depolymerization of pectic polysaccharides and XG and overall decreases in hemicelluloses and cellulose occurred during the early stages of softening in hard green berries nearing the veraison stage (Yakushiji et al., 2001). These modifications are finely regulated and are the result of highly coordinated actions of cell wall modifying enzymes, plant growth hormones like auxin and cell wall loosening proteins such as expansins (Cosgrove, 2016). Cell wall modifying enzymes play a critical role in the modification of cell wall composition (Barnavon et al., 2000; Huang et al., 2005). Aside from development-dependent physiological factors, the changes in cell walls can also be attributed to external factors such as the adaptative response to environmental stress and pathogen attack.

According to Nunan et al. (1998), cell wall polysaccharides isolated from the mesocarp of Muscat Gordo Blanco berries consist of 30 - 35 mol % of cellulose, approximately 10 mol % of hemicelluloses and 48 - 53 mol % of pectic polysaccharides, with varying proportions at different ripening stages. During development, the changes in cell wall composition of climacteric fleshy fruits such as papaya (*Carica papaya*) and tomato (*Lycopersicon esculentum*), from early ripening to over-ripening when senescence occurs, have been studied widely (Saladié et al., 2007; Shiga et al., 2009;

Karlova et al., 2014). However, little is known about the changes in the SCWCs of grape berries during the late ripening stages, when commercial harvest for winemaking commonly occurs. It has been reported that while cell wall polysaccharide content remained constant, modifications of specific wall polysaccharides such as a decrease in AG I from approximately 20 mol % to close to 4 mol %, occurred when ripening Muscat Gordo Blanc grape berries softened (Nunan et al., 1998). Similarly, Vicens et al. (2009) reported that the polysaccharide content of the exocarp of ripening Shiraz grape berries remained constant, but a decrease in the Gal residues of the side chains of pectic polysaccharides occurred. In avocado and tomato cell walls, it is common that pectic polysaccharides become more soluble during ripening and have lower molecular weights due to the removal of neutral side chains such as AGs (Huber and O'Donoghue, 1993).

Understanding the changes in the solubility of wall polysaccharides during grape berry ripening is critical to the present study because juice-soluble polysaccharides, which are resistant to fermentation conditions and soluble in the wine matrix, constitute the precursors of the wine complex carbohydrates that are potentially important for mouthfeel (Barnavon et al., 2000; O'Neill et al., 2004). Barnavon et al. (2000) reported that changes in grape cell wall composition during ripening could have considerable impact on the release of soluble polysaccharides during the processing of grape musts. This is in agreement with Ducasse et al. (2010a), who suggested that the maturity levels of grape berries could affect the release of polysaccharides during winemaking processes.

The solubilization of pectic polysaccharides is known to be involved in fruit softening (Paniagua et al., 2014). Pectic polysaccharides undergo chemical modification and their content can vary as a result of berry expansion (Yakushiji et al., 2001). According to Silacci and Morrison (1990), water-soluble pectin content increased in Cabernet Sauvignon grape berries during ripening. Similarly, in Muscat Gordo Blanco grape berries, Nunan et al. (1998) reported an increase of water-soluble polysaccharides during ripening, primarily attributed to the increased solubility of galacturonan. In Syrah grape berries exocarp, the degree of methyl-esterification of pectic polysaccharides decreased during ripening from 52 % to 38 % (Vicens et al., 2009). Vidal et al. (2001) reported that the pulp tissue

of Grenache Blanc berries contained twofold more buffer-soluble pectic polysaccharides and arabinogalactan-protein than the skin tissue. Accordingly, Vicens et al. (2009) also reported that the content of water-soluble polysaccharides increased more than twofold during the ripening period of Shiraz berries.

In conclusion, cell walls are never static structures. The synthesis, modification and degradation of cell wall polysaccharides are complex biochemical events that occur during grape berry ripening and a change in the turnover rate of a particular component will affect the overall wall composition. Therefore, it is critical to understand the changes in SCWCs during grape berry ripening to optimize grape harvesting decisions and select for desirable grape SCWC mouthfeel precursors. The presence of these naturally occurring mouthfeel precursors in abundance is highly valuable, especially when potential exogenous sources of mouthfeel ingredients are either lacking or not desired for certain wine styles such as “unoaked” or “unwooded” white wines.

1.4. The Chemistry of Wines

A central objective of the present study was to investigate the role of SCWCs in the mouthfeel attributes of wine. However, wine consists of a number of other components that could potentially contribute to mouthfeel and, as a result, current information on the composition of wines is presented below.

1.4.1. The General Composition of Wines

The universal classes of compounds found in dry table wines were previously determined and are listed in Table 1.1. In each of these categories, many individual compounds are often present, but not all of them play an important role in wine quality. Therefore, it is useful to tabulate in detail known components that play an important role in defining wine style and sensory attributes. Apart from the polysaccharides in wine, which will originate both from berry and yeast cell walls, other components such as phenolics, nitrogenous compounds, lipids and terpenoids, acids and other components might

Table 1.1. Estimates of the typical percentages by weight of components of dry table wines (Boulton et al., 2010)

Class of compounds	From juice fermented	From juice and pomace ^a fermented
Water	86	85.0
Inorganic salts	0.2	0.2
Carbohydrates	3.0	4.0
Alcohols	10.0	10.0
Acids	0.7	0.6
Phenols	0.01	0.1
Nitrogenous compounds	0.1	0.05
Lipids	0.01	0.02
Terpenoids	0.01	0.015
Other volatiles	0.1	0.1
Miscellaneous	0.5	0.1
Total	100	100

^a The solids (grape skins and seeds especially) left after juice or wine is drained and pressed from the whole must or young wine

be expected to make some contribution to the mouthfeel of both red and white wines. Current information on these components and their potential contributions to mouthfeel is summarized in the sections below.

1.4.2. Soluble Cell Wall Carbohydrates from Grape Berries

Grape cultivars, yeast strains and winemaking techniques influence the content and composition of SCWCs present in wine. White wine is made by fermenting juice, whereas red wine is made by fermenting juice and pomace together for an extended period of time (Del Barrio-Galán et al., 2011). Here, pomace is defined as the solids (grape skins and seeds especially) left after juice is drained and pressed from the whole must (Boulton et al., 2010). Importantly, the profile of wine SCWCs is determined by the solubility of cell wall polysaccharides and, subsequently, their resistance to change throughout the winemaking process (Vidal et al., 2001). It has been reported that some wine SCWCs from grapes and yeasts are highly stable and can remain at their initial levels 10 years after bottling (Doco et al., 1999).

Yeast mannoprotein, AG, RG I and RG II were the main SCWCs found in a Carignon Noir red wine and were present at 35 %, 45 %, 4 %, and 19 %, respectively (Vidal et al., 2003a). In a similar study, Ayestarán et al. (2004) reported that the SCWCs and related compounds present in a Tempranillo red wine were AG and arabinogalactan-proteins (AGPs) (38 - 41 %), RG II (38 – 46 %) and yeast mannoprotein (14 - 19 %). In contrast, a different proportion of polysaccharide types was

observed in a Verdejo white wine, which consisted of AGPs (20 %), RG II (22 %) and yeast mannoprotein (58 %) (Del Barrio-Galán et al., 2011).

RG II was first isolated from Carignan Noir wine and consisted of Rha, Fuc, Ara, apiose, Gal, GalA and GlcA, as well as rare diagnostic monosaccharides of 2-O-methylfucose, Dha and Kdo (Doco and Brillouet, 1993). Structurally complex RG II from grapes is conserved in wine because it resists winemaking processes such as enzymatic degradation during fermentation (Vidal et al., 2003a). It has been reported that RG II is found in red wines and white wines at levels of 100 - 150 mg/L and approximately 20 mg/L, respectively (O'Neill et al., 2004). The amount of RG II in red wine is much higher because red wine is produced by fermenting juice and pomace together and thus contains SCWCs from both pulp and skin. Four RG II fractions that differ in chain length and degree of methyl-esterification were isolated and purified from a Carignon wine by a series of chromatography techniques, including anion exchange chromatography, Concanavalin A affinity chromatography and size exclusion chromatography (Pellerin et al., 1996). In this case, two of the four RG II fractions contained boron, and it is postulated that they were likely present as a borate di-ester, which potentially cross-linked two RG II molecules together (Pellerin and O'Neill, 1998). Pellerin and O'Neill (1998) suggested that the ratio of RG II monomers and dimers in wine depends on the boron levels in grapes. Further studies show that wine RG II cross-linked with borate forms a dimer stable at wine pH (Pellerin et al., 1996; Pérez et al., 2003). According to O'Neill et al. (2004), RG II acts as a chelator and can complex trace heavy metal ions such as lead, barium, and strontium in wine to form a dimer. It is known that wine RG IIs are well conserved because the major structural features of wine RG II are similar to those found in the cell walls of sycamore (Pellerin et al., 1996).

Even though RG I with side chains comprising characteristic monosaccharides such as Ara and Gal is more abundant than RG II, as reported in Grenache Blanc grape berries, the understanding of wine RG I is limited (Vidal et al., 2001). This is partly attributable to the diversity of RG I side chains, namely AG I, AG II and arabinan, which are present in low abundance with varying degree of polymer length and substitution. The arabinan side chain of RG I represents only 2 - 3 % of the total SCWC

composition of wine and is found to be solubilized in the ethanol fraction during cell wall isolation (Villettaz et al., 1981; Vidal et al., 2003a). However, the presence of short-chain AG II lacking 3-linked Gal residues indicate ramified side chains on the RG I backbone (Vidal et al., 2003b). Furthermore, it has been reported that wine RG I linked to xyloglucan-like polysaccharides becomes difficult to isolate and characterize (Zabackis et al., 1995; Vidal et al., 2003b; Ducasse et al., 2010b).

Pectic polysaccharides were reported to be negatively charged and stable in model wine solutions at around pH 3.5 and therefore to establish electrostatic and ionic interactions with other charged compounds in wine (Vernhet et al., 1996). Another finding reported that oligomers of RG and HG, containing between three to fifteen monosaccharide residues, are important structural characteristic of SCWCs in wine (Apolinar-Valiente et al., 2015).

1.4.3. Soluble Cell Wall Carbohydrates from Yeast

The presence of yeast SCWCs in wines comes from these three sources: endogenous yeast on grape berries, commercial yeast inoculated into juice for fermentation and commercial yeast derivatives added into wine. The overall polysaccharide profile released from yeast into wines is influenced by the winemaking technique and commercial yeast strain (Doco et al., 1999; Vidal et al., 2003b). Grape berry surfaces provide a physical environment on which a complex microbial ecosystem of diverse species and strains of yeasts, bacteria and fungi can grow (Setati et al., 2012). Liu et al. (2015) isolated 93 different yeast species of 30 different genera from 49 different grape varieties in 22 countries from grape berries during ripening. Significant yeast species heterogeneity and distributions among grape samples in the same vineyard at the same stage of berry ripeness have been reported to be subject to significant intra-vineyard spatial fluctuations and the presence of different microbiota in different sections of the vineyard (Setati et al., 2012).

Commercial yeast, *Saccharomyces cerevisiae*, is often used for winemaking and represents another source of wine SCWCs. It has been reported that the overall polysaccharide profile released from yeast into wines is influenced by the winemaking technique and commercial yeast strain (Doco et al., 1999).

Mannoproteins are the main polysaccharides released by yeast. Doco et al. (2003a) reported that yeast SCWCs, predominantly neutral polysaccharides with a size range of 5 – 800 kDa, were liberated during the alcoholic fermentation and subsequent aging of wines on yeast sediments (or lees). The aging of wines on lees is described as “*sur lie* aging”. *Sur lie* aging is a well-known vinification technique that improves wine flavor and mouthfeel for many years. *Sur lie* aging for periods ranging between 2 – 15 months is commonly applied to white wine production, especially in barrel-fermented Chardonnay wine (Patynowski et al., 2002). According to Pati et al. (2012), Cabernet wine aged on red grape-derived lees led to an increase in the wine polysaccharide content because red grape-derived lees contained an enriched source of grape polysaccharides arising from the maceration and fermentation steps of the wine making process. The increase in Gal and Ara residues, which are constituents of pectic polysaccharides, in a Verdejo wine that was aged on commercial yeast derivatives, is indicative of the presence of grape derived SCWCs in the lees (Del Barrio-Galán et al., 2011). The presence of these compounds could be related to the manufacturing and purification processes of the yeast derivatives.

1.4.4. Phenolics

Phenolic compounds (or phenolics) encompass all compounds with hydroxyl groups attached to aromatic rings. Phenolic compounds in wine consist mainly of anthocyanins, flavan-3-ols, flavanols, phenolic acids and stilbenes (Li et al., 2011), and they play a key role in determining wine color and astringency perception. These compounds are synthesized in the berry and concentrated in the skin and seed (Table 1.2). The fruit of red grape varieties contains much higher concentrations of phenolic compounds than white grape varieties, since the latter contain no anthocyanins. Hydroxycinnamates are located in the pulp of the grape, whereas flavan-3-ols (catechins) are found in the seeds. The hydroxycinnamates, predominantly caffeic acid, represent the vast majority of the phenolic pool present in white wines. Quercetin glucosides are important indicators of red grape berry maturity and quality. Sun light exposure increases quercetin glucosides accumulation in the berries. The deglycosylation of quercetin glucosides takes place during winemaking.

Tannin is generally used in the wine industry to describe polyphenols. Condensed tannin (proanthocyanidins), which is found in grapes, and hydrolysable tannin (ellagitannins), which is found in oak, are two major sources of tannins in commercial wines. Anthocyanins will combine with tannins to form stable polymeric pigments.

Table 1.2. Total phenol distribution in *Vitis vinifera* Wine Grapes^a (Winkler, 1974)

Portion	Red Varieties	White Varieties
Skins	1859	904
Pressed pulp	41	35
Juice	206	176
Seeds	3525	2778
Total	5631	3893

^aIn gallic or tannic acid equivalents, mg/kg berries

Tannin structures undergo modifications due to the oxidation and rearrangement reactions that occur during fermentation and wine aging. The phenolic profile of wines can be affected by grape varieties, environmental factors, grape ripeness levels, winemaking variables and aging conditions. For example, Pérez-Trujillo et al. (2011) reported that most of the Canary Islands wines that were made from the autochthonous grape varieties showed characteristically high contents of stilbenes.

1.4.5. Other Compounds

°Brix and reducing sugars

Total soluble solids in juice are expressed as °Brix. The °Brix measurement is performed by refractometry, where 1 °Brix = 1 % w/w soluble solids as sucrose. In the wine industry, °Brix is more commonly used to predict the maturity of the fruit than individual sugars in juices. In California, the °Brix of grape varieties at harvest usually ranges between 18 - 26 °Brix, depending upon variety and desired wine style.

Glucose and fructose are the primary fermentable reducing sugars present in the berries, with the former being a preferred carbon source for yeasts, and together usually represent > 95% of the total soluble solids. The term 'residual sugar' refers to all forms of sugar, both the fermentable reducing sugars and non-fermentable pentoses, remaining after fermentation. In most cases, the amount of

non-fermentable pentoses remaining in wines is very low. Therefore, glucose and fructose in wines are generally held to be synonymous with 'residual sugar'. A dry wine is generally described as a wine with residual sugar less than 2 g/L and without a detectable sweet taste (Boulton et al., 2010).

Ethanol

Wine ethanol is the natural product of the fermentation of berry glucose and fructose by yeast. The amount of ethanol is dependent on the sugar concentration of juice (Formula 1).

$$\text{Formula 1: } \% \text{ potential alcohol (v/v)} = 0.57 \times \text{°Brix}$$

The ethanol levels of white and red wines typically range from 11.6 - 13.2 % v/v and 12.4 – 15.9 % v/v, respectively (Ohkubo and Ough, 1987; Hjelmeland et al., 2013). Ethanol also acts as a solvent to extract color and phenolic compounds from the must of red grapes during fermentation.

Organic Acids

In grape berries, some citric acid is found with the more abundant tartaric and malic organic acids. These acids carry over into the resulting wine. Lactic, acetic and succinic acids are produced by yeast and bacteria during the fermentation process (Mattick, 1983). Tartaric acid is present as a free acid and as salts such as potassium bitartrate (Boulton, 1980).

The actual acid composition and concentration of organic acids within the must or wine vary greatly and are influenced by factors such as variety, climatic conditions and cultural practices. At veraison, the acids in the fruit decrease, with the reduction in malic acid more prominent than the reduction in tartaric acid. At harvest, the fruit usually contains more tartaric acid than malic acid, and the exact concentrations and ratios to one another are again cultivar specific and harvest date dependent (Dokoozian and Kiewer, 1996). Generally, in ripe grape berries, the organic acid levels are lower in grapes grown in a warm region compared to those from a cooler region.

These organic acids contribute to the stability of wine color and pH, and their preservative properties can reduce the risk of microbial contamination (Ribéreau-Gayon et al., 2006). The acidity contributed by these compounds is expressed as titratable acidity (TA). In wineries, TA is the best practical expression of the organic acid concentration in must and wine. In many wine producing

regions, tartaric acid can be added to either must or wine to increase TA, while potassium bicarbonate can be added to decrease TA, in order to modulate the sensory perceptions and chemical properties of the wine.

pH

pH is a logarithmic measure of the free hydrogen ion concentration of a solution and is regarded as the most important measure of juice and wine acidity. In wines, malic and tartaric acid are primarily present in their protonated and associated conjugate base forms. The pH values of wines typically range from 3.0 – 3.7. Red wines generally have higher pH values than white wines because more potassium is extracted during maceration. Thus, more tartaric acid exists in a salt form and this decreases the concentration of free tartaric acid (Jackson, 2008b). Wine pH plays an important role in the equilibrium of tartrate salts, the solubility of grape proteins and the microbiological and color stability of the wine (Boulton et al., 2010).

Potassium

Inorganic minerals in the soil are taken up by the roots of grapevines and transported to the fruit. The main mineral cations found in grape berries are potassium, calcium and sodium, whereas the most common mineral anions are phosphate and chloride. In grapes and wine, potassium is the principal mineral, and grape skin is the major source of potassium. The potassium concentration in wine ranges from 200 - 2000 mg/L (Margalit, 2004). The interaction between tartaric acid and potassium results in the formation of largely-insoluble potassium bitartrate, and once saturated, potassium bitartrate will precipitate from the wine. This may result in an increased pH and may negatively impact wine quality (Gawel et al., 2000). Potassium bitartrate sediments may be present in amounts ranging from several grams of crystals at the bottom of a bottle to just a few crystals adhering to the cork (Zoecklein et al., 1995). In red wines, the crystals are dark in color due to the adsorption of pigments into the crystal lattice. This significantly reduces the aesthetic value of the product, often leading to consumer rejection.

Color

Color is one of the most important quality attributes of red wines. Anthocyanins, which are water-soluble flavonoid pigments that accumulate in grape cell vacuoles, are the principal source of the red color in wine. Visual detection of red color is due to the absorption of green visible light at 520 nm. In red wine, anthocyanins readily polymerize with tannins to form pigmented tannins, and these stable pigments are required to give red wine its long-term color stability.

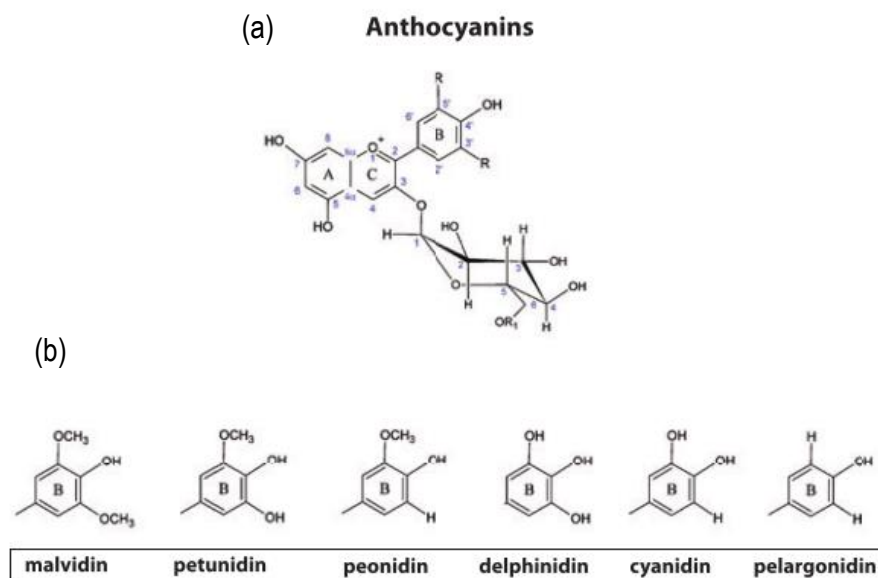


Figure 1.7. Overview of chemical structures (a) the 3-O-glucoside form of anthocyanins, (b) the five versions of the B-ring found in grape anthocyanins. This diagram is from Casassa and Harbertson (2014).

Anthocyanin refers to glycosylated anthocyanidins, which is the term for a simple flavonoid ring system. Anthocyanins are classified by the position of hydroxyl and methyl groups on the second phenyl ring. In grape berries, the common anthocyanidins are malvidin, petunidin, peonidin, delphinidin, cyanidin and pelargonidin (Figure 1.7). Malvidin-3-glucoside is the primary contributor to the red color and represents about 40 % of the total anthocyanins (He et al., 2010). In grape berries, the content and composition of anthocyanins depends on varieties, environmental conditions and viticulture practices. Throughout the wine making and aging processes, the color is determined by the anthocyanin chemical profile as well as the availability of the complexing agents and the pH of the solution.

Color is also an important quality attribute of white wines. Browning negatively impacts white wine quality. Browning of white wines results from the oxidation of phenols to quinones, which in turn polymerize to form macromolecules with a yellow-brown hue (Singleton et al., 1999). The optical density of wine at 420 nm is used as an indicator of browning. At the early stages of winemaking, browning is caused by enzymatic reactions; whereas following fermentation, wines with an abundance of phenolic compounds are susceptible to oxidative browning (Cheynier et al., 1989).

Aroma compounds

Volatile compounds are responsible for wine aroma quality. These compounds accumulate during the latter stages of grape ripening and exist as free volatile forms as well as non-volatile glycosidically bound aroma precursors that can be released through chemical reactions during winemaking and aging. Both acid and enzymatic hydrolysis can liberate bound aroma compounds to their odor-active forms (Gunata et al., 1990; Williams et al., 1995). The type and concentration of aroma compounds found in grapes varies widely among varieties. Most varieties share a common group of aroma compounds that contribute to the basic neutral aroma of the wines. But, there are varieties that contain specific potent aroma compounds that give the wines its characteristic aroma profile. For example, grape varieties such as Muscat of Alexandria, which contain high levels of monoterpenes, produce fruit with a distinguished floral aroma, while others such as Pinot Gris produce fruit with a neutral aroma (Williams et al., 1980). Monoterpenes, C₆ compounds, methoxypyrazines and norisoprenoids represent a large diversity of aroma nuances (herbaceous, fruity and floral) in grapes and wine, and they are generally present at low concentrations. Some of these aroma compounds impact odorants because of their very low sensory thresholds. For example, in Chardonnay wines, the reported concentration of linalool, which has an odor (fruity and floral) detection threshold of 25 µg/L, is generally in the range of 2 - 142 µg/L (Gambetta et al., 2014).

The aroma compounds formed during fermentation and aging also contribute to the final wine aroma profile. The best-known aroma compounds from the metabolic by-products of yeasts are esters, which contribute to the 'fruity' character of wines when present above their detection thresholds. For

example, ethyl butanoate when present above 20 µg/L can contribute to the 'strawberry-like' aroma character of Chardonnay wines (Gambetta et al., 2014).

Vineyard management practices, yeast strains, enzymes and oak types are important grape growing and winemaking factors that influence the wine aroma. Mendez-Costabel et al. (2014) reported that a reduced water and nitrogen application at the vineyard decreased the 3-isobutyl-2-methoxypyrazine (bell pepper-like aroma) concentration in Merlot grape berries at harvest.

1.5. Sensory and Mouthfeel of Wines and Impact of Wine Constituents on Sensory Parameters

Wine consumption is driven by consumer expectations of quality and sensory attributes. Because consumers have expectations of unique and differentiated products in a competitive market, the sensory aspects of wine have become integral in discerning quality criteria for consumers. According to Cox (1999), the sensory evaluation of wine is simply - "what happens first with the eyes, then the nose, and then the senses of taste and feel in the mouth." In a scientific manner, Pickering and Demiglio (2008) described this phenomenon as the integrated sensory perceptions from visual (hue, intensity and clarity), olfactory (ortho- and retro-nasal aroma), taste (sourness, sweetness, bitterness, saltiness) and tactile stimulant (mouthfeel). A cognitive model indicating the sequential processing of unimodal food properties is presented in Figure 1.8.

Olfactory has been the best known sensory system, and is responsible for the aroma perception of wine. Wine aroma is the dominant factor in the production and sales of wines, as stated by Bruwer et al. (2011). Since the 1980s, many research efforts have focused on developing wine aroma sensory vocabularies that are free of hedonics and judgments based on the extrinsic factors, in order to reliably describe wines. The original Wine Aroma Wheel, containing a list of standardized wine aroma terminology, was published by the Department of Viticulture and Enology at the University of California, Davis and the American Society for Enology and Viticulture in 1984 (Noble et al., 1984). The hierarchical wheel structure has proven to be a useful tool to provide standardized vocabularies

to facilitate communication among winemakers, wine researchers, wine marketing personnel and wine consumers. The wheel is made of three tiers, with general terms located in the center (e.g. fruity) and specific terms in the outer tier (e.g. grapefruit). These terms represent ones that are frequently encountered or recognized across different types of wines. In 1987, a modified version of the Wine Aroma Wheel (Figure 1.9) was published, with the major changes including the reorganization of the order of the terms, the addition of "nutty" aroma, and the use of reference standards, such as guaiacol for "smoky", to define each term (Noble et al., 1987). These aroma terms are currently widely incorporated into the sensory evaluation of commercial and research wines.

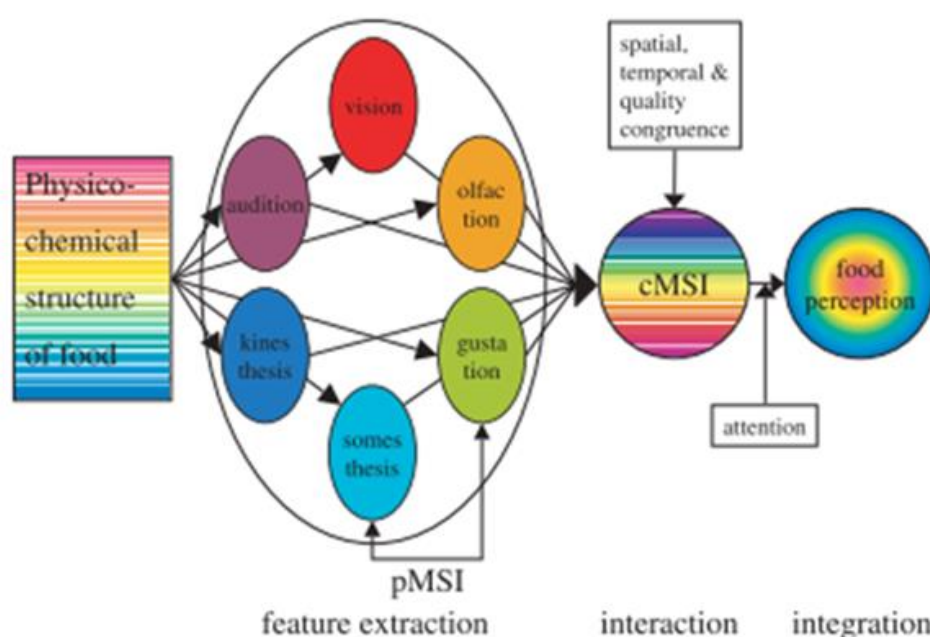


Figure 1.8. A cognitive model indicating the sequential processing (extraction, interaction and integration) of unimodal food properties (Verhagen and Engelen, 2006). pMSI, peripheral multisensory integration; cMSI, central peripheral multisensory integration.

Wine sensory science is extremely complex. Therefore, to formulate successful products, it is essential to focus on both the aroma and mouthfeel of the wine. Mouthfeel sensations are a consequence of oral-tactile stimulations derived from the stimulation of one or more of the four types of trigeminal receptors in the mouth, namely mechanoreceptors, thermoreceptors, nociceptors and proprioceptors (Jackson, 2008a). Lawless and Heymann (1998) described mouthfeel as “sensations

together for an extended period of time. The influence of mouthfeel, which has a strong effect on consumer preferences, is also reported in beers and soft drinks (Langstaff et al., 1991; Redondo et al., 2014). Despite its commercial importance, there is little consensus among academia, industry and consumers regarding the sensory vocabularies that reliably describe mouthfeel. This could be attributed to the difficulties in identifying and measuring the typically subtle sensations it encompasses, and disagreements on universal sensory descriptors (Pickering and Demiglio, 2008). Thus, mouthfeel is generally considered as the least understood aspect of wine sensory attributes.

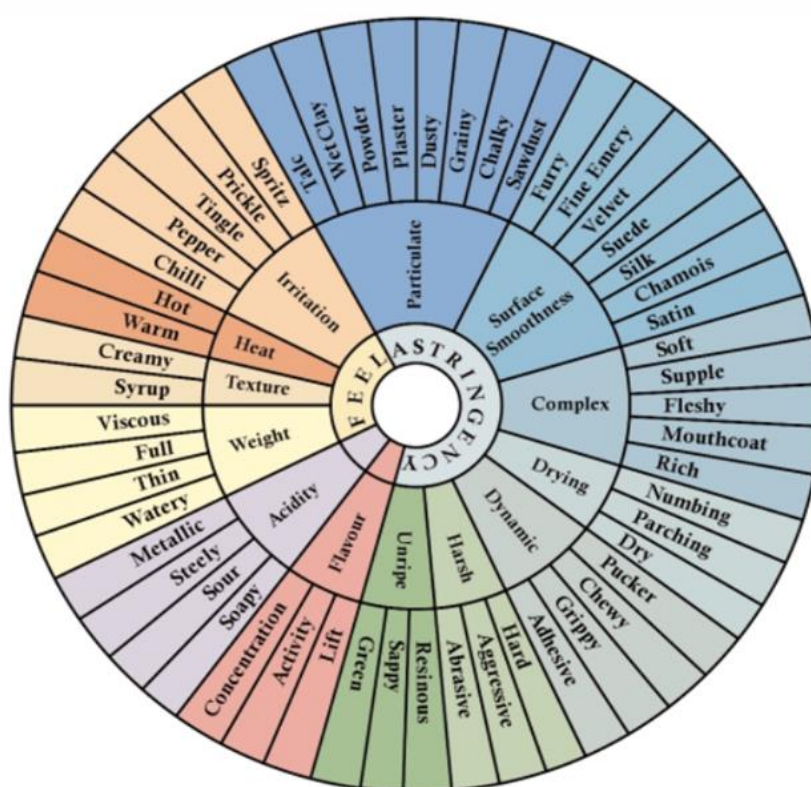


Figure 1.10. The Red Wine Mouthfeel Wheel (Gawel et al., 2000)

Gawel et al. (2000) developed a Red Wine Mouthfeel Wheel (Figure 1.10) in light of the popularity of the Wine Aroma Wheel that emerged in the 1980s. This Red Wine Mouthfeel Wheel follows the same principal as the Wine Aroma Wheel in that the vocabularies for communicating the mouthfeel characteristics of red wine are presented in a hierarchical structure. Notably, these terms and their definitions were generated by consulting the opinions of experienced wine tasters following exposure to an extensive range of commercial red wines (Gawel et al., 2000).

As mentioned earlier, white wines elicit mouthfeel sensations that are qualitatively different to those elicited by red wines. Gawel et al. (2000) and Pickering and Demiglio (2008) developed a new set of white wine mouthfeel terms and definitions, along with an evaluation protocol and reference standards by consulting the opinions of experienced wine tasters. The tasters were exposed to a wide variety of commercial white wines, which included table, sparkling, dessert and fortified styles. In addition to the mouthfeel sensations, the taste sensations of white wine are also included in the White Wine Mouthfeel Wheel (Figure 1.11).

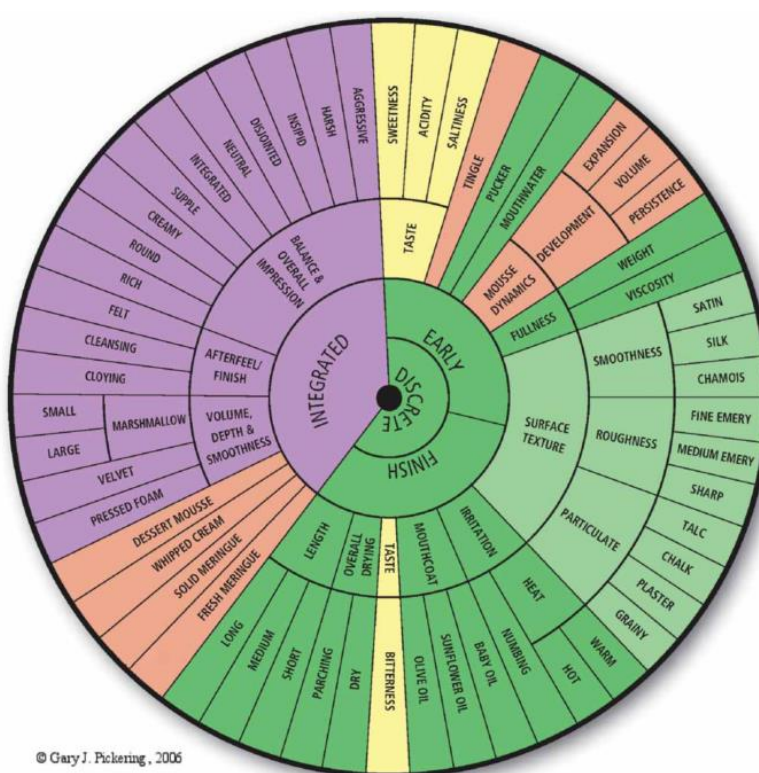


Figure 1.11. The White Wine Mouthfeel Wheel (Pickering and Demiglio, 2008).

At E. and J. Gallo Winery, a Wine Mouthfeel Wheel has been developed to support the production of commercial wines in order to meet the sensory preference of wine consumers and an adaption of this wheel is represented in Figure 1.12. The development of this wheel is almost entirely based on the sensory data collected from wine consumers. The opinions of these wine consumers

are translated into a measurable sensory lexicon. In addition to the mouthfeel attributes, the taste sensations of wine are also included in the Wine Mouthfeel Wheel. Unlike the hierarchal wheel structures presented in Figures 1.9 – 1.11, this mouthfeel wheel presents only the general sensory descriptors, and each descriptor is supported by detailed explanations along with a specific reference standard (Table 1.3).



Figure 1.12. A representation of the E. and J. Gallo Winery Mouthfeel Wheel.

Table 1.3. E. & J. Gallo Mouthfeel (M) and taste (T) attribute list with definitions and reference standards

Attribute	Definition	Reference Standard
M-Astringency	Drying sensation on tongue and pulling in and tightening of cheeks and mouth surfaces, sensed without moving tongue over surfaces	Grape seed extract ^a
M-Burn	Burning/warming sensation on surfaces of mouth; heat	Grain neutral spirits ^b
M-Chalky	Subcategory of mouth coating, chalky, powdery coating perceived after expectoration.	Milk of Magnesia
M-Coating	Any sensation that a coating has been left on the surfaces of the mouth after expectoration, especially sensed by moving tongue over surfaces of the mouth, roof, teeth, and gums	
M-Roughness	Roughness of mouth surfaces, sensed by running tongue over surfaces, especially roof of mouth, includes roughening of tongue's surface	Concentrated juice from Zinfandel ^a
M-Viscosity	Thickness, weight of wine, increase or decrease in force necessary to move tongue through liquid, must be evaluated while wine is in mouth. Includes syrupy mouthfeel	Gum Arabic ^b
M- Overall Sensation in Mouth	Includes the mouth feel sensations of chemical feeling factors (alcohol burn, astringency and spritz) and texture (mouth roughness, viscosity and mouth coating); may also include basic taste sensations but not the actual taste; what you feel when wine is in your mouth, what sensations you have on the surfaces of your mouth after expectoration; an overall perception of what is felt in the mouth	
T-Sweet	Sweet taste	Sucrose ^b
T-Sour	Sour/acidic taste	Tartaric acid ^b
T-Bitter	Bitter taste	Caffeine ^b

^aStandards are presented in a E & J Gallo Winery's commercial white wine.

^bStandards are presented in a E & J Gallo Winery's commercial red wine.

Tannins

Tannins are widely believed to be the key compounds affecting astringency and bitterness of wines, and this relationship has been studied extensively over the past three decades (Casassa and Harbertson, 2014). Wine astringency is mainly a tactile sensation due to the interaction of tannins with salivary glycoproteins (Peleg et al., 1999; Brossaud et al., 2001), whereas bitterness is a taste sensation (Robichaud and Noble, 1990). The correlation between phenolic compounds and mouthfeel agrees with previous studies suggesting phenolic compounds are important contributors to wine mouthfeel (Hufnagel and Hofmann, 2008b; Versari et al., 2013).

It is widely known that red wine astringency increases with higher phenolic content and molecular size of the phenolics (Kennedy et al., 2006; Soares et al., 2015). Robichaud and Noble (1990) reported that gallic acids, in particular flavan-3-ols exhibiting molecular masses less than 500 Dalton, elicit astringent and bitter tastes in red wines. (Kennedy et al., 2006; Soares et al., 2015).

Monomeric phenols contribute to the taste of wine and are generally more bitter and astringent than larger molecular weight derivatives, such as polymeric tannins (Brossaud et al., 2001). According to Robichaud and Noble (1990), bitterness increased linearly with the concentrations of catechin, gallic acid and grape seed tannins in blended white wines. The Syrah wines from shaded berries, which contained lower concentrations of tannins compared to wines from berries exposed to dappled sunlight, were found to be significantly lower in overall astringency, and less 'coarse' and 'grainy' (Ristic et al., 2007). The structure of phenolic compounds can also impact the perception of astringency. Vidal et al. (2003a) reported that, in a wine-like medium, the overall astringency and the related attributes such as drying, chalky and puckering positively correlated with increased chain length of proanthocyanidins.

The relationship between tannins and bitterness in red wines is not always clear. The human taste bitterness recognition thresholds are significantly higher than the astringency taste recognition thresholds of the same red wine phenolic compounds, as determined by Hufnagel and Hofmann (2008a) (Table 1.4). According to Vidal et al. (2003a), all wines spiked with tannins, irrespective of types, were rated very low in bitterness by a group of experienced sensory panelists, and the proanthocyanidin chain length did not affect the bitterness. The monomeric flavonoid phenols tend to exhibit a stronger relationship with bitterness. Peleg et al. (1999) indicated that catechin and epicatechin were significantly higher in bitterness than their respective dimers and trimers. However, epicatechin was found to have a greater maximum intensity and longer persistence of bitterness and astringency than its stereoisomer catechin. The structure of tannins also contributes to the bitterness perception. Notably, the linkage of monomeric phenols had an effect on bitterness, the catechin-catechin dimer linked by a 4→6 bond is more bitter than the catechin-(4→8)-catechin dimer (Peleg et al., 1999). While further studies are warranted, these studies show that, the composition and structure of tannins have an effect on the relative bitterness and astringency of wine.

Table 1.4. Human taste recognition thresholds (Hufnagel and Hofmann, 2008a).

Compounds isolated from red wine	Taste threshold ^a (mg/L)	
	Astringency	Bitterness
<i>Velvety astringent compounds</i>		
quercetin-3-O- β -d-Galp	0.2	non-detect
syringetin-3-O- β -d-Glcp	0.1	non-detect
<i>Pickering astringent compounds</i>		
gallic acid	50	non-detect
caffeic acid	72	non-detect
(E)-caftaric acid	5	non-detect
polymeric fraction (>5 kDa)	22	non-detect
<i>Bitter and astringent compounds</i>		
(-)-epicatechin	270	270
(+)-catechin	119	290
procyanidin C1	260	347
caffeic acid ethyl ester	58	229
gallic acid ethyl ester	37	438

^a Taste threshold concentrations were determined in bottled water by means of a triangle test for bitterness and by means of the half-tongue test for astringency

Hydroxycinnamates and Other Phenolics

The hydroxycinnamates are the most abundant class of phenolics in white wines and are thought to be important contributors to wine astringency and bitterness. However, Vèrette et al. (1988) concluded that hydroxycinnamates did not play any direct role in the taste of wines, particularly bitterness in a Chardonnay base wine. Furthermore, Gawel et al. (2014a) postulated that white wine bitterness was not directly related to hydroxycinnamates, but rather to the overall phenolic concentration and relative concentrations of phenolic compounds present in the wine. Australian winemakers perceived that higher quality Riesling wines have lower phenolic content (Gawel et al., 2014b). The addition of phenolics isolated from a Gewurtztraminer wine into a Riesling base wine resulted in significantly higher bitterness, astringency and viscosity than the control wines (Smith and Waters, 2012).

The sensory effects of the phenolic compounds are likely to be matrix-dependent. In a model white wine solution, gallic acid and catechin were found to be astringent and bitter; the latter was more pronounced (Robichaud and Noble, 1990). The bitter taste was associated with caffeic acid, whereas the burning and astringent sensations were associated with caftaric acid in Verdicchio wines (Boselli et al., 2006). In another study, the bitterness of white wines was positively associated with the summed

presence of free hydroxycinnamic acids as well as their tartaric acid and ethyl esters (Gawel et al., 2014a). This is in agreement with Keast et al. (2003) who reported that the perceived bitterness in the mouth is an additive effect of combinations of similar bitter compounds.

The chemistry and sensory characteristics of white wine are influenced by pre-fermentation juice extraction techniques. Free run juice is primarily comprised of the hydroxycinnamates from the pulp, whereas pressed juice or juice with extensive skin and seed contact contain a relatively larger amount of flavonoids (Patel et al., 2010). Hernanz et al. (2007) also reported that the pre-fermentative maceration increased the levels of phenolic compounds in the Zalema wines.

Phenolic Compounds and Mouthfeel

The majority of attributes used to evaluate mouthfeel of white wines differs from those used to evaluate red wines. White wines contain significantly lower amounts of tannins, which are reported to be mouthfeel constituents, when compared with red wines (Gawel et al., 2014a). The sensory threshold estimates of common astringent phenolic compounds found in white wines differ greatly, and these compounds are typically present in white wines below their detection thresholds (Gawel, 1998).

Tannins might not be the only contributor to the mouthfeel properties of wines. Wine mouthfeel is highly complex, and the sensory descriptors should not be limited to astringency. Indeed, Yonker (2012), showed that tannins and other phenolics are not major contributors to mouthfeel in red and white wines and this is supported by other published work (Scollary et al., 2012; Wollmann and Hofmann, 2013). Many studies have suggested that organic acids, proteins, polysaccharides, glycerol, and ethanol can impact wine mouthfeel (Lawless et al., 1996; Sowalsky and Noble, 1998; Vidal et al., 2004b; Gawel et al., 2007; Hufnagel and Hofmann, 2008b; King and Heymann, 2014).

Polysaccharides and Sensory Characteristics

The SCWCs have been reported to be correlated with wine mouthfeel (Table 1.5) and shown to increase the 'fullness' sensation of a model wine comprised of ethanol/water (13:87 v/v) saturated with potassium hydrogen tartrate at pH 3.6 (Vidal et al., 2004b). In white wines of high pH (3.6), Gawel

et al. (2016) reported that the polysaccharides generally caused a significant increase in viscosity when compared to wines at low pH (3.3). In red wines, a negative correlation between the intensities of astringency and the levels of polysaccharides was elucidated (Boulet et al., 2016). Whereas AGP lowered bitterness perception, RG II was shown to significantly decrease the sensory ratings associated with astringency and its sub-attributes, namely chalky, pucker and coarse (Vidal et al., 2004a; Vidal et al., 2004b). The addition of RG II in the model wine caused a significant decrease of overall astringency without contributing to bitterness, which has been attributed mainly to changes in mouth lubrication and the formation of complexes with astringent compounds.

Table 1.5. Mean attribute ratings for the samples scored by the panel and the estimates of significance^a for the comparison between each fraction^b and the model wine (Vidal et al., 2004b).

Attributes	Model wine	Mannoprotein + AGP	RG II
<i>In mouth</i>			
Roughness	0.57	0.45 ns	0.34 *
Chalky	0.3	0.21 ns	0.15 *
Dry	0.75	0.7 ns	0.55 **
Fullness	1.34	1.82 ***	1.67 **
<i>After spitting</i>			
Roughness	0.87	0.72 ns	0.55 ***
Chalky	0.33	0.34 ns	0.26 ns
Dry	1.29	1.08 *	1.02 **
Astringency	1.16	1.07 ns	0.98 *
Bitter	0.32	0.37 ns	0.32 ns

^a Significance calculated with contrast tests for each fraction versus the model wine: ns, not significant;

* $P < 0.1$; ** $P < 0.05$; *** $P < 0.01$

^b Mannoprotein + AGP isolated from ethanol precipitated wine colloids; RG II isolated from apple juice by-products

In a study of 13 different commercial Tempranillo wines, Quijada-Morín et al. (2014) reported that all polysaccharides families negatively correlated with perceived astringency because they are located on the loading plot opposed to astringency along the principle component (PC) 1, which represents 54.5 % of the variation (Figure 1.13.). This effect is relatively stronger for RG II and mannoproteins since they are opposed to astringency in both PC 1 and 2. Notably, alum was used as the reference standard for astringency in this study. In Monastrell wines, the presence of glucose and xylose indicated that wines contain hemicelluloses such as XG, but their abundance is lower than

pectic polysaccharides and their mouthfeel contributions to wines are not well established (Apolinar-Valiente et al., 2013; Apolinar-Valiente et al., 2015).

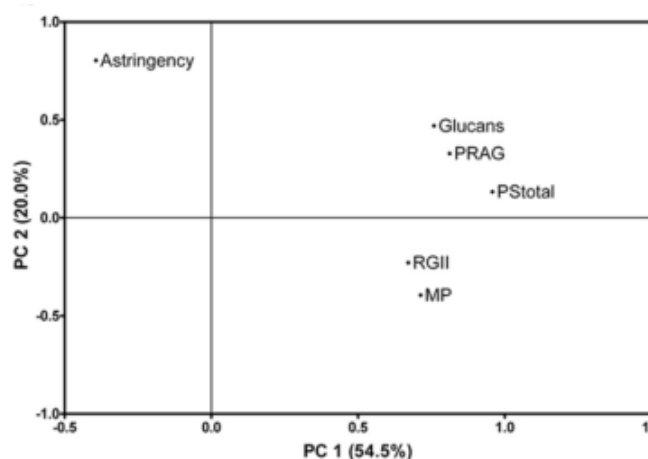


Figure 1.13. Loading plot of the principal component analysis relating to the perceived astringency and the polysaccharide composition. PRAG, polysaccharides rich in arabinose and galactose; PStotal, total polysaccharide content; RG II, rhamnogalacturonan II; MP, mannoprotein. This diagram is from Quijada-Morín et al. (2014).

It has been suggested that polysaccharides can also combine with other macromolecules in the wine matrix to modulate sensory characteristics. For example, salivary protein-tannin interactions may be inhibited by the presence of polysaccharides in solution, thus reducing astringency (Carvalho et al., 2006). Polysaccharides can either encapsulate tannins to increase aggregation or form soluble ternary complexes with the protein-tannin aggregates (Luck et al., 1994; Boulet et al., 2016). It was observed that wine RG II might cause the self-aggregation of proanthocyanidins in model wine, while AGP tends to inhibit proanthocyanidin aggregation (Riou et al., 2002). In contrast, wine oligosaccharides exhibited the opposite sensorial effect. Recently, physiochemical properties of wine oligosaccharides, such as the ability to chelate cations, have been reported to positively correlate with the astringency perception in Tempranillo wines (Apolinar-Valiente et al., 2015). Boulet et al. (2016) suggested that there was a direct impact of oligosaccharides, which consisted of mainly mannose and galactose, on the astringency of red wines. This may be attributed to the oligosaccharides being either too small to prevent protein-tannin aggregations or having a low affinity for complex tannins (Luck et

al., 1994; de Freitas et al., 2003). The importance of the three dimensional structures and sizes of carbohydrates in astringency remains to be studied (Quijada-Morín et al., 2014).

According to Pati et al. (2012), the red grape-derived lees arising from the maceration and fermentation processes and the autolysis of dead yeast cells can improve wine “roundness”. In this study, the SCWCs were comprised of Ara, Rha, Man, Gal and GalA. In contrast, Del Barrio-Galán et al. (2012) indicated that commercial yeast derivatives contain mostly neutral polysaccharides high in mannose and can reduce the acidity in Verdejo white wines, while in red wines, yeast derivatives can reduce “green tannins” mouthfeel and increase “softness” on the palate. Consistent with previous studies, all *sur lie* aged Verdejo wines had higher values of mouthfeel, less bitterness and lower values of acidity and astringency than the control wines (Del Barrio-Galán et al., 2011).

These results imply that the mode of action of yeast SCWCs are similar to those of grape SCWCs in modulating wine mouthfeel attributes. The yeast derivatives released polysaccharides in both model wines with and without proanthocyanidines, but the latter was higher in polysaccharide concentrations (González-Royo et al., 2017). *Sur lie* aging is useful, especially in young red wines that are more astringent and typically perceived as ‘unbalanced’. However, this conventional process is slow and the results may be inconsistent due to the concentration and composition of the SCWCs in the lees. In order to attain the aged character of the wines more rapidly and meet the market demand, *sur lie* aging with the supplement of purified commercial yeast derivatives can be considered.

It has been postulated that polysaccharides could modulate bitterness in wines (Gawel et al., 2016). However, there is limited knowledge around the mechanism and effects of interactions between polysaccharides and other wine compounds on taste and mouthfeel. A study by Wang et al. (2013) revealed that polyphenol monomers, such as gallic acid, and oat β -glucan formed a complex via hydrogen bonding, thus hindering the interaction between gallic acid and the taste receptor and reducing the free bitter-tasting phenolic compounds in the solution. This information provides insights into the function of polysaccharides on bitterness perception in wines.

SCWCs are also known to influence the rate and intensity of aroma release in the food matrix, but this knowledge has been largely overlooked by the wine industry. Polysaccharides can affect flavor or aroma molecules through a “salting out” effect that consists of covalent chemical interactions and non-covalent, physical entrapment of these molecules within the food matrix. For example, lactones can form non-covalent inclusion complexes with starch (Hansson et al., 2001; Heinemann et al., 2003; Mitropoulou et al., 2011). Studies demonstrate that the perception of aroma can be influenced by varying the SCWC-dependent viscosity levels of food while the in-mouth volatile concentrations remained unaffected (Verhagen and Engelen, 2006; Bult et al., 2007). In recent studies, the increase in aroma compounds such as terpenes, norisoprenoids and esters in Cabernet, Airen and Albariño wines are positively correlated with the concentrations of mannoprotein from lees (Pati et al., 2012; Juega et al., 2015). To a lesser extent, wine polysaccharides can prevent protein haze and reduce tartaric acid precipitation. However, this area requires more rigorous studies (Pellerin et al., 1993; Gerbaud et al., 1996; de Freitas et al., 2003; Ayestarán et al., 2004).

Besides tannins and SCWCs, other chemical components of wines have been reported to contribute to wine mouthfeel. Sensory studies indicated that ethanol can contribute to hotness, body, bitter, viscosity and astringent-like drying in wines (Gawel et al., 2007; Pickering and Demiglio, 2008). Wine organic acids impart “tartness” and “sourness” as well as elicit astringent-like sensations of wines (Sowalsky and Noble, 1998; Pickering and Demiglio, 2008). According to Lawless et al. (1996), wine astringency and sour taste decreased with an increased pH. Potassium is believed to contribute to undesirable wine sensory attributes such as astringency, ‘metallic’, saltiness and sourness (Waterhouse et al., 2016)

1.6. Proposed Research

It should be noted that the data summarized in the sections above, and particularly in Table 1.5 and Figure 1.13, were obtained from relatively incomplete studies, in which the effects of just a limited selection of polysaccharides from either berries or yeast were investigated, often in model or reconstituted wines. No comprehensive studies have been undertaken on the sensory effects of a complete set of well-defined berry and yeast SCWCs that are actually present in the final wine. In a previous study performed jointly by E & J Gallo Winery and The University of Adelaide, Yonker (2012) investigated the effects of tannins and polyphenols on the important mouthfeel characteristic of wines. This study reflected the central importance of the mouthfeel characteristic in commercial wine production. In the event, Yonker (2012) could show no convincing correlation between polyphenolics in wine and the mouthfeel of that wine. This shifted attention to other possible contributors to mouthfeel, with a particular focus on the SCWCs from berries and yeast. This analysis of the potential effects of cell wall polysaccharides and oligosaccharides derived from them during the wine-making process therefore formed the basis of the current research project.

The current knowledge gap regarding the potential role of SCWCs in wine mouthfeel was primarily due to the lack of rigorous studies linking polysaccharide chemistry and the resulting wine sensory profiles, in a 'real wine' experimental system. The difficulty of isolating and characterizing wine SCWCs was one of the limiting factors. Moreover, the mechanism by which SCWCs affect wine mouthfeel properties can be complex, because SCWCs may contribute directly to wine "fullness" or indirectly via binding to proanthocyanidins to modulate astringency (Vernhet et al., 1996; Vidal et al., 2004a), as described in the sections above. Additionally, there is a lack of fundamental understanding of the content and composition of grape berry SCWCs throughout ripening from the veraison to late maturity stage. Nunan et al. (1998) monitored the composition of cell walls isolated from grape berries during ripening and maturation, but did not analyse the soluble carbohydrates from either the berries or from yeast that survived the winemaking process and actually contributed to sensory characteristics in the final wine. It is likely that requirements for laborious fieldwork from the veraison to late maturity

stages and limited fruit availability over an extensive study period have seriously restricted the ability to address these important research questions. The latter stage of grape berry maturity, when commercial harvest for winemaking occurs, is extremely important for the development of sensory characteristics in the final wine. Knowledge of the changes that occur in SCWCs during ripening, which are potential precursors of wine mouthfeel compounds, would enable winemakers to harvest grapes at times that would maximize these precursors. During winemaking, these precursors can be affected by enzymes, heat and processing techniques. Therefore, only those that resist winemaking conditions have the potential to impact wine mouthfeel attributes. Obtaining additional knowledge in this particular area, namely the SCWCs and derived oligosaccharides that survive the process and are found in the final wine, will allow winemakers to manipulate fermentation conditions and processing techniques to retain and enhance the SCWCs important for mouthfeel.

Sensory evaluation of wine is another challenging aspect in which a wider array of mouthfeel descriptions, such as fullness, need to be measured rather than being limited to astringency (Gawel et al., 2000; Vidal et al., 2004b). A broad suite of sensory assay systems was available through E & J Gallo Winery for this study.

The goal of the work described in this thesis was therefore to investigate if there was a clearly demonstrable relationship between grape or yeast SCWCs and wine mouthfeel. The research was focused on the effects of SCWCs from Chardonnay and Cabernet Sauvignon grapes (*Vitis vinifera* L.) on wine sensory characteristics because of the commercial importance of these two wines. Chardonnay and Cabernet Sauvignon are the most-planted white and red wine grape varieties in California, occupying over 92,634 acres and 82,243 acres in 2015, respectively (United States Department of Agriculture Statistics Service, 2015). In Australia, Chardonnay and Cabernet Sauvignon made up almost 38 % of the total national wine production in 2015 (Australian Bureau of Statistics, 2015). As previously mentioned, Chardonnay is made by fermenting juice, whereas Cabernet Sauvignon is made by fermenting juice and pomace together for an extended period of time. The outcome of this study would also provide insights into the yield of different SCWCs and their

distributions in wines made with different techniques and from grape sources with different traits.

Against this overall research goal, the more specific aims of the project were to:

1. develop and adapt primary methods to isolate SCWCs and oligosaccharides from wines and to quantitate the levels, compositions and chemical structures of these SCWCs
2. use the analyses to estimate the proportions of key classes of polysaccharides and derived oligosaccharides in the final wine
3. define the changes that occur in these SCWCs during fruit maturation.
4. use the extensive array of wine sensory evaluation methods available through E & J Gallo Winery to define the major mouthfeel, taste and aroma characteristics of the wines
5. apply principle component analyses to find correlations between major SCWCs (and derived oligosaccharides) and key sensory characteristics and, in particular, to investigate and compare the potential link between the soluble polysaccharides and oligosaccharides from both grape berries and yeast with the mouthfeel attributes in red and white wines.
6. investigate the potential for developing assays for specific polysaccharide classes in grapes, with a view to developing rapid predictive assays for key sensory characteristics, such as mouthfeel.

Chardonnay and Cabernet Sauvignon grapevines grown in a commercial vineyard near Lodi, California were used in the study. These vineyards are managed using standard viticulture practices for the varieties and region. The weekly fruit sampling for micro-vinification was executed from 16 °Brix to 24 °Brix and 18 °Brix to 26 °Brix for Chardonnay and Cabernet Sauvignon, respectively. The extended study period captured the time window of commercial harvest, which has logistic and winemaking implications.

Several analytical methods were developed and adapted during the course of the study to determine the content and composition of the SCWCs and these are described in Chapter 2. In particular, SCWC size distribution was analyzed using the method described by Chong et al. (2009), but modifications of the method were expected to suit the sample matrix. The glycosyl linkage analysis

of SCWCs was performed at the Complex Carbohydrate Research Center (CCRC) at the University of Georgia using the method described by York et al., 1985 to deduce the composition of SCWCs. Prior to the glycosyl linkage analysis, grape and wine samples must go through a series of isolation and purification procedures to remove interference compounds such as proteins. Previously published cell wall preparation procedures for grape berries were evaluated and tested to generate a thorough process suitable for the grape and wine sample matrix in this study (Nunan et al., 1997; Yakushiji et al., 2001; Deng et al., 2005). A professionally trained sensory panel at E & J Gallo Winery evaluated the sensory characteristics of the wine, which encompassed a wider array of mouthfeel attributes including astringency and all other relevant sensory descriptors applicable for white and red wines, such as viscosity and mouth coating.

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Chapter 2

Development and Adaptation of Methods

2.1. Chemicals Used in This Study and Their Source

(-)-Epicatechin	Sigma-Aldrich E1753
(+)-Catechin	Sigma-Aldrich C1251
2-heptanol	Sigma-Aldrich H3003
20 % Sodium dodecyl sulfate	USB Corporation 75832
50 % Sodium hydroxide solution	Fisher
200 proof ethanol	Gold Shield Chemical Co.
Acetic anhydride	Sigma-Aldrich
Acetone	Alfa Aesar 67-64-1
Acetonitrile	EMD Millipore
Acetyl chloride	Sigma-Aldrich
Ammonium formate	Fluka 17843
Anhydrous methanol	Sigma-Aldrich
α -Ionone	Fluka 58170
β -Damascenone	Advanced Biotech 2254
β -Damascone	Sigma-Aldrich 23726-91-2
β -Ionone	Sigma-Aldrich 1-1260-3
Caffeic acid	Sigma-Aldrich C0625
Chloroform	Alfa Aesar 865-49-6
cis-3-Hexenol	Sigma-Aldrich H-1265
Citric acid monohydrate	Sigma-Aldrich C1909
Concentrated hydrochloric acid	Sigma-Aldrich 320331
Dextran 410,000	Sigma-Aldrich 00895
Dextran 150,000	Sigma-Aldrich 00893
Dextran 50,000	Sigma-Aldrich 00891
Dextran 25,000	Sigma-Aldrich 00271

Dextran 12,000	Sigma-Aldrich 00270
Dextran 5,000	Sigma-Aldrich 00269
Dichloromethane	EMD Millipore or Sigma-Aldrich
Dimethyl sulfoxide	Sigma-Aldrich
Ethyl butanoate	Sigma-Aldrich E15701
Ethyl decanoate	Sigma-Aldrich 14897
Ethyl dihydroxycinnamate	Sigma-Aldrich W24360
Ethyl heptanoate	Sigma-Aldrich 11,236-4
Ethyl hexanoate	Sigma-Aldrich 148962
Ethyl Isobutyrate	Sigma-Aldrich 246085
Ethyl Isovalerate	Sigma-Aldrich W246301
Ethyl nonanoate	SAFC W244708
Ethyl octanoate	Sigma-Aldrich 112321
Ethyl-2-methyl butanoate	Sigma-Aldrich 306886
Folin Ciocalteu Reagent 2N	Shape Products 6041
Gallic Acid	MPBIO 101750
Geraniol	Sigma-Aldrich 106-24-1
Glacial acetic acid	Fisher
Hexanal	Sigma-Aldrich 115606
Hexanal-d12	CDN Isotopes D-6961
Hexanol	Sigma-Aldrich 224715
Hexanol-d13	CDN Isotopes D-3982
Iodomethane	Sigma-Aldrich
Isoamyl acetate	Mallinkrodt 2491-57
Isopropanol	Baker
Linalool	Fluka 62138

Linalool-d3	CDN Isotopes D-6961
Malvidin-3-O-glucoside chloride	Indofine Chemical Company 020911S
Methanol	BDH 20864
Phenol saturated with Tris-HCl buffer	Invitrogen 15513-047
Phosphoric Acid 85%	EMD PX0996-6
Potassium (K)	Inorganic Ventures CGK10-5
Potassium metabisulfite	Sigma-Aldrich P2522
Propyl propionate	Sigma-Aldrich 112267
Quercetrin	Sigma-Aldrich Q3001
Sodium (salt)	Inorganic Ventures CGNA10-5
Sodium borodeuteride	Sigma-Aldrich
Sodium Carbonate	Mallinckrodt 7527 or Baker
Sodium Hydroxide	WR 1310-73-2
Tartaric Acid	Amresco 0501
Trifluoroacetic acid	Pierce
trans-2-Hexenal	Sigma-Aldrich 132659
trans-2-Hexenol	Sigma-Aldrich 132667
trans-3-Hexenol	Sigma-Aldrich 224715
Water (18 MΩ)	
Yttrium (Y)	Inorganic Ventures CGY1-5

2.2. Introduction

In this Chapter, detailed protocols for the experimental procedures used throughout the project are presented. Reference to these detailed protocols is made in the following chapters, where shorter summaries of the methods are provided. Of particular importance are the methods used for linkage analyses of the soluble cell wall carbohydrate (SCWC) fractions from grapes and wine. In summary, the University was keen for me to learn and understand the methods for linkage analysis, and for this reason I visited the laboratory of Professor Tony Bacic at the University of Melbourne, where I was tutored in the procedures while analyzing nine SCWC samples. However, there were 76 samples to analyse overall, and our University of Melbourne colleagues did not have the throughput capacity to analyse so many samples in the timeframe required for the completion of this thesis. As a result, the 76 samples, which included the nine samples analysed at the University of Melbourne, were sent to the Complex Carbohydrate Research Center (CCRC) at the University of Georgia for analysis.

2.3. Materials

2.3.1. Plant Materials

Vineyard Location and Field Trials

The Cabernet Sauvignon grapevines (*Vitis vinifera* L.) used in this study were grown in a commercial vineyard near Lodi, California (38° 10' 36.43" N, 121° 14' 27.51" W). The vines were planted in 1995 on 5C rootstock, spaced 1.8 m x 3.3 m (within row x between row spacing) and trellised to a horizontally divided training system. Standard viticulture practices for the variety and region were followed. The field trial was conducted in 2012 and employed a randomized complete block design. Eleven different harvest dates (treatments) were replicated four times using seven-vine plots. Twenty clusters were randomly selected weekly from from the middle five vines in each replicate on each harvest date beginning at seven weeks after veraison (WAV) to two weeks post-commercial harvest. Fruit was stored in ziplock bags and transported to the laboratory in insulated ice

coolers for chemical analyses. One set of vines (treatment) was harvested weekly beginning at nine WAV, which at this point grapes achieved suitable maturity level with 22 °Brix in juice for red wine micro-vinification, to two weeks post-commercial harvest for micro-vinification. Approximately 50 kg of fruit from each replicate plot was collected for micro-vinification and was stored overnight at 4 °C for processing the next day.

The Chardonnay grapevines (*Vitis vinifera* L.) used in this study were grown in a commercial vineyard near Lodi, California (38° 10' 36.00" N, 121° 14' 28.00" W). The vines were planted in 1991 on Freedom rootstock, spaced 2.1 m x 3.4 m (within row x between row spacing) and trellised to a horizontally divided training system. Standard viticulture practices for the variety and region were followed. The field trial was conducted in 2012 and employed a randomized complete block design. Nine different harvest dates (treatments) were replicated four times using seven-vine plots. Twenty clusters were randomly selected weekly from the middle five vines in each replicate on each harvest date beginning at four WAV to two weeks post-commercial harvest. Fruit was stored in ziplock bags and transported to the laboratory in insulated ice coolers for chemical analyses. One set of vines (treatment) was harvested weekly beginning at six WAV, which at this point grapes achieved suitable maturity level with 20 °Brix in juice for white wine micro-vinification, to two weeks post-commercial harvest for micro-vinification. The weekly fruit sampling procedure for chemical analyses and micro-vinification was the same as Cabernet Sauvignon.

Berry Sample Preparations

For the sample preparations, representative portions of machine de-stemmed whole berries from each collected fruit sample (20 clusters) were transferred into a sealed ziplock bag and hand-pressed to prepare juice, while other portions were machine pulverized using a Knife Mill Grindomix GM 200 (Retsch, Haan, Germany) into berry homogenates.

One 45 mL sample of juice from each variety was centrifuged at 2,791 x g for 15 min using an Allegra 6 centrifuge (Beckman Coulter Inc., CA, USA) to remove solids prior to measurements of °Brix, pH, titratable acidity (TA), tartaric acid and malic acid. For the Chardonnay grapes, an

additional juice sample was prepared by the addition of 45 mL of freshly pressed juice into a 50 mL tube, which was prefilled with 0.5 mL 9 g/L sulfur dioxide solution to prevent juice browning until the spectral evaluation of white juice phenolic compounds had been performed. This mixture was centrifuged at $2,791 \times g$ for 15 min using an Allegra 6 centrifuge (Beckman Coulter Inc., CA, USA) to remove solids before the analysis. For the analysis of Chardonnay total six-carbon (C6) volatile aroma content, 45 mL of juice was added into a 50 mL tube, which was prefilled with 0.5 mL of 20 % sodium dodecyl sulfate (SDS) solution, and mixed thoroughly.

Two 50 mL homogenate samples from each variety were centrifuged at $6577 \times g$ for 10 min in a Spinchron™ 15 centrifuge (Beckman Coulter Inc., CA, USA) and the supernatant was collected for the isolation of alcohol insoluble residue (AIR) and for the analysis of linalool precursor. For the analysis of Cabernet Sauvignon C6 volatile aroma content, 35 mL of homogenate was poured into a 50 mL tube prefilled with 0.5 mL of 20 % SDS solution and mixed well following the addition of 14.5 mL of saturated salt solution. For the Cabernet Sauvignon grapes, one gram of homogenate sample was also used for color and phenolic composition analyses.

2.3.2. Wines

Cabernet Sauvignon red winemaking

Cabernet Sauvignon berries from each replicate were crushed with a Magitec A15DC destemmer-crusher (Helmut Amos, Paarl, South Africa) into an 80 L stainless steel fermentation tank with an adjustable-height lid designed for a submerged cap system. Sulfur dioxide at a rate of 60 mg/L was added to the must immediately after destemming. To standardize the alcohol level for wines made from grapes of all ripening levels, the must sugar levels were either increased to 24 °Brix with the addition of a 2 parts glucose:1 part fructose mixture (approximately 333 g of glucose and 168 g of fructose dissolved in 1 L of water) or diluted to 24 °Brix with water. Diammonium phosphate was added to supplement the Yeast Assimilable Nitrogen to 300 mg/L and tartaric acid was added to adjust the pH to 3.6 before inoculation with D254 commercial yeast (Scott Laboratories, CA, USA) at a rate of 0.18 g/L. Fermentation was conducted at 25 °C and the grape skins mixed were resubmerged into

the fermenting juice once a day, commonly referred to as “punch down”. The must was pressed to separate out pomace when the reducing sugar reached below 2 g/L, using a Diemme GB00C299181 membrane press (Diemme Enologia, Emilia-Romagna, Italy). The resulting wine was cold settled at 2 °C for two weeks prior to filtration using a 1.0 µm pad filter followed by a Meissner 0.8 µm and a 0.45 µm cartridge filters in series, and bottled into 750 mL screw cap glass bottles, which were stored at 13 °C.

Chardonnay white winemaking

Chardonnay berries from each replicate were crushed with a Magitec A15DC destemmer-crusher (Helmut Amos, Paarl, South Africa) into a 100 L plastic bin. Sulfur dioxide at a rate of 40 mg/L was added to the must immediately after destemming, followed by the addition of a 0.3 mL/L commercial Rohavin MXpectolytic enzyme preparation mixture (AB Enzymes, Darmstadt, Germany) into the must at about 10 °C to increase juice yield. After 2 h of incubation time at 10 °C, the must was immediately pressed using a Diemme GB00C299181 membrane press (Diemme Enologia, Emilia-Romagna, Italy), and the resulting juice was transferred into a 50 L jacketed fermentation tank for cold settle at 2 °C. Skins and seeds were discarded following pressing. After two days, the juice was racked into another 50 L jacketed fermentation tank and the juice sugar level was either increased to 22.5 °Brix with the addition of a 2 parts glucose:1 part fructose mixture (approximately 333 g of glucose and 168 g of fructose dissolved in 1 L of water) or diluted to 22.5 °Brix with water, in order to standardize the alcohol level for wines made from grapes of all ripening levels. Diammonium phosphate was added to supplement the yeast assimilable nitrogen to 300 mg/L, and tartaric acid was added to adjust the titratable acidity to 6.7 g/L before inoculation with Vin13 commercial yeast (Scott Laboratories, CA, USA) at a rate of 0.12 g/L. Fermentation was conducted at 18 °C. The jacketed tank was set to 2 °C when the reducing sugar reached below 2 g/L in order to settle out solids before racking the wine into a 30 L stainless steel canister can for cold storage at 4 °C. The resulting wine was filtered within a week of cold storage using a 1.0 µm pad filter, a Meissner 0.8 µm cartridge filter

and a 0.45 µm cartridge filter in series, and bottled into 750 mL screw cap glass bottles, which were stored at 13 °C.

2.4. Standard Grape and Wine Analyses

The °Brix, pH, titratable acidity, tartaric acid and malic acid of each juice sample were analyzed by WineScan FT-120 Fourier Transform Infrared Spectroscopy (FTIR) (FOSS North America, MN, USA). About 30 mL juice was transferred into a plastic vial before loading on the auto-sampler. Juice samples were irradiated with broad spectrum infrared light and the resulting spectra characteristic of the measured analytes were mathematically applied to E & J Gallo Winery's calibration models developed from primary methods, which analyzed each analyte of interest employing thousands of samples. This operating procedure also applied to wine samples for the measurement of the percent alcohol, titratable acidity, malic acid and pH. A Gallo Winery's standard red wine with known levels of these attributes was used as a control sample.

The anthocyanin content of Cabernet Sauvignon grape samples was analyzed using an Agilent 8453 Spectrophotometer that was equipped with a photodiode array detector and a cuvette with a path length of 5 mm (Agilent, CA, USA). A total of 10 mL 50 % v/v ethanol (adjusted to pH 2 using 12 M HCl) was added into the tube containing 1 g Cabernet Sauvignon grape homogenate. The reaction mixture was placed on a shaker for 1 h and centrifuged at 2,791 x g for 15 min using an Allegra 6 centrifuge (Beckman Coulter Inc., CA, USA). Thereafter, 1 mL of extract (supernatant) was transferred into a fresh tube and diluted with 10 mL 1 M HCl. An aliquot of 8 mL of the well-mixed diluted extract was used for the analysis and the remaining volume was used for phenolic composition analysis (described in section 2.5.3). The reading at 520 nm was converted to mg anthocyanin per gram of berry by the equation:

$$(A_{520}/50) * 11 \text{ mL total volume} * [(10.5 \text{ mL}) / (\text{weight of homogenate g})] * (10 \text{ mm standard path length} / 5 \text{ mm actual path length}) = \text{mg anthocyanin} / \text{g berry}$$

Potassium (K) levels of wines were determined using a PerkinElmer Optima 7000 DV inductively coupled plasma optical emission spectroscopy (ICP-OES) (PerkinElmer Inc., MA, USA). The standard curve was generated for juice and wine samples in the concentration range of 100 to 2000 mg/L. In this analysis, 7.5 mL of Yttrium (Y) internal standard (2 mg/L) was added to 2.5 mL juice sample, whereas 5 mL of the same internal standard was added to 5 mL wine sample. Each reaction mixture was inverted at least five times. Thereafter, the mixture was aspirated through a nebulizer into the spray chamber of the ICP-OES. The resulting vapor was introduced into the argon plasma of the instrument. The element specific emission spectra were dispersed by a grating spectrometer and the intensities were measured by a Charged–Coupled Device (CCD) detector. The results are reported in mg/L.

2.5. Phenolic analysis

2.5.1. Total Phenols Content of White Wine and Red Wines

The total phenol (commonly referred to as “tannins”) content of wines was determined using the Folin–Ciocalteu Assay (Singleton et al., 1999), which is a simple, rapid and inexpensive analysis. It is a standardized method used in the wine industry. The solution mixture of 10 μ L wine, 150 μ L sodium carbonate and 74 μ L water became blue after 10 min in the presence of 50 μ L Folin–Ciocalteu reagent. The phenol content was measured at 751 nm and the reading was compared against a standard curve produced using gallic acid at concentrations of 50 to 500 mg/L. The results determined by this procedure represent the total phenols content, expressed as the gallic acid equivalent in mg/L.

2.5.2. Phenolic Composition of White Juice and White Wine

The method used to quantify the phenolic composition of Chardonnay white juice and white wine was adapted from Somers and Ziemelis (1985). This spectroscopy method is widely used in the wine industry to measure the typically low level phenolic compounds that are present white juice and white wine. The absorbances at 280 nm (index for phenols), 320 nm (index for hydroxycinnamates) and 420 nm (index for ‘brown color’) of samples were measured using an Agilent 8453

spectrophotometer that was equipped with a photodiode array detector (Agilent, CA, USA). Each juice sample was filtered through a 0.45 μm syringe filter into a 15 mL centrifuge tube, loaded on the auto-sampler and irradiated by a combination of a deuterium-discharge lamp for the ultraviolet (UV) wavelength range and a tungsten lamp for the visible and short wave near-infrared (SWNIR) wavelength range. The readings at 420 nm, 280 nm and 320 nm were collected as the samples flowed through the cuvette (Agilent, CA, USA), path length of 10 mm and 1 mm. This operating procedure was also applied to the wine samples, as filtration prior to the analysis was not required. An E & J Gallo Winery's standard white wine with known concentrations of analytes and resulting spectra was used as a control sample to ensure the precision of the method. The results are reported in absorbance units, AU.

2.5.3. Phenolic Composition of Red Grape and Red Wine

The method used to determine the phenolic composition of red grape homogenate and red wine was adapted from Waterhouse et al. (1999). The phenolic compounds were analyzed using an Agilent 1200 reversed phase high-performance liquid chromatography (HPLC) with a diode array detector and a 4.6 X 50 mm Zorbax Eclipse Plus C18 column (Agilent, CA, USA). The standard curves for gallic acid, (+)-catechin, (-)-epicatechin, caffeic acid, quercetrin, quercetin and malvidin-3-O-glucoside chloride were all generated at concentrations of 1, 5, 10, 25, 50, and 100 mg/L. The analysis was performed using a gradient mobile phase system consisting of mobile phase A acidified water (0.2 % v/v phosphoric acid) and mobile phase B acetonitrile at a flow rate of 1 mL/min. The elution conditions of mobile phase B were as follows: 5 % - 19 % from 0 to 10 min; 19 % - 33 % from 10 - 10.25 min; hold at constant 33 % for 10.25 – 12.5 min; 33 % - 95 % at 12.5 - 13 min; hold constant at 95 % for 13 – 15 min, and finally decrease to 5 % for 15 - 15.5 min. The eluted peaks were monitored at absorbances of 280 nm, 320 nm, 360 nm, 420 nm and 520 nm. The phenolic compounds were identified and quantitated based on the absorbance, retention time and peak area of the chemical standards (Table 2.1).

Table 2.1. Phenolic compounds and chemical standards detected at different wavelengths

Wavelength (nm)	Detection	Chemical Standards
230	Polymeric Tannins	(+)-Catechin
280	Gallic Acid	Gallic Acid
280	Catechin	(+)-Catechin
280	Epicatechin	(-)-Epicatechin
320	Caffeic Acid	Caffeic Acid
360	Quercetin	Quercetin
360	Quercetin	Quercetin
360	Quercetin glycoside	Quercetin
520	Malvidin glucoside	Malvidin-3-O-glucoside chloride

2.6. Aroma Analyses

2.6.1. Grape Total C6 Aroma Content of White Juice and Red Homogenate

The grape total C6 volatile aroma content was determined using the aroma method adapted from Siebert et al. (2005), with the modifications described below. Each sample of the treated Cabernet Sauvignon homogenate (10 g) and Chardonnay juice (10 mL) was transferred into a Gas Chromatograph (GC) headspace vial (Microliter, GA, USA), spiked with 10 μ L internal standard solution (Hexanal- d_{12} , Hexanol- d_{13} and 2-heptanol) and capped immediately. The GC vials used for Chardonnay juice samples were each prefilled with 3 g of NaCl.

The C6 volatile compounds were analyzed using an Agilent 7890B Series GC that was equipped with a 5973 Series Mass Selective (MS) Detector and a 30 m x 0.25 mm x 0.5 μ m DB-wax Ultra Inert capillary column (Agilent, CA, USA). The GC was linked with a Gerstel multipurpose sampler that was coupled with a CIS 4 inlet system (Gerstel Inc., MD, USA). During the solid phase microextraction (SPME), a Supelco DVB/CAR/PDMS fiber (Sigma-Aldrich Corp., MO, USA) was inserted into the headspace of the vial, heated to 70 °C and held for 20 min to adsorb volatiles. It was subsequently injected into the GC inlet port for the thermal desorption of volatiles, with helium gas acting as the carrier gas. The oven temperature was started at 35 °C and was increased linearly to 275 °C within the 24 min run time. For data acquisition, selective ion mode (SIM) was used and the qualifying ions that were monitored were based on characteristic m/z values of each volatile compound (Table 2.2). The aroma compounds were identified and quantified (expressed in parts per

billions, ppb) by comparison to the retention time, qualifying ion and peak area in relation to each chemical standard. The calculation used to determine the total C6 aroma compounds (ppb) in a sample is as follows:

$$\text{Total C6 aroma compounds (ppb)} = \text{hexanol (ppb)} + \text{hexanal (ppb)} + \text{trans-2-hexenol (ppb)} + \text{trans-3-hexenol (ppb)} + \text{cis-3-hexenol (ppb)} + \text{trans-2-hexenal (ppb)}$$

Table 2.2. The characteristic *m/z* values and the chemical standards of each C6 aroma compound

C6 compounds	<i>m/z</i> values	Calibration range (ppb)
hexanal	56,72,82	50 -15000
trans-2-hexenal	98,69,83	200 - 30000
trans-3-hexenol	67,82,69	1 - 500
cis-3-hexenol	55,67,82	1 - 3000
trans-2-hexenol	82,57,67	40 - 6000
hexanol	69,55,56	60 - 8000

2.6.2. Grape Linalool Precursor Content of White and Red Grape Homogenates

The method used to determine the linalool precursor level in red and white grapes was adapted from Singh et al. (2011) and Chong and Cleary (2012), with the following modifications. In this study, hydrolysis of samples at 100 °C for 45 min in 0.2 M citric acid (pH 3) was found to be the optimum condition to liberate linalool from its precursor. The purpose of using the mild acid hydrolysis was to mimic the potential acid hydrolysis effect that occurs during fermentation and wine aging. The linalool content was analyzed using a Agilent 6890 GC equipped with a 5973 series MS detector and a 25 m x 0.25 mm x 0.25 µm DB-5ms Ultra Inert capillary column (Agilent, CA, USA). The SPME and the data acquisition procedures were the same as the grape total C6 aroma content method (described in Section 2.6.1). The calibration curve of linalool (*m/z* 12,93) ranged from 0.5 to 150 mg/L and linalool d₃ was used as internal standard. The oven temperature was started at 40 °C and was increased linearly to 250 °C within the 45 min run time.

2.6.3. Chardonnay Wine Aroma Composition Analysis

The method used to determine the Chardonnay wine aroma composition was adapted from Siebert et al. (2005), with some modifications. Wine aroma samples were analyzed using an Agilent 6890 series GC equipped with a 5975 series mass selective detector and a 25 m x 0.25 mm x 0.25 μ m DB-5ms Ultra Inert capillary column (Agilent, CA, USA). The SPME procedure (described in section 2.4.1) was performed at 50 °C for 20 min. The data acquisition procedure was the same as the grape total C6 aroma content method (described in Section 2.6.1). Each wine sample was diluted with Milli Q water to achieve a 10 mL final sample volume at 8.5 % (v/v) ethanol, before dispensing into a 20 mL headspace vial (Wheaton, NJ, USA). After the addition of 100 μ L internal standards solution (ethyl nonanoate, β -damascone, propyl propionate, ethyl heptanoate and d₁₃-hexanol), the vial was capped and mixed gently before analysis. The oven temperature was started at 40 °C and was increased linearly to 275 °C within the 45 min run time. The characteristic *m/z* values and the chemical standards of each wine compound are shown in Table 2.3.

Table 2.3. The characteristic *m/z* values and the chemical standards of each volatile compounds

Aroma compounds	<i>m/z</i> values	Calibration range (ppb)
<i>β-damascenone</i>	190,175,121	0.04 - 80
<i>β-ionone</i>	177,135,192	
Ethyl decanoate	88,101,155	
Ethyl dihydrocinnamate	178,91,104	
Ethyl butanoate	71,88,43	0.4 - 400
Ethyl isobutyrate	43,71,116	
Ethyl isovalerate	88,85	
Ethyl hexanoate	88,99,60	4 - 1200
Ethyl octanoate	88,101,127	
Isoamy acetate	43,70,55	30 – 2400
Hexanol	56,43,55	500 - 40000

2.7. SCWC Analyses

2.7.1. Grape and Wine SCWC Preparation

The method was adapted from Doco et al. (1999), Vidal et al. (2000) and Ayestarán et al. (2004), with the modifications described below.

Rotary evaporation or ultrafiltration has been commonly used in previous studies to concentrate a juice or wine volume in order to reduce the subsequent solvent usage (Pellerin et al., 1996; Vidal et al., 2003; Apolinar-Valiente et al., 2013). However, in the present study, sample loss was noticed throughout the concentration process due to the multiple volume transfers. This concentration step was therefore omitted. The starting sample volumes of 25 mL for juice and 200 mL for wine were selected because these volumes generated adequate amounts of AIR for subsequent SCWC analyses. Five volumes of 100 % cold ethanol were added to one volume of each sample, to generate a final ethanol concentration of about 83 %. It is important to note that alcohol insoluble residues (AIR) fractions are generally prepared using 4 vol ethanol, or a final ethanol concentration of 80 % (v/v). In the current study, the relative volumes of ethanol and the sample solution (5:1) were adjusted to those used by Vidal et al. (2000) in their studies of selected wine polysaccharides. Furthermore, there are indications that some arabinogalactan-peptides of the type found in wheat endosperm are at least partly soluble in 80 % ethanol (Fincher, 2013), so the final ethanol concentration was increased slightly to 83 % (v/v) to minimize loss of these types of polysaccharides. The studied materials were naturally acidic, so acidified ethanol was not required.

The reaction mixtures were stored overnight at 4 °C. Thereafter, the AIR of each sample was recovered by centrifugation at 6577 x g for 10 min using a Spinchron™ 15 centrifuge (Beckman Coulter Inc., CA, USA) washed twice with 83 % ethanol, dried under a stream of nitrogen, dissolved in 30 to 35 mL of Milli Q water, transferred into a 100 to 500 MWCO dialysis membrane (Spectrum Lab Inc., CA, USA) and dialyzed overnight at 22 °C against water. The relatively low cutoff dialysis tubing was used because the primary objective of this step was to remove ethanol from the samples,

without losing any low molecular weight polysaccharides or oligosaccharides that might be precipitated by 83 % ethanol. Each dialyzed sample was transferred into a 50 mL tube, kept in a -40 °C freezer for at least 4 h, freeze-dried and weighed. The dried AIR content was referred to as “soluble cell wall carbohydrate content” according to Vidal et al. (2000), Ayestarán et al. (2004), Apolinar-Valiente et al. (2013) and Gawel et al. (2014), but it is also likely that it included some oligosaccharide material that had been precipitated by 83 % ethanol.

The method used in this study was adapted from Nunan et al. (1998) and Vidal et al. (2001), with modifications. In particular, Nunan et al. (1998) developed a method to remove proteins and lipids associated with the cell walls of grape berries. Their method was adapted to remove these compounds from the SCWC fractions isolated in the present study.

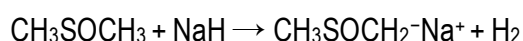
About 50 mg of SCWC sample was transferred to a 50 mL Nalgene round bottom chemical resistant centrifuge tube (Thermo Fisher, MA, USA), followed by the addition of 30 mL phenol solution saturated with 500 mM Tris-HCl, pH 7.4 (Invitrogen, CA, USA). The sample was capped and placed on the shaker for 60 min to remove proteins. The suspension was centrifuged at $6863 \times g$ for 10 min using an Allegra 25R centrifuge (Beckman Coulter Inc., CA, USA) and the supernatant was removed carefully with a capillary glass pipette. This phenol extraction treatment was repeated twice. The pellet was washed with ethanol (30 mL) and acetone (40 mL) to remove phenol, some colored compounds, tannins and other lipids. Each suspension mixture was stirred for 60 min before centrifugation. Thereafter, the pellet was washed twice with 30 mL chloroform:methanol (1:1, v/v), the suspension was mixed for 60 min on the shaker, centrifuged to recover the pellet. Following the addition of 30 mL of acetone, the pellet was mixed for 45 min on the shaker and filtered through a sintered glass filter. The recovered pellet was dried overnight in a vacuum oven at 45 °C and stored in a desiccator prior to analysis.

A significant sample yield loss was observed during the purification process. The yield loss was partly due to the changes in the physical properties of the SCWCs and partly due to multiple liquid sample transfers between different labware. The original SCWC material, which was light and fluffy,

became a loose pellet when treated with the phenol solution and re-suspended easily into the phenol fraction after centrifugation. During the ethanol/acetone treatment, some pellets adhered to the wall of the sintered glass filter. In order to reduce yield loss, 'chemically resistant' centrifuge tubes were used in conjunction with sintered glass filters, because this only required a single transfer. Each sample was washed three times with the phenol solution to ensure the complete removal of proteins. A brown layer, which might have included polyphenols or degraded proteins, separated from the pellet into the phenol fraction after centrifugation of the first two phenol washes. The addition of acetone to the sample further helped to remove color and tannins that could potentially precipitate with SCWCs during the ethanol precipitation procedure. The purification of SCWCs was repeated several times to identify the manageable range of the solvents at each step of the process and the amount of starting material required for good final yields of the SCWC fractions.

2.7.2. Glycosyl Linkage Analysis

The glycosyl linkage composition determined by methylation analysis is important for the characterization of polysaccharide structures. In general, the first key reaction of this analytical procedure is to ionize or de-protonate all the free hydroxyl groups on the polysaccharide. Because of the high pK_a values of these hydroxyl groups, very strong bases are required to remove the protons. This has been achieved via two major approaches. Hakomori (1964) used methyl sulfinyl carbanion as the base for de-protonation. This carbanion is generated by dissolving sodium hydride in dimethylsulphoxide (DMSO), as follows:



The methyl sulfinyl carbanion so generated is a very powerful base that will readily ionize hydroxyl groups of polysaccharides, usually in a single step (Hakomori, 1964). However, because the carbanion is unstable and can form explosive mixtures with oxygen or water, strong solutions of NaOH are usually preferred for polysaccharide linkage analysis (Ciucanu and Kerek, 1984; Pettolino et al.,

2012). In this second approach, a slurry of solid NaOH can be prepared in DMSO, or a solution of 50 % NaOH can be mixed with DMSO. The NaOH/DMSO mixture is used to de-protonate the polysaccharide hydroxyl groups, but because the NaOH base is not as powerful as the methyl sulfinyl carbanion, it is sometimes necessary to repeat the methylation procedure. Once the free hydroxyl groups of the polysaccharide are ionized, they are methylated through the addition of methyl iodide. The partially methylated polysaccharide is hydrolysed and this releases partially methylated monosaccharides in which the carbon atoms involved in the glycosidic linkage are now present with attached hydroxyl groups. To distinguish the hydroxyl groups on carbon atoms originally involved in the glycosidic linkage from the methylated carbon atoms of the polysaccharide backbone, they are reduced with sodium borohydride and acetylated with acetic anhydride. The resultant partially methylated alditol acetates (PMAAs) are analyzed by GC-MS. In the presence of acidic polysaccharides, reduction of the carboxyl groups of uronic acids to their sugar counterparts is necessary before methylation is undertaken (Figure 2.1).

The interpretation of methylation data can be subject to errors of a considerable magnitude when undermethylation occurs in the analysis (Pettolino et al., 2012). Undermethylation occurs when polysaccharides are not completely solubilized in DMSO or when methylation of the hydroxyl groups of the polysaccharide is incomplete. This can be overcome by swelling the samples in DMSO overnight or longer, heating the samples in DMSO or by performing double methylations. Although this analysis can determine the composition of the glycosyl residues of the polysaccharides, it is limited by an inability to define anomeric configuration (α/β), which is lost during the reduction step, and the sequential arrangement of the glycosyl residues of the polysaccharides. Complementary analyses are required to determine the complete primary structure of polysaccharides. For example, glycosyl residue sequence can be revealed by a combination of techniques, such as liquid chromatography mass spectrometry and nuclear magnetic resonance spectroscopy. However, in the present study the major objective was to estimate the relative proportions of different, commonly occurring polysaccharides and oligosaccharides in the various SCWC samples. These can be

estimated through methods that take advantage of known structures of these commonly occurring polysaccharides (Nunan et al., 1998; Caffall and Mohnen, 2009; Burton et al., 2010; Pettolino et al., 2012).

As mentioned in the Introduction to this Chapter, at an early stage in the present study, a small set of purified wine SCWC samples was subjected to methylation analysis and linkage composition under the supervision of Professor TonyBacic and Mrs. Cherie Beahan at The University of Melbourne. The procedure used was exactly as described by Pettolino et al. (2012), in which solid NaOH in DMSO was the basic agent. These experiments were performed because my supervisors believed it was important for me to obtain hands-on experience with the methylation procedure and with subsequent calculations and estimates of polysaccharide compositions of the SCWC samples. However, as the study proceeded it became apparent that a total of 76 samples required linkage analysis. This number of samples could not be accommodated by the University of Melbourne group at that time, so the 76 samples, including the sample set originally analyzed at the University of Melbourne, were sent for analysis at the Complex Carbohydrate Research Center (CCRC) at the University of Georgia in the USA.

The methylation procedure used by the CCRC followed reduction of the carboxylic acid groups of uronic acids with sodium borodeuteride and used NaOH as the base (Ciucanu and Kerek, 1984; York et al., 1985; Heiss et al. 2012). To ensure the complete solubility of the samples in DMSO, the CCRC elected to stir the samples in DMSO for a week. In contrast, samples were incubated in a warm water bath and sonicated twice in the method of Pettolino et al. (2012). For the base preparations, CCRC added 50% aqueous NaOH solution to DMSO, whereas Pettolino et al. (2012) prepared a slurry by grinding solid NaOH in DMSO. The CCRC method is relatively easy to execute. However, the solid NaOH is hygroscopic and can scavenge the water produced in the methylation reaction. Unlike Pettolino et al. (2012), the CCRC method cannot determine the degree of methyl-ester content of the samples.

The following is a description of the methylation analysis procedures used by the CCRC to analyze the wine SCWCs:

Uronic Acid Reduction

Methanolic HCl was used to methyl esterify the carboxyl group of the uronic acid residues with the release of a water molecule. About 1 mg of sample was methyl esterified in 400 μ L of 0.5 M methanolic HCl at 80 °C for 20 min, dried down twice with methanol, and reduced with 400 μ L sodium borodeuteride at room temperature for 2 h (Figure 2.1a and Figure 2.1b). In this process, the uronic acids of the purified SCWCs were reduced to their corresponding 6,6-dideuterio glycosyl residues before methylation and these derivatives could be separated from their neutral sugar counterparts by GC-MS analysis. The excess sodium borodeuteride was decomposed with acetic acid, the samples were dried under nitrogen and borate, which was formed in the decomposition, was removed by the repeated addition of methanol and evaporation under nitrogen.

Although the methanolic HCl treatment might be expected to partially hydrolyze acid-labile linkages, such as those adjacent to Araf residues, this would probably be minimal given the relatively low temperature and short time period of the esterification process; this has not been reported as a problem in the literature.

Base Preparation

In a glass tube, 100 μ L 50% NaOH was mixed with 100 μ L anhydrous methanol. The NaOH precipitate was collected by centrifugation, washed five times with DMSO and mixed with 2 mL DMSO before use.

Permethylation

The reduced samples were suspended in excess DMSO, stirred for one week until dissolved completely and methylated twice with about 20 drops of NaOH base and 100 μ L methyl iodide. Methylation is achieved by the substitution of the hydroxyl groups of monosaccharide residues with a methyl group (Figure 2.1c). As noted above, the first step of the methylation method consists of the deprotonation of the high-pK_a hydroxyl group with NaOH (strong base), in the presence of the DMSO (aprotic solvent). This reaction is an equilibrium process but the reverse reaction can be at least partly

inhibited not only by using the aprotic solvent, which has a greater charge in the transition state than the monosaccharide residues, but also by increasing the rate of removal of the nucleophilic alkoxides. The second step involves the addition of the methylating reagent (MeI) to the solution in order to yield methyl ethers. The iodide ion is a good leaving group because it is a weak base. The permethylated samples were extracted with dichloromethane and washed with water; each top layer was removed and dried.

Hydrolysis and Reduction

The dried partially methylated polysaccharides were hydrolyzed with 400 μ L 2 M trifluoroacetic acid (TFA) at 121°C for 2 h and dried (Figure 2.1d). Isopropanol was added during subsequent drying to remove residual TFA. The hydrolysates were reduced with sodium borodeuteride for a minimum of 3 h, treated with glacial acetic acid and dried following two additions of methanol. In the reduction, a deuterium atom will tag the anomeric C atom of the alditol during the opening of the sugar ring and this can be detected by MS.

Acetylation

The partially methylated alditols were acetylated with 250 μ L acetic anhydride in the presence of concentrated TFA, incubated at 45 °C for 25 min, dried down with methanol and treated with sodium carbonate until effervescence ceased (Figure 2.1e). About 1.5 mL dichloromethane (DCM) was added, vortexed and centrifuged to separate the organic phase from the aqueous phase. The organic phase, which contained the partially methylated alditol acetates (PMAAs), was transferred into a fresh tube and evaporated to remove the methylene chloride. The acetylation process increases the volatility of the derivatives for GC analysis.

GC analysis of the PMAAs

The resulting PMAAs were analyzed using an Agilent 7890A GC that was equipped with a 5975C MS detector. The PMAAs were separated on a 30 m x 0.32 mm x 0.20 μ m Rtx-2330 fused silica capillary column (Restek Corp., PA, USA) and identified based on their peak elution times relative to *myo*-inositol, coupled with their mass spectra, which were matched against mass spectra of known standard compounds. These mass spectra are presented in “The CCRC mass spectral

database for PMAAs at <https://www.ccruc.uga.edu/specdb/ms/pmaa/pframe.html> and in Supplementary Table 1 of Pettolino et al. (2012).

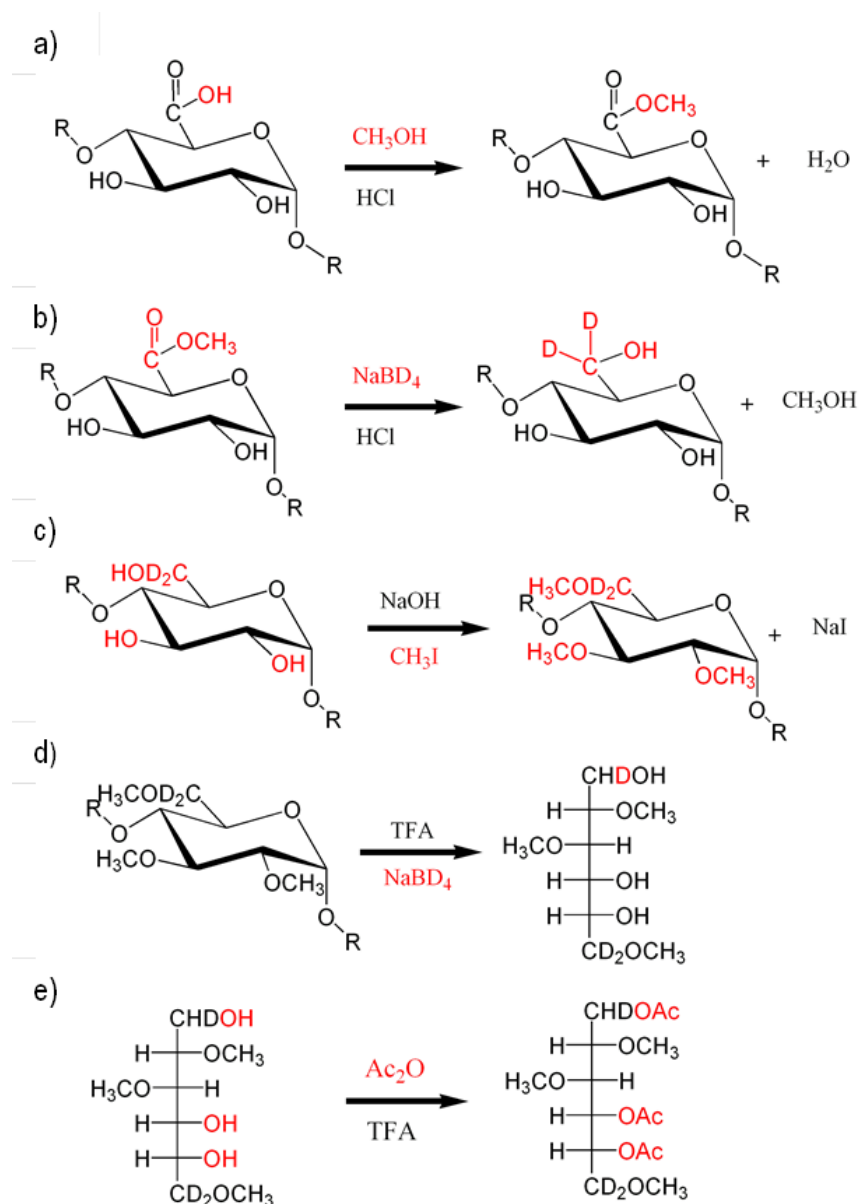


Figure 2.1. Methylation analysis of SCWCs with a mixture of neutral and acidic glycosyl residues (York et al., 1985; Pettolino et al., 2012). a) esterification of uronic acid, b) reduction of uronic acid, c) permethylation, d) acid hydrolysis and reduction, e) acetylation. For SCWCs without uronic acid glycosyl residues, step a) and b) can be omitted.

The general rules of electron-impact (EI) fragmentation patterns in mass spectra of PMAAs are as follows (York et al., 1985):

Rule 1: Primary fragments are generated by cleaving the alditol backbone.

Rule 2: The fragment with a methoxy-bearing carbon atom adjacent to the cleavage point carries the charge.

Rule 3: Fragmentation between two adjacent methoxy-bearing carbon atoms is favored

Rule 4: Secondary fragment-ions are formed by the loss of methanol or acetic acid.

Rule 5: When the partially O-acetylated, partially O-methylated alditols are labeled at C-1 with a deuterium atom, the m/z of a fragment ion that contains C-1 is even and the m/z of a fragment ion that does not contain C-1 is odd.

The *myo*-inositol was used as the internal standard of choice because it is not present in cell wall polysaccharides and has a different retention time from the glycosyl residues released from wall polysaccharides. In this study, the method used to prepare the standard compounds was to undermethylate a pure methyl glycoside. The quantitated peak areas were corrected to mol % using the calculations described by Pettolino et al. (2012). Polysaccharide assignment (Table 2.4) was based on the linkage structures of well-characterized SCWCs from grapes (Nunan et al., 1998), wines (Doco and Brillouet, 1993; Pellerin et al., 1996) and yeast (Kwiatkowski et al., 2009; Cuskin et al., 2015), as well as information in (Pettolino et al., 2012).

Calculation of polysaccharide composition based on linkage analysis

The levels of specific polysaccharides in the SCWCs were estimated from the linkage analyses on the basis of known polysaccharide structures (Table 2.4). It must be emphasized that the calculated polysaccharides compositions of the SCWC samples are estimates only and that unusual polysaccharides or minor variations in polysaccharide structures might not be detected in these estimations. The information in Table 2.4 is from the literature and includes, in addition to the major plant cell wall polysaccharide derivatives, a more detailed analysis of RG I and RG II than was provided by Pettolino et al. (2012), together with methods for calculating the amounts of yeast glucan and mannan.

Table 2.4. Calculation of polysaccharide composition based on linkage analysis^a

Polysaccharide	Expected Linkages	Monosaccharide Linkage Assignment
Arabinan (Pettolino et al., 2012)	5-Ara	All 5-Araf
	2,5-Ara	All 2,5-Araf
	3,5-Ara	All 3,5-Araf
	t-Araf	Equivalent to branch points; subtract value from t-Araf
		Total Arabinan
Arabinogalactan Type I (AG I) (Pettolino et al., 2012)	4-Gal	All 4-Gal
	3,4-Gal	All 3,4-Gal
	4,6-Gal	All 4,6-Gal
	t-Araf	t-Araf not already assigned to arabinan (must be $\leq$ total branch points); subtract value from t-Araf
		Total AG I
Arabinogalactan Type II (AG II) (Pettolino et al., 2012)	3-Gal	All 3-Gal
	6-Gal	All 6-Gal
	2-Ara	All 2-Ara
	3,6-Gal	All 3,6-Gal
	3,4,6-Gal	All 3,4,6-Gal
	t-Rha	All t-Rha assigned to RG I/II**
	t-Araf	Equivalent to branch points; subtract value from t-Araf
	t-Gal	Equivalent to branch points less t-Araf; subtract value from t-Gal
	t-GlcA	Equivalent to branch points less t-Araf and t-Gal; subtract value from t-GlcA
		Total AG II
Homogalacturonan (HG)*	4-GalA	All 4-GalA
	t-GalA	All t-GalA
		Total HG

Table 2.4. Calculation of polysaccharide composition based on linkage analysis^a (continued)

Polysaccharide	Expected Linkages	Monosaccharide Linkage Assignment
Rhamnogalacturonan I/II (RG I/II)**	2-Rha	All 2-Rha
	3-Rha	All 3-Rha
	2,4-Rha	All 2,4-Rha
	2,3-Rha	All 2,3-Rha
	2,3,4-Rha	All 2,3,4-Rha
	2-GlcA	All 2-GlcA
	2,4-GalA	All 2,4-GalA
	3,4-GalA	All 3,4-GalA
	2,3,4-GalA	All 2,3,4-GalA
	2,4-Gal	All 2,4-Gal
	3,4-Gal	All 3,4-Gal
	4-Gal	All 4-Gal
	2-Ara	All 2-Ara
	2,3-Ara	All 2,3-Ara
	3,4-Fuc	All 3,4-Fuc
	4-Ara	All 4-Ara
	t-Rha	All t-Rha
	t-Araf	t-Araf not already assigned to arabinan and AG I/II. Equivalent to branch points; subtract value from t-Araf
	t-Ara	t-Ara not already assigned; Equivalent to branch points less t-Araf; subtract value from t-Ara
	t-Gal	t-Gal not already assigned to AG II; Equivalent to branch points less t-Araf and t-Ara; subtract value from t-Gal
	t-GlcA	t-GlcA not already assigned to AG II; Equivalent to branch points less t-Araf, t-Ara and t-Gal; subtract value from t-GlcA
	t-Fuc	Equivalent to branch points less t-Araf, t-Ara, t-Gal and t-GlcA; subtract value from t-Fuc
	t-Xyl	Equivalent to branch points less t-Araf, t-Ara, t-Gal and t-GlcA and t-Fuc; subtract value from t-Xyl
		Total RG I/II
Heteroxylan (HX) (Pettolino et al., 2012)	4-Xyle	All 4-Xyl
	2,4-Xyle	All 2,4-Xyl
	3,4-Xyle	All 3,4-Xyl
	2,3,4-Xyle	All 2,3,4-Xyl
	t-Glu A ^e	t-GluA not already assigned to AG II and RG I/II (must be ≤ total branch points); subtract value from t-GluA
	t-Araf ^e	t-Araf not already assigned to Arabinan, AG I and RG I/II (must be ≤ total branch points); subtract value from t-Araf
		Total HX

Table 2.4. Calculation of polysaccharide composition based on linkage analysis^a (continued)

Polysaccharide	Expected Linkages	Monosaccharide Linkage Assignment
Heteromannan (HM) (Pettolino et al., 2012)	4-Man	All 4-Man
	4,6-Man	All 4,6-Man
	4-Glc	Equivalent to 4-Man (must be $\leq$ total 4-Glc); subtract from 4-Glc
	4,6-Glc	Equivalent to 4,6-Man (must be $\leq$ total 4,6-Glc); subtract from 4,6-Glc
	t-Gal	t-Gal not already assigned to AG II and RGI/II (must be $\leq$ total branch points); subtract value from t-Gal Total HM
Xyloglucan (XG) (Pettolino et al., 2012)	4,6-Glc	All remaining 4,6-Glc
	4-Glc	Equivalent to 4,6-Glc (must be $\leq$ total 4,6-Glc remaining)
	2-Xyl	All 2-Xyl (must be $\leq$ branch points)
	2-Gal	All 2-Gal (must be $\leq$ 2-Xyl)
	t-Fuc	t-Fuc not already assigned to RG I/II (must be $\leq$ 2-Gal); subtract from t-Fuc
	t-Xyl	t-Xyl not already assigned to RG I/II; Equivalent to branch points less t-Fuc; subtract from t-Xyl Total XG
Yeast Glucan (Kwiatkowski et al., 2009)	3-Glc	All 3-Glc
	4-Glc	All remaining 4-Glc
	6-Glc	All 6-Glc
	3,6-Glc	All 3,6-Glc
	t-Glc	Equivalent to branch points; subtract value from t-Glc Total Glucan
Yeast Mannan (Cuskin et al., 2015)	6-Man	All 6-Man
	2,6-Man	All 2,6-Man
	3,6-Man	All 3,6-Man
	2-Man	All 2-Man
	3-Man	All 3-Man
	t-Man ^g	Equivalent to branch points; subtract from t-Man Total Mannan
Unassigned	Sum of unassigned linkages	

^aLinkages found in literature

*This calculation differs from Pettolino et al. (2010): 1) Pettolino et al. (2012). assigned 4-GalA to HG and RGs, 2) Here, all 4-GalA were assigned to HG because there are too many linkages specific to RGs found in grapes and wine. These linkages are not reported in Pettolino et al. (2010).

**This is difficult to calculate. There are a lot of details around the RGs linkages that are specific to grapes and wine (Doco and Brillouet, 1993; Pellerin et al., 1996; Vidal et al., 2001; Vidal et al., 2003). Therefore, all non-terminal linkages that are previously identified in the literature were assigned to RGs in our study. The order of the terminal glycosyl residues calculation is from most abundant to least abundant.

2.7.3. Size Exclusion Chromatography

The method used was adapted from Chong et al. (2009), with modifications. High-performance size exclusion chromatography (HPSEC) was performed on an Agilent 1200 isocratic liquid chromatograph that was equipped with a refractive index detector and a 7.8 mm x 300 mm Ultrahydrogel 250 column (Waters Corp., MA, USA), which gave the best separation of the SCWCs in the wine samples. The total run time was 40 min at a flow rate of 0.4 mL/min, using an eluant of 50 mM ammonium formate, pH 5.5. The polysaccharide size distribution profile of the sample was compared with the elution time of peaks of dextran calibration standards (Sigma-Aldrich, MO, USA) with molecular weights of 410,000, 150,000, 50,000, 25,000, 12,000 and 5,000. For best peak resolution, 1 mg of the dextran standards and 2 mg of the samples were dissolved in 1 mL of the eluant, filtered through a 0.45 µm syringe filter into a 1.8 mL auto-sampler vial (VWR, PA, USA), capped and loaded on the auto-sampler for analysis.

2.8. Wine Sensory Evaluation

2.8.1. Preliminary Tasting

A preliminary tasting of the wines was conducted in an open-plan sensory laboratory, to ensure that the wines were free of faults and met the criteria for descriptive analysis established by Consumer and Product Insights of E & J Gallo Winery, CA, USA. Wines were assessed by a panel of five employees, who had extensive wine tasting experience. During the preliminary tasting session, the employees evaluated all wines in order of increasing ripening levels. Based on the outcome of the bench tasting, 24 Chardonnay white wines from seven subsets of different ripening levels (each subset had four technical replicates) with detectable sensory differences were selected for wine descriptive analysis. These Chardonnay white wines were from WAV 6, 7, 8, 9, 11 and 12. The subsets of wines from WAV 10 were excluded from the wine descriptive analysis because none of the employees were able to detect any differences compared with wines of ripening levels a week shorter or longer. The same evaluation procedure was applied to the Cabernet Sauvignon red wines, and

based on the outcome of the bench tasting, 28 Cabernet Sauvignon red wines from nine subsets of different ripening levels (each subset had four technical replicates) with detectable sensory differences were selected for wine descriptive analysis. These Cabernet Sauvignon red wines were from WAV 9, 11, 13, 14, 15, 16 and 17. The subsets of wines from WAV 10 and 12 were excluded.

2.8.2. Wine Descriptive Analysis

The wine descriptive analysis at E & J Gallo Winery was performed by professionally trained sensory panelists

Table 2.5. Mouthfeel (M), taste (T) and aroma (A) attribute list with definitions and reference standards*

Attribute	Definition	Reference Standard*
M-Astringency	Drying sensation on tongue and pulling in and tightening of cheeks and mouth surfaces, sensed without moving tongue over surfaces	Grape seed extract
M-Burn	Burning/warming sensation on surfaces of mouth; heat	Grain neutral spirits
M-Chalky	Subcategory of mouth coating, chalky, powdery coating perceived after expectoration.	Milk of Magnesia
M-Coating	Any sensation that a coating has been left on the surfaces of the mouth after expectoration, especially sensed by moving tongue over surfaces of the mouth, roof, teeth, and gums	
M-Roughness	Roughness of mouth surfaces, sensed by running tongue over surfaces, especially roof of mouth, includes roughening of tongue's surface	Concentrated juice from Zinfandel ^a
M-Viscosity	Thickness, weight of wine, increase or decrease in force necessary to move tongue through liquid, must be evaluated while wine is in mouth. Includes syrupy mouthfeel	Gum Arabic ^b
M-Overall Sensation in Mouth	Includes the mouth feel sensations of chemical feeling factors (alcohol burn, astringency and spritz) and texture (mouth roughness, viscosity and mouth coating); may also include basic taste sensations but not the actual taste; what you feel when wine is in your mouth, what sensations you have on the surfaces of your mouth after expectoration; an overall perception of what is felt in the mouth	
T-Sweet	Sweet taste	Sucrose
T-Sour	Sour/acidic taste	Tartaric acid
T-Bitter	Bitter taste	Caffeine
A-Total Fruit	Fruit	
A-Fruit	Fruit	
A-Citrus	Lemon, lime, orange	Lemon juice, lime juice, orange juice
A-Tree/Stone Fruit	Pear, apple, peach, apricot	Pear nectar, apple juice, peach nectar, apricot nectar
A-Green	Fresh green, stemmy, cut grass, bell pepper	wheatgrass, tomato stems, green bell pepper

*Standards are presented in E & J Gallo Winery commercial white and red wines

*Standards are presented in E & J Gallo Winery commercial white and red wines

In this study, nine professional sensory panelists were trained over 32 sessions (4 h each) during nine weeks to identify and rate wine sensory attributes comprising mouthfeel, taste and aroma through the presentation of reference standards and tasting a series of wines with different sensory profiles. All panelists attended a mandatory orientation session the day before the descriptive analysis for introduction to representative samples from the wine set and to calibrate the mouthfeel and taste descriptors using the reference standards (Table 2.5). All 52 wines (Chardonnay and Cabernet Sauvignon) were evaluated in two repetitions, yielding a total of 104 tasting samples, which were evaluated within a two-week period. Approximately 60 mL of each wine sample was served at room temperature in a black glass covered with a plastic lid an hour before the evaluation. Each glass was labeled with a random three-digit code and presented blind to the panelists in a balanced rotation, one at a time. Water and unsalted crackers were available for palate cleansing between samples. Intensity scores were assigned by the panelists to the attributes of each sample based on the universal wine ballot 15-point universal scale (anchored). Sensory data were recorded using SIMS software (Solana Beach, CA, USA).

2.9. Statistical Analysis

Chemical and sensory data were subjected to one-way analysis of variance (ANOVA) with a post hoc Tukey's test using SPSS version 21 (IBM, 2012). Principal component analysis (PCA) was performed using XLSTAT version 2015.1.03.16174 (Addinsoft, 2015).

2.10. Summary

As noted in the Introduction to this Chapter, detailed protocols used throughout the thesis have been described here. However, minor variations to these methods were sometimes required and special calculations were found to be necessary when dealing with very low molecular weight SCWCs. The variations in methods are outlined in Chapters 3, 4 and 5.

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Chapter 3

Soluble Cell Wall Carbohydrates and Their Relationship with Sensory Attributes in Cabernet Sauvignon Wine

Preface

In accordance with University of Adelaide thesis guidelines, this Chapter is presented in manuscript format. Here, the Materials and Methods section, together with calculations associated with linkage analyses, are described at the level expected in common plant and food journals. Full details of the materials and precise descriptions of the methods used are provided in Chapter 2 of the thesis (Development and Adaptation of Methods). Some minor repetition was therefore necessary between the two chapters in these materials and methods sections. Formats for the text, referencing, figures and tables have been standardized throughout the thesis. Sections and subsections have been numbered as required for a thesis.

Soluble Cell Wall Carbohydrates and Their Relationship with Sensory Attributes in Cabernet Sauvignon Wine

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Keywords: grapes; mouthfeel; red wine; polysaccharides; sensory evaluation; taste; yeast

Abstract

The chemical and sensory profiles of wines prepared from Cabernet Sauvignon grapes at different ripening stages vary greatly. Here, the soluble cell wall carbohydrate (SCWC) and phenolic profiles of wines were analyzed in parallel with the sensory evaluation of their mouthfeel and taste characteristics. Both SCWCs and phenolic compounds correlated with wine mouthfeel. Ten types of grape- and yeast-derived SCWCs were identified by linkage analysis and the sensory data indicated that mouthfeel was affected similarly by SCWCs and phenolic compounds. When analyses were extended to specific classes of cell wall carbohydrates, it was shown that rhamnogalacturonan I/II, arabinan, arabinogalactan types I and II and xyloglucan from grapes were the key determinants of overall mouthfeel descriptors, particularly viscosity, astringency and roughness, whereas heteromannan from grapes was associated with mouth coating and chalkiness. A perceived sour taste was notably associated with higher homogalacturonan contents. This finding provides insights into the contributions of non-phenolic compounds to wine mouthfeel. The data provide opportunities for the development of simple monosaccharide marker assays, such as galacturonic acid content, to monitor major mouthfeel characteristics in red wines.

3.1. Introduction

Wine consumption is driven by consumer expectations of quality and sensory attributes. The sensory aspects of wine, particularly mouthfeel, have become integral discriminating quality criteria for consumers in a competitive market (Gawel et al., 2000; Vidal et al., 2004b; Bruwer et al., 2011). Astringency is a common wine mouthfeel sensory descriptor and is associated with descriptions conveying a wide range of sensations, including drying, roughing, harsh and puckering (Vidal et al., 2004a). Tannins are believed to be the key compounds affecting astringency, and this relationship has been studied extensively over the past three decades (Casassa and Harbertson, 2014). However, complex sensory experience and tannins cannot exclusively explain sensations such as 'body', 'smoothness' or 'fullness' (Bruwer et al. (2011) experienced during wine consumption, which suggests that other factors contribute to these sensations.

Soluble cell wall carbohydrates (SCWCs) in wine, which are derived from the cell walls of both grapes and yeast, are of great interest because of their organoleptic properties (Vidal et al., 2000; Vicens et al., 2009). Research on wine SCWCs has recently increased with the recognition that the abundance of SCWCs in wine is similar to that of tannins (Ducasse et al., 2010a; Quijada-Morín et al., 2014). In addition, some characteristics of wine SCWCs are similar to those of commercial polysaccharides, such as pectin isolated from citrus, whose hydrocolloid properties improve the mouthfeel and texture of foods (Vidal et al., 2003b) and beverages (Gil et al., 2012). SCWCs in wine are mostly non-cellulosic polysaccharides and include complex and heterogeneous polymers such as rhamnogalacturonan I (RG I), rhamnogalacturonan II (RG II), xyloglucans, arabinogalactan (AG), and arabinogalactan-protein (AGP) (Vidal et al., 2003b). These polysaccharides significantly increase the 'fullness' sensation and can potentially add 'mellowness' to wines (Vidal et al., 2004b). Soluble wine polysaccharides from grape are stable and can remain at their initial levels 10 years after bottling (Doco et al., 1999). It is also important to point out that some SCWCs will be derived from yeast used during wine fermentation. Yeast cell wall polysaccharides include mannans, mannoproteins and a variety of glucans. While many of the polysaccharides from grape and yeast cell walls will be insoluble

or only partly soluble in the cell wall itself, it is essential to take these into account, because their partial depolymerization during wine production could decrease their molecular size and hence render them soluble in aqueous media, and hence in the wine.

While both the composition and structure of polysaccharides are believed to correlate with wine mouthfeel (Vidal et al., 2004a), the elucidation of the role of SCWCs has been limited by a lack of rigorous studies linking polysaccharide chemistry and wine sensory profiles, as well as the difficulty of isolating and characterizing wine polysaccharides and derived oligosaccharides. In addition, more comprehensive sensory evaluations of wine must be conducted using a wider array of mouthfeel descriptions rather than being limited to astringency (Gawel et al., 2000). Thus, further work is needed to assess and better understand the role of SCWCs in wine mouthfeel.

Phenolic compounds, which include anthocyanins, flavanols, phenolic acids and stilbenes (Li et al., 2011), have also been implicated in the mouthfeel of wines, together with a variety of other wine components (Hufnagel and Hofmann, 2008b; Versari et al., 2013). However, no comprehensive studies have been undertaken on the sensory effects of a complete set of well-defined grape and yeast compounds that are actually present in the final wine.

The goal of this study was therefore to investigate any correlations between grape and yeast compounds with mouthfeel and taste, with a focus on the individual constituents of the SCWCs found in Cabernet Sauvignon wine. The data show clear correlations between various mouthfeel descriptors and certain key classes of specific SCWCs.

3.2. Materials and Methods

3.2.1. Winemaking

Cabernet Sauvignon grapevines (*Vitis vinifera* L.) grown in a commercial vineyard near Lodi, California (38° 10' 36.43" N, 121° 14' 27.51" W) were used in this study, which was performed in 2012. The vines were planted in 1995 on 5C rootstock, spaced 1.8 m x 3.3 m (within row x between row spacing) and trellised to a horizontally divided system. Standard viticulture practices for the variety

and region were followed. The field trial employed a randomized complete block design. Nine different harvest dates (treatments) were replicated four times using seven-vine plots. Randomly selected 20 middle grape clusters from the middle five vines in each plot of each replicate were collected weekly beginning at nine weeks after veraison (WAV) to two weeks post-commercial harvest. Fruit was contained in ziplock bags and transported to the laboratory in insulated ice coolers for basic chemical analysis. One set of vines (treatment) was harvested weekly for micro-vinification. Approximately 50 kg of fruit from each replicate plot was collected at the same time for micro-vinification and was stored overnight at 4 °C for processing at the research winery the next day.

Fruits from each replicate were crushed with a Magitec A15DC destemmer-crusher (Helmut Amos, Paarl, South Africa) into an 80 L stainless steel fermentation tank with an adjustable-height lid designed for a submerged cap system. Sulfur dioxide was added to the must at a rate of 60 mg/L immediately after destemming. To standardize the alcohol level for wines made from grapes of different ripening levels, the must sugar levels were either increased to 24 °Brix with the addition of a 2 parts glucose:1 part fructose mixture (approximately 333 g of glucose and 168 g of fructose dissolved in 1 L of water) or diluted to 24 °Brix with water. Diammonium phosphate (DAP) was added to supplement the Yeast Assimilable Nitrogen (YAN) to 300 mg/L, and tartaric acid was added to adjust the pH to 3.6 before inoculation with D254 commercial yeast (Scott Laboratories, CA, USA) at a rate of 0.18 g/L. Fermentation was conducted at 25 °C, and the grape skins were resubmerged into the fermenting juice once a day, commonly referred to as “punch down”. The must was pressed to separate out pomace when the reducing sugar decreased below 2 g/L, using a Diemme GB00C299181 membrane press (Diemme Enologia, Emilia-Romagna, Italy). The resulting wine was cold settled at 2 °C for two weeks prior to filtration using a 1.0 µm pad filter, and a Meissner 0.8 µm and 0.45 µm dual-cartridge filter system, and bottled into 750 ml screw cap glass bottles, which were stored at 13 °C.

3.2.2. Chemical Analysis

The percent alcohol, titratable acidity (TA), malic acid and pH of each wine were analyzed using a WineScan FT-120 Fourier Transform Infrared Spectroscopy (FTIR) (FOSS North America, MN, USA). Potassium (K) levels were determined using a PerkinElmer Optima 7000 DV inductively coupled plasma with optical emission spectroscopy (ICP-OES) (PerkinElmer Inc., MA, USA).

3.2.3. Phenolic Analysis

The total phenol content was determined by the Folin–Ciocalteu Assay (Singleton et al., 1999) and the phenol composition was analyzed using an Agilent 1200 reversed phase high-performance liquid chromatography (HPLC) with a diode array detector and a 4.6 X 50 mm Zorbax Eclipse Plus C18 column (Agilent, CA, USA). HPLC was performed using a gradient mobile phase system of acidified water (0.2 % v/v phosphoric acid) and acetonitrile at a flow rate of 1 mL/min (Waterhouse et al., 1999). These methods were described in detail in Chapter 2.

3.2.4. SCWC Preparation

An aliquot of 200 mL wine from each replicate was mixed with 1 L of ethanol and stored at 4 °C overnight. The precipitate, which is referred to as the alcohol-insoluble residue (AIR), was recovered by centrifugation at 6577 x g for 10 min washed twice with ethanol and dried under nitrogen. The dried AIR was dissolved in water and dialyzed (100-500D MWCO tubing, Spectrum Laboratories, CA, USA) against water for 24 h at 22 °C before lyophilization and weighing.

A composite sample was prepared from 10 mg AIR materials from each of the four replicates. The sample was suspended in phenol saturated with 500 mM Tris-HCl buffer, pH 7, agitated for 60 min and centrifuged at 6863 x g for 10 min to recover the pellets. This procedure was repeated twice and the pellets were successively treated with ethanol, acetone and chloroform:methanol (1:1, v/v) with continuous mixing for 60 min each. A sintered glass funnel (Ace Glass Inc., NJ, USA) was used to remove the solvents from the pellets. The treated pellets were finally washed with acetone, dried under nitrogen and stored in a desiccator for glycosyl linkage analysis.

3.2.5. Glycosyl Linkage Analysis

This analysis was performed by the Complex Carbohydrate Research Center (CCRC) (Georgia, USA) according to the methods of Ciucanu and Kerek (1984), York et al. (1985) and Heiss et al., (2012). The method is described in detail in Chapter 2. The SCWC sample (1 mg) was treated with 0.5 M methanolic HCl at 80 °C for 20 min, followed by the addition of NaBD₄ at room temperature. The sample was methylated using NaOH suspended in DMSO, and methyl iodide. Following methylation, the sample was extracted into dichloromethane, hydrolyzed using 2 M TFA at 121 °C for 2 h and reduced with NaBD₄. The hydrolyzed, reduced sample was acetylated with acetic anhydride. The partially methylated alditol acetates (PMAAs) were analyzed on a capillary column by GC-MS. The quantification of each PMAA was converted to mol %, which was used for polysaccharide assignment as described in Pettolino et al. (2012) and in Chapters 2. Polysaccharide assignments were calculated with reference to well-characterized grape, wine and yeast SCWCs (Pellerin et al., 1996; Nunan et al., 1998; Kwiatkowski et al., 2009; Cuskin et al., 2015).

3.2.6. Size Exclusion Chromatography

High-performance size exclusion chromatography (HPSEC) was performed on an Agilent 1200 liquid chromatograph with a refractive index detector and a 7.8 mm x 300 mm Ultrahydrogel 250 column (Waters Corp., MA, USA) as described in Chapter 2. The flow rate was 0.4 mL/min with an eluent of 50 mM ammonium formate, pH 5.5. The polysaccharide size distribution profile of the samples was compared with the elution time of peaks of dextran calibration standards (Sigma-Aldrich, St. Louis, MO, USA) with molecular weights ranging from 410,000 to 5,000.

3.2.7. Wine Sensory Evaluation

A preliminary tasting was conducted in an open-plan sensory laboratory to ensure that the wines were free of faults and met the criteria for descriptive analysis established by Consumer and Product Insights of E & J Gallo Winery, CA, USA. Wines were assessed by five employees from E & J Gallo Winery who had wine tasting experience. During the preliminary tasting session, the employees evaluated all wines in order of increasing ripening levels. Based on the outcome of the

bench tasting, 28 wines from seven subsets of different ripening levels (each subset had four technical replicates) with detectable sensory differences were selected for wine descriptive analysis. These wines were from WAV 9, 11, 13, 14, 15, 16 and 17. The subsets of wines from WAV 10 and 12 were excluded from the wine descriptive analysis because none of the employees were able to detect any sensorial differences compared with wines of ripening levels a week shorter or longer.

The wine descriptive analysis at E & J Gallo Winery was performed by professional sensory panelists. Nine sensory panelists were trained over 32 sessions (4 h each) over 9 weeks to identify and score wine sensory attributes comprising mouthfeel, taste and aroma through the presentation of reference standards and tasting wines with different sensory profiles. All sensory panelists attended an orientation session the day before the wine descriptive analysis (DA) for introduction to representative samples from the Cabernet Sauvignon wine set and to calibrate the mouthfeel and taste descriptors using the reference standards (Supplementary Table S3.1). All 28 wines were evaluated in two repetitions, yielding a total of 56 tasting samples. Approximately 60 mL of each wine sample was served at room temperature in a black glass covered with a plastic lid. These samples were prepared an hour before the evaluation. Each glass was labeled with a random three-digit code and presented blind to the panelists in a balanced rotation one at a time. Water and unsalted crackers were required for palate cleansing between samples. Intensity scores were assigned by the panelists to the attributes of each sample based on the universal wine ballot 15-point universal scale (anchored). Sensory data were recorded using SIMS software (Solana Beach, CA, USA).

3.2.8. Statistical Analysis

Chemical and sensory data were subjected to one-way analysis of variance (ANOVA) with a post hoc Tukey's test using SPSS version 21 (IBM, 2012). Principal component analysis (PCA) was performed using XLSTAT version 2015.1.03.16174 (Addinsoft, 2015).

3.3. Results and Discussion

3.3.1. Wine Chemistry

Commonly used chemical analyses of the wines are presented in Table 3.1. The alcohol levels and pH values did not differ significantly among wines from any of the stages of berry development examined here. The alcohol levels remained the same for all wines because their must sugar levels were adjusted to the same level before fermentation, to minimize the influence of the alcohol concentration on the sensory evaluations (Fischer et al., 1994). Similarly, the pH of the must was adjusted by adding tartaric acid and thus there were differences in titratable acidity (TA) but not pH among the wines (Table 3.1). Malic acid decreased from 1.88 g/L on WAV 9 to 1.18 g/L on WAV 14 but increased slowly thereafter to a final value of 1.45 g/L. The general decline in the malic acid content during ripening was consistent with other studies (Hrazdina et al., 1984). Potassium concentrations fluctuated between 1.41 g/L and 1.49 g/L from WAV 9 to WAV 14 but increased to greater than 1.60 g/L at WAV 15 and remained the same until WAV 17. The potassium concentrations of wines were comparable with the reported concentration range of commercial Californian red wines (Berg et al., 1979).

Table 3.1. Wine chemistry

WAV	Alcohol (% v/v)	TA (g/L)	pH	Malic Acid (g/L)	K (g/L)
9	13.6 ± 0.2	5.6 ± 0.1ab	3.72 ± 0.04	1.88 ± 0.14c	1.41 ± 0.09a
10	13.6 ± 0.1	5.7 ± 0.2ab	3.78 ± 0.02	1.53 ± 0.15b	1.57 ± 0.03ab
11	13.7 ± 0.2	6.0 ± 0.5b	3.74 ± 0.04	1.45 ± 0.12ab	1.54 ± 0.07ab
12	13.6 ± 0.2	5.9 ± 0.2ab	3.76 ± 0.04	1.41 ± 0.16ab	1.57 ± 0.10ab
13	13.7 ± 0.1	5.8 ± 0.1ab	3.76 ± 0.02	1.39 ± 0.18ab	1.55 ± 0.02ab
14	13.7 ± 0.2	5.8 ± 0.2ab	3.70 ± 0.08	1.18 ± 0.17a	1.49 ± 0.12ab
15	13.7 ± 0.2	6.1 ± 0.1b	3.78 ± 0.04	1.35 ± 0.13ab	1.62 ± 0.07b
16	13.6 ± 0.1	6.0 ± 0.2b	3.75 ± 0.02	1.46 ± 0.11ab	1.62 ± 0.06b
17	13.6 ± 0	5.4 ± 0.1a	3.74 ± 0.06	1.45 ± 0.03ab	1.63 ± 0.03b

Values are the mean of 4 technical replicates. Values followed by different letters indicate a significant difference ($P \leq 0.05$, one-way ANOVA, Tukey's posthoc)

3.3.2. SCWC Content and Total Phenols

In this experiment (Figure 3.1), neither the SCWC content nor the total phenols varied with respect to soluble solids or TA (data not shown), the general parameters commonly used to assess

ripeness. The SCWC content remained at approximately the same level for the first three weeks and dropped to its lowest level at WAV 12 before increasing steadily to peak on WAV 16. By contrast, the total phenols increased rapidly from WAV 9 to 10, dropped to the lowest level on WAV 12, and peaked at WAV 15 (Figure 3.1). In general, the SCWC content exhibited greater variation than the total phenols during ripening and might therefore be expected to have a greater impact on differences in wine sensory characteristics. Notably, both the SCWC content and the total phenols dropped drastically in the last wine sample to a level nearly identical to that in the first wine sample, suggesting that ripening of the grapes did not translate to higher SCWC availability. Previous studies have reported that, during fruit ripening, the molecular mass, solubility and structure of various polysaccharides frequently change without a significant change in cell wall polysaccharide content (Fischer and Bennett, 1991; Nunan et al., 1998).

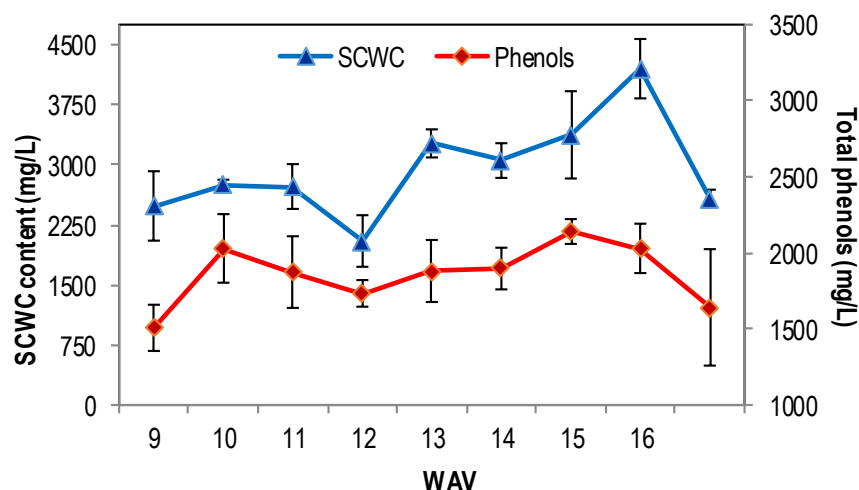


Figure 3.1. Comparing SCWC content and total phenols in wines

3.3.3. Quantification of Phenolic Compounds

Of the phenolic compounds measured, only polymeric tannin and quercetin glycosides occurred at concentrations above their sensory recognition thresholds for astringency; these thresholds are 22 mg/L and 1 mg/L, respectively (Supplementary Table S3.2) (Hufnagel and Hofmann, 2008a). The concentrations of the polymeric tannins were the highest by far, with levels of 576 – 746

mg/L between WAV 10 and WAV 16. In contrast, the levels of quercetin glycosides ranged from 14 – 23 mg/L during the same period. Both compounds increased rapidly in the wine early in the season and thereafter remained at a relatively constant level before decreasing markedly during the final two weeks of ripening. Differences in the wine phenolic profile were affected by the physiological stages of the fruit, generally resulting in changes in extractable phenolic compounds, solubility and degradation (Gil et al., 2012).

3.3.4. Monosaccharide Composition and Linkage Analysis

The monosaccharide compositions of the wine polysaccharides were calculated from the methylation data (Table 3.2; Pettolino et al., 2012) and showed that GalA, Rha, Ara and Gal were the most abundant monosaccharide residues. These results are consistent with those previously reported for red wines (Vidal et al., 2003b; Ducasse et al., 2010b).

Table 3.2. Glycosyl residue composition (mol %) of the wine polysaccharides

Glycosyl Residue	WAV								
	9	10	11	12	13	14	15	16	17
Gal	7.1	10.6	10.9	6.1	5.0	7.6	14.0	13.9	20.7
Glc	5.1	3.6	3.5	3.2	2.9	3.7	4.7	10	9.6
Rham	6.3	7.8	8.5	11.9	16.8	12.3	10.8	10.9	17.9
Xyl	0.6	0.2	0.1	0	0.4	0	0.4	0.5	0.3
Ara	0	2.6	2.0	6.3	15.2	7.0	3.9	2.6	4.1
Man	5.2	4.9	6.1	2.9	2.3	2.9	8.1	6.7	10.7
GalA	75.5	68.2	66.8	65.4	56.0	65.0	55.1	52.5	31.9
GlcA	0.2	1.6	1.7	1.1	1.4	1.5	2.4	2.5	4.0
Fuc	0	0.5	0.4	3.1	0	0	0.6	0.4	0.8

0, not detected

Methylation data were used to calculate the molar percentages (mol %) of individual glycosyl residues and their linkage types, as outlined in Pettolino et al. (2012), and these are presented in Table 3.3. The most abundant residues are derivatives of Rha and GalA, which are clearly derived from pectic polysaccharides in the grape berries (Table 3.3). In this study, 4-Ara and 5-Ara residues co-eluted in the chromatogram of the linkage analysis. Here, the value is attributed to 5-Ara residues because it is known that arabinan containing 5-Ara residues is commonly found in the side chains of pectic polysaccharides (Mohnen, 2008). Analysis of the Gal residues indicates that 4-Galp residues

are low, while 3-Galp, 6-Galp and 3,6-Galp residues are relatively abundant. This suggests that type II arabinogalactans (AG II) of the AGP type are abundant in the wine samples. The high levels of t-Araf residues (where t- indicates non-reducing terminal residues) are consistent with the abundance of pectic polysaccharides and type II AGs. The origin of the Araf residues is not known at this stage.

Table 3.3. Glycosyl residue linkage analysis of SCWCs from Cabernet Sauvignon wines

Glycosyl Residue	Deduced Glycosidic Linkage	Linkage Composition (mol %) / WAV								
		9	10	11	12	13	14	15	16	17
Galactopyranosyl (Galp)	2-	0	0.1	0.1	0.2	0	0	0.1	0.1	0
	3-	0	0.6	0.6	0.5	0	0.3	0.8	0.5	0.4
	4-	0	0	0	0	0	0	0	0	2.4
	6-	0	2.2	1.9	0.4	0	0.7	3.3	2.6	2.4
	2,4-	0	0.1	0.2	0.4	0.2	0	0.1	0.3	0.3
	3,6-	0	1.4	1.1	0	0	0	1.6	1.1	2.1
	4,6-	0	0.2	1.1	0.3	0	0	0.2	0.4	0.4
	Terminal	7.1	6.0	5.8	3.9	4.6	6.3	7.6	8.5	12.4
Glucopyranosyl (Glc p)	2-	0	0.1	0.1	0	0	0	0.1	0.2	0.4
	3-	0	0	0.1	0	0	0	0.2	0.7	0.2
	4-	2.0	0.9	0.6	0.8	0.6	0.7	0.3	3.7	2.6
	6-	0	0.4	0.6	0.6	0.3	0.3	0.6	1.1	0.9
	2,3-	0	0	0	0	0	0	0	0	0.1
	3,4-	0	0	0	0	0	0	0	0.4	0.2
	3,6-	0	0	0.1	0	0	0	0.2	0	0.6
	4,6-	0	0	0.1	0	0	0	0	0.8	0.6
Rhamnopyranosyl (Rhap)	Terminal	3.1	2.2	1.9	1.8	2.0	2.7	3.3	3.1	4.0
	2-	6.3	4.7	5.5	5.7	9.9	7.6	6.6	7.7	11.8
	4-	0	0.3	0.4	1.2	0.5	0.2	0.3	0.6	0.8
	2,3-	0	0.3	0.4	1.2	0.6	0.3	0.4	0.6	0.6
	2,4-	0	0.6	0.8	1.0	1.2	0.4	0.7	0.6	1.7
	Terminal	0	1.9	1.4	2.8	4.6	3.8	2.8	1.4	3.0
Xylopyranosyl (Xylp)	4-	0.6	0.2	0.1	0	0.4	0	0.2	0.5	0.3
	Terminal	0	0	0	0	0	0	0.2	0	0
Arabinopyranosyl (Arap)/ Arabinofuranosyl (Araf) ^a	4- / 5-	0	0.3	0.2	0	0	0.2	0.3	0.3	0.3
	Terminal(p)	0	1.5	1.2	4.8	10.6	3.5	2.1	1.6	2.6
	Terminal(f)	0	0.8	0.6	1.5	4.6	3.3	1.5	0.7	1.2
Mannopyranosyl (Manp)	4-	0	0	0.3	0	0	0	0.1	0.4	0.3
	6-	0	0	0.1	0	0	0	0.1	0.1	0.3
	2- / 3-	2.2	1.7	2.2	0	0	0.5	2.9	2.0	2.9
	2,3-	0	0	0.3	0	0	0	0.2	0	0.4
	2,6-	0	0.2	0.2	0	0	0	0.2	0	0.3
	3,6-	0	0	0	0	0	0	0.1	0	0.1
	Terminal	3.0	3.0	3.0	2.9	2.3	2.4	4.5	4.2	6.4
	Galacturonopyranosyl ^b (GalAp)									
	2-	0	0.2	0.2	0.6	0.3	0	0.1	0.2	0
	3-	0	0.8	0.8	1.0	0	0	0.3	0.7	0.3
	4-	46.1	38.2	41.8	41.0	38.7	35.2	29.2	32.4	19.7
	2,4-	0	0.3	0.4	1.5	0.9	0.5	0.2	0.4	0.4
	3,4-	2.2	1.7	2.7	6.1	3.1	2.1	0.9	2.3	2.1
	Terminal	25.9	25.5	20.1	13.4	11.8	25.8	22.0	15.7	7.7

^a 4-Arap and 5-Araf peaks co-eluted. The value here was represented by 5-Araf

^b Uronic acids were reduced to 6,6'-dideuterio neutral sugars before methylation

0, not detected

Table 3.3. Glycosyl residue linkage analysis of SCWCs from Cabernet Sauvignon wines (continued)

Glycosyl Residue	Deduced glycosidic linkage	Linkage Composition (mol %) / WAV								
		9	10	11	12	13	14	15	16	17
Glucuronopyranosyl ^b (GlcAp)	2-	0	0.1	0.1	0	0	0	0.1	0.1	0.4
	3-	0	0	0	0	0	0	0	0.2	0
	4-	0.2	0.2	0.2	0.3	0	0.1	0.2	0.5	0.5
	Terminal	0	1.3	1.4	0.8	1.4	1.4	2.1	1.7	3.1
Fucopyranosyl (Fucp)	Terminal	0	0.5	0.4	3.1	0	0	0.6	0.4	0.8
Galactofuranosyl (Gal _f)	Terminal	0	0	0.1	0.4	0.2	0.3	0.3	0.4	0.3
Galacturonofuranosyl (GalAf)	Terminal	1.3	1.5	0.8	1.8	1.2	1.4	2.4	0.8	1.7

^a 4-Arap and 5-Araf peaks co-eluted. The value here was represented by 5-Araf

^b Uronic acids were reduced to 6,6'-dideuterio neutral sugars before methylation

0, not detected

Examination of the Man residues shows that the major contributors to mannans in the wine are yeast (1,2/1,3;1,6)- α -linked mannans (as described in Chapter 1), although some (1,4)-mannans from the grape cell walls are likely to be present (Table 3.3). The high levels of 2-Man_p and 3-Man_p are indicators of the presence of oligo-mannans attached to the 6-mannan backbone, as described in Chapter 1.

It is also clear from these data that the levels of some plant cell wall polysaccharides and their oligosaccharide degradation products are quite low in the SCWC samples. Thus, the low levels of 4-Xyl_p suggest that the levels of heteroxylan products are low. Similarly, the low levels of t-Xyl_p indicate that products derived from xyloglucans (XG) are also in low abundance in the SCWC samples.

However, 4-Glc_p residues are present, albeit in relatively low abundance (Table 3.3), and these might have originated from yeast (1,4)- α -glucan. It has been reported that this polysaccharide is present on the outer layer of fungal cell walls and is more soluble compared with the β -glucans that form the rigid core of fungal cell walls (Barreto-Bergter and Figueiredo, 2014). Kwiatkowski et al. (2009) indicated that (1,4)- α -glucan covalently bound to (1,6)- β -glucan in *Saccharomyces cerevisiae* cell walls. Therefore, the 4-Glc_p residues are assigned to yeast glucan (Table 3.4), along with 3-Glc_p, 6-Glc_p and 3,6-Glc_p residues. It is acknowledged that 4-Glc_p residues might also have originated from either degraded XG or cellulose.

Finally, it is clear from the methylation analyses in Table 3.3 that terminal residues of many monosaccharides are relatively high. This suggests that the SCWCs of the wine are of low molecular weight and/or highly branched. This is discussed in more detail below in the size exclusion chromatography section 3.3.6.

3.3.5. Estimation of Polysaccharide Composition

One key advantage of the linkage analyses performed here is that they allow the estimation of the classes of polysaccharides present in the wine and/or the origin of oligosaccharides, using methods that take advantage of known structures of commonly occurring cell wall polysaccharides (Nunan et al., 1998; Caffall and Mohnen, 2009; Burton et al., 2010; Pettolino et al., 2012). Thus, estimates of polysaccharide compositions of the SCWCs in the Cabernet Sauvignon wine were deduced from the linkage analyses based on the summation of the mol % of individual glycosyl residues using the linkage structural characteristics described in the literature (Doco and Brillouet, 1993; Nunan et al., 1998; Kwiatkowski et al., 2009; Pettolino et al., 2012). In particular, the estimates were based on the procedure detailed by Pettolino et al. (2012). This method is also described in Chapter 2 of this thesis. The results are shown in Table 3.4.

Some of the glycosyl linkage types could not be readily assigned to well-characterized cell wall polysaccharides from yeast and plants, and are therefore listed as “Unassigned” in Table 3.4. This “Unassigned” component shown in Table 3.4 is commonly observed when glycosyl linkage analyses are used to estimate polysaccharide composition, although values of about 10 % or less are usually found (Pettolino et al., 2012). The higher values listed in Table 3.4 could result from the extreme complexity of the SCWC mixture, from under-methylation, or from artifacts generated during the chemical steps of the analysis.

During the course of the initial calculations, based on Pettolino et al. (2010), there were an unusually high number of unassigned residues, in particular *t*-Galp, *t*-Glc_p, *t*-Man_p and *t*-GalAf. The source of the *t*-GalAf is not known at this stage, and may be an artifact of the method. For the unassigned *t*-Galp, *t*-Glc_p and *t*-Man_p residues, these suggest that the parent polysaccharides were

subjected to extensive depolymerization during the winemaking process. For example, if *Araf* residues were removed from type II AGPs and endo- β -galactanases partially degraded the galactan backbone and branches of the polysaccharide, many additional t-Galp residues would be exposed during the methylation analyses. Similarly, if the polysaccharides chains or oligosaccharide substituents of yeast mannans and mannoproteins were degraded by mannanases during the winemaking process, a large number of t-Manp residues would be exposed. As a result, it is believed that the t-Galp and t-Manp residues originated in the relatively abundant type II AGPs and yeast mannans/mannoproteins, respectively. In the case of the unassigned t-Glcp, it is currently less clear where this originated, although it seems likely that it is a degradation product of yeast glucan.

Table 3.4. Probable sources of SCWCs in Cabernet Sauvignon wines

Polysaccharide ^a	Relative Amount (mol %) / WAV									
	9	10	11	12	13	14	15	16	17	
G-Arabinan	0	0.3	0.2	0	0	0.2	0.3	0.3	0.3	
G-Type I Arabinogalactan (AG I)	0	0.4	1.7	0.6	0	0	0.4	0.8	3.2	
G-Type II Arabinogalactan (AG II)	7.1	11.6	10.5	4.8	4.6	7.3	14.9	13.8	19.4	
G-Homogalacturonan (HG)	72.0	63.7	61.9	54.4	50.5	61.0	51.2	48.1	27.4	
G-Rhamnogalacturonan I/II (RG I/II)	8.5	12.2	13.4	28.6	26.5	18	14.1	16.3	25.4	
G-Heteroxylan (HX)	0.6	0.2	0.1	0	0.4	0	0.2	0.5	0.3	
G-Heteromannan (HM)	0	0	0.6	0	0	0	0.2	0.8	0.6	
G-Xyloglucan (XG)	0	0	0.2	0	0	0	0	1.6	1.2	
Y-Glucan	5.1	3.5	2.9	3.2	2.9	3.7	4.5	7.4	7.4	
Y-Mannan/Mannoprotein	5.2	4.9	5.5	2.9	2.3	2.9	7.8	6.3	10.0	
Unassigned ^b	1.5	3.2	3	5.5	12.8	6.9	6.4	4.1	4.8	

^a G - denotes grape derived SCWCs; Y - denotes yeast derived SCWCs

^b Sum of linkage types that cannot be assigned to well-characterized SCWCs from plants and yeast; 0, not detected

The specific unassigned residues referred to in Table 3.4 are listed in Supplementary Table S3.3. It should be stated again that the methylation data indicate that the SCWCs in wine predominantly represent extensively degraded oligosaccharides from the yeast and grape cell wall polysaccharides. It is also important to point out that many wines have low but measurable levels of bacterial contamination, in particular, lactic acid bacteria such as *Lactobacillus spp.*, and that some of the unassigned residues might originate from these bacteria.

It should also be noted that the complexity and heterogeneity of cell wall polysaccharides from both the grape berry and yeast complicated the unequivocal identification of the types and proportions of the various 'source polysaccharides' in the test samples; the values presented in Table 3.4 should therefore be viewed as estimates only. The complexity also prevented the accurate, independent estimation of the RG I and RG II polysaccharides, so the abundances of these were merged into a single value for rhamnogalacturonans (Table 3.4).

In summary, a total of ten different types of polysaccharides, predominantly acidic pectic polysaccharides, were identified in the wines, namely grape-derived RG I/II and HG, together with smaller amounts of grape- and yeast-derived neutral polysaccharides, such as arabinan, AG I, AG II, HX, HM, XG, yeast glucan and yeast mannan (Table 3.4). The structures of these yeast and grape cell wall polysaccharides are shown in Chapter 1 of this thesis.

On a mol % basis, pectic polysaccharides constituted more than 50 % of the total polysaccharides in the SCWC fractions of all wines and, in all cases, the proportions of HG were greater than those of RG I/II (Table 3.4). Notably, HG decreased steadily from 72 mol % on WAV 9 to 27.4 mol % eight weeks later. By contrast, a temporary increase in RG I/II was observed in the beginning of the sampling period, from 8.5 mol % to a maximum of 28.6 mol % on WAV 12; it decreased steadily thereafter before reaching 25.4 mol % on the last week. The changes in RG I/II content observed in this study following veraison are in agreement with a previous study of a Carignan Noir wine (Vidal et al., 2003b). Wine RG II, which is structurally similar to the RG II found in the primary cell wall of sycamore suspension cultured cells, has been shown to be highly resistant to fermentation conditions and winemaking generally (O'Neill et al., 2004) and is stable at wine pH. The RG II component that is common in red wines can exist as a monomer or a dimer that is cross-linked with borate (Pellerin et al., 1996).

Ara and Gal are the predominant neutral glycosyl residues of the side chains of RG I. The side chains of RG I are composed of 5-arabinan and AG I, and it has been suggested that AG II might also be covalently linked with pectic polysaccharides (Mohnen, 2008; Tan et al., 2013). The AG II was

more abundant than AG I and was present in all wine samples (Table 3.4). Both Ara and Gal residues increased in wines during the later stages of ripening. The side chain ratio of RG I may vary with the physiological state of the plants (Mohnen, 2008) and RG I with fewer side chains is more soluble (Vidal et al., 2001). Arabinan, which has been identified in Carignan Noir, was present in low concentrations in these wines, probably because it is susceptible to enzymic degradation during the winemaking processes (Vidal et al., 2003a).

Table 3.4 shows that only small amounts of the hemicelluloses HM, HX and XG were present in wines and that these remained at very low levels from WAV 9 to WAV 15 before increasing slightly in WAV 16 and WAV 17. Apolinar-Valiente et al. (2015) also reported the presence of only trace amounts of hemicelluloses in red wines. Nunan et al. (1998) also detected HM, HX and XG in grape berries but at a much higher mol %, but these clearly did not survive the winemaking process. There is evidence to suggest that the structure of wine XG is typical of XG in the primary cell walls of dicotyledonous plants and likely cross-links with RG I in the wine matrix (Zabackis et al., 1995; Vidal et al., 2003b).

A significant proportion of the constituents of the SCWC fractions originated from yeast during the fermentation stage of the winemaking process (Table 3.4). Yeast glucan and yeast mannan/mannoprotein contents remained relatively constant in wines initially, but increased greatly after WAV 14. Yeast cell wall polysaccharides accounted for approximately 2 – 10 % of total polysaccharides in the wines. Although the glucan content of the wines was similar to reported values for other red wines such as Carignan Noir and Tempranillo, the overall yeast polysaccharide content was lower than previously reported, presumably because polysaccharide released from yeast is influenced by the specific winemaking technique used here and by the yeast strain (Doco et al., 1999; Vidal et al., 2003b).

3.3.6. Size Exclusion Chromatography

As mentioned above, the high levels of non-reducing terminal residues observed in the linkage analyses (Table 3.3), indicate that the carbohydrates found in the Cabernet Sauvignon wines

studied here were probably derived from oligosaccharides of low molecular weight. For example, for an essentially linear polysaccharide molecule such as cellulose or its derived cellodextrins, the number of 4-Glcp residues divided by the number of t-Glcp residues in the analysis provides an estimate of the degree of polymerization (DP) of the molecule being analysed. However, when a polysaccharide such as the highly branched and substituted RG II is subjected to methylation analysis, it has a very large number of terminal residues that will, if simply divided into the number of backbone residues, lead to the calculation of a very low DP. This value should be more correctly referred to as the average internal chain length of the polysaccharide. The RG II structure shown in the Introduction (Vidal et al., 2001; <https://www.ccruc.uga.edu/~mao/rg2/intro.htm>) has approximately 12 non-reducing terminal residues in a polysaccharide of approximately 29 residues.

To address these points, the molecular sizes of the SCWCs from the Cabernet Sauvignon wines were subjected to high-performance size exclusion chromatography (HPSEC). The elution profiles revealed that, although there were low levels of high molecular weight polysaccharides in the samples, the molecular sizes of the two major populations were much smaller (Figure 3.2).

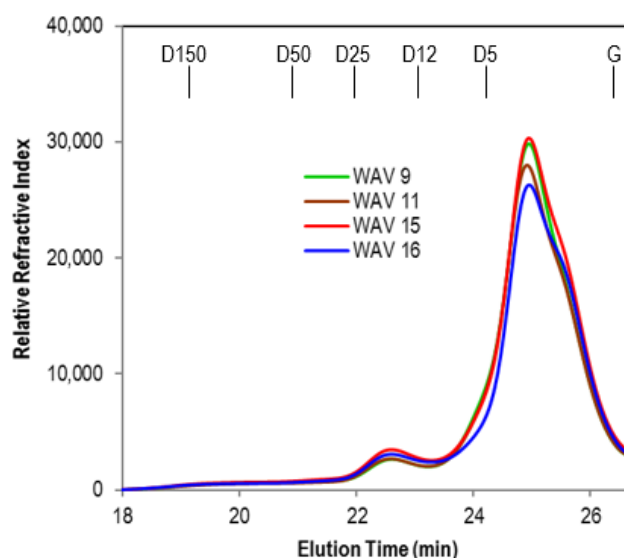


Figure 3.2. The elution profiles of SCWCs from WAV 9, 11, 15 and 16 (other data not shown). Inserts show elution times of dextran standards (D x 1000 MW); G, glucose.

One of these peaks is eluted just before the dextran of 12,000 molecular size. Assuming that the SCWCs and the dextrans have about the same overall conformation and that the anhydro-molecular weight for GalA is 178, this enables a very rough estimate of 60 residues for this peak. However, the second peak was much larger and its elution time was considerably later than the elution time of the smallest dextran standard of 5,000 MW, suggesting that oligosaccharides were present in relatively high abundance in this second peak. If the molecular sizes of these SCWCs were 1,000, then the estimated DP would be about 5. It is important not to over-interpret these data, but they are nevertheless in general agreement with Apolinar-Valiente et al. (2015), who reported that wine oligosaccharides contain between three and 15 monosaccharide residues.

In this study, SCWC in all wines had similar size distribution profiles, with relatively high levels of oligosaccharides. This observation is consistent with the high proportion of t-GalAp in relation to 4-GalAp, which suggests the presence of oligomers of HG (Table 3.3). The relatively high number of terminal glycosyl residues such as t-Galp, t-Manp and t-Glcp may be attributable to the presence of various oligomers of different polysaccharides or to the high substitution levels of the SCWCs in the wines with these terminal glycosyl residues (Figure 3.2), as discussed above. It is noteworthy that the second major peak of WAV 15 and 16 has a small trailing shoulder, which presumably results from an additional sub-population of oligosaccharides. Hemicellulose and HG have been reported to be depolymerized during grape ripening (Nunan et al., 1998; Yakushiji et al., 2001). However, the elucidation and confirmation of the oligosaccharide structures detected here in the SCWCs from Cabernet Sauvignon wines clearly require advanced analytical techniques such as matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry and dynamic light scattering.

3.3.7. Relationship Between the Sensory and Chemical Properties of Wine

Following the linkage analyses and the subsequent estimation of polysaccharide and oligosaccharide contents in the SCWC samples, statistical analyses were performed to investigate any correlations between SCWC composition and taste sensory attributes, with an emphasis on any

relationships with mouthfeel characteristics. The mean intensity ratings of the mouthfeel and taste sensory attributes for the wines can be found in the Supplementary Table S3.4. Analysis of variance (ANOVA) of the DA data indicated that all sensory attributes were significantly different ($p < 0.1$).

PCA is a powerful multivariate tool used to understand the correlations between instrumental and sensory data (Preys et al., 2006). Only the chemical attributes that differed significantly were overlaid on the principal component (PC) plot for matrix analysis. The PCA was performed on the mean intensity ratings of the significant sensory attributes for all wines from different ripening stages, with PC1 and PC2 accounting for 91.6 % and 5.3 % of the variation, respectively (Figure 3.7). The PC 1 can be best explained by viscosity (used here as a sensory descriptor), astringency, overall sensation in the mouth, sweet, mouth roughness, chalky, mouth coating, sour and bitter. It is obvious that PC 1 mainly differentiates the wines on the basis of taste and mouthfeel. Overall, the variation in the wines was nearly entirely explained by PC 1. As the ripening level of the fruit increased, the wines were progressively perceived to have an improved overall mouthfeel and a sweeter taste, as indicated by the shift of the WAV from the left quadrant towards the mouthfeel attributes (Figure 3.7). Thus, wines from the later harvest were clustered together in the right quadrants, whereas wines from the earlier harvest were clustered together in the left quadrants. As reported previously, mouthfeel was higher while acidity, astringency and bitterness were lower in both Cabernet Sauvignon and Tempranillo wines made from riper grapes (Gil et al., 2012).

PC1 suggests that the separation is primarily driven by the types of phenolic and polysaccharide compounds. The correlation between phenolic compounds and mouthfeel agrees with previous studies suggesting phenolic compounds are important contributors to wine mouthfeel (Hufnagel and Hofmann, 2008a; Versari et al., 2013). It is not surprising that total phenols, polymeric tannins and pigmented polymers correlated with mouthfeel attributes in the right quadrants. It is widely known that red wine astringency increases with higher phenolic content and polyphenolic size (Kennedy et al., 2006; Soares et al., 2015). Comparatively, the correlation of monomeric phenols with mouthfeel attributes on PC1 was slightly stronger. Among monomeric phenols, gallic acid and caffeic

acid were highly associated with mouth coating, chalkiness, astringency, mouth roughness, burn and overall mouthfeel, whereas caftaric acid was related to both sour and bitter tastes in the opposite quadrant (Figure 3.3).

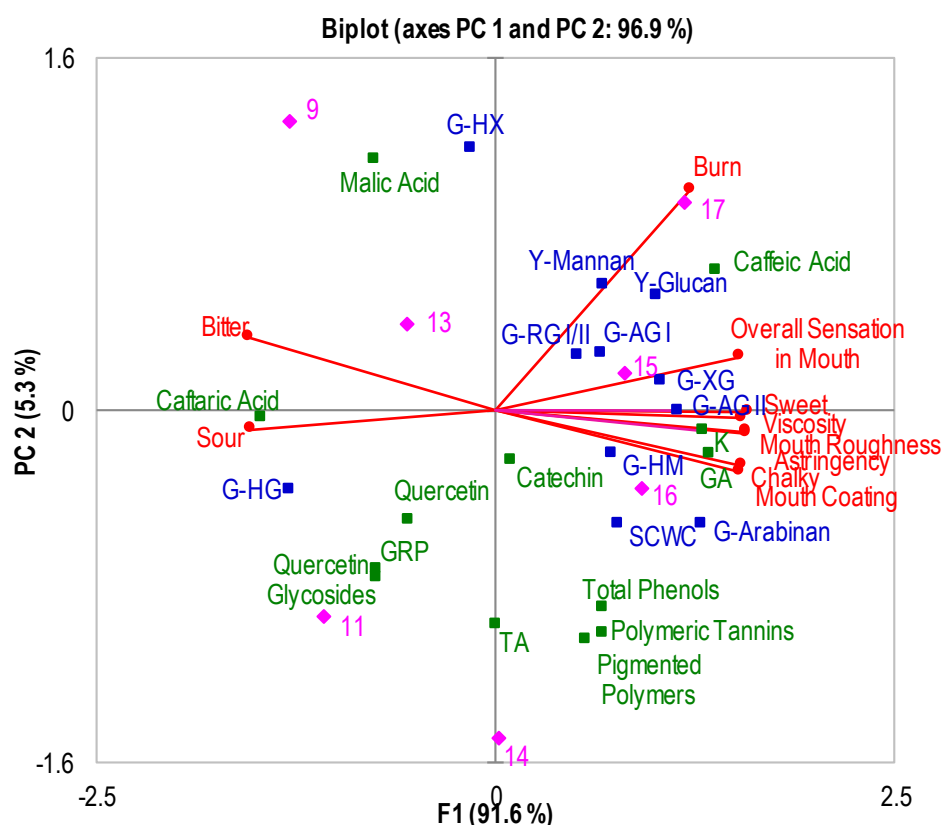


Figure 3.3. Principal Component Analysis of chemistry and mouthfeel sensory attribute ratings of Cabernet Sauvignon wines. Only variables that showed a significant difference between wine samples across treatments, represented by WAV (indicated in pink diamonds) are shown ($p \leq 0.05$; $p \leq 0.1$) with the exception for polysaccharide composition. Sensory metrics (indicated in red circles); polysaccharides (indicated in blue squares); and other chemical metrics (indicated in green squares). G- denotes grape derived polysaccharide; Y- denotes yeast derived polysaccharide. GA – gallic acid

Monomeric phenols contribute to the taste of wine and in general are relatively more bitter and astringent than larger molecular weight derivatives such as polymeric tannins (Brossaud et al., 2001). Based on the clustering of various chemical variables on the PCA, SCWCs and phenolic compounds appear to have a similar impact on mouthfeel (Figure 3.3). This finding provides insights into the contributions of non-phenolic compounds to wine mouthfeel. It has been reported that SCWCs, which are both acidic and neutral in nature, significantly increase the 'fullness' and, to some extent,

the 'mellowness' of wines (Vidal et al., 2004b), although the contributions of specific polysaccharides or oligosaccharides has not been reported previously. Thus, no comprehensive studies have been undertaken on the sensory effects of a complete set of well-defined and yeast SCWCs that are actually present in the final wine.

A distinct separation was observed between HG, which was isolated in the left quadrant, and all other polysaccharides, which were predominantly located in the right quadrants. HG was clearly negatively correlated with mouthfeel and was positively correlated with sour taste in this set of wines. This is not unexpected, given the acidic nature of this component. Wine polysaccharides do not contribute to bitter taste whether naturally present or added back to base wines and can actually reduce bitter taste (Vidal et al., 2004a). The PCA clearly confirms that polysaccharides have no effect on bitterness (Figure 3.3).

In the right quadrants, mixtures of the polysaccharides arabinan, AG I, AG II, RG I/II, XG, HM, yeast glucan and yeast mannan, irrespective of type, positively correlated with mouthfeel. Among them, AG II correlated most strongly with mouthfeel in this study, although arabinogalactan-proteins themselves do not usually form aqueous solutions of high viscosity (Fincher et al., 1983). AG II is a polysaccharide consisting of a branched backbone of (1,3, 1,6)-linked β -Galp residues substituted with α -Araf residues that has been statistically correlated with astringency (Quijada-Morin et al., 2014). The results suggest that the correlation of the AG II with mouthfeel attributes is not because of the physical viscosity of the SCWCs themselves. It is more likely that this polysaccharide or oligosaccharide is detected at low concentrations by specific taste receptor cells, or gustatory cells, which provide the sensory mouthfeel attribute.

Potassium, the most abundant inorganic element in wine, was closely associated with a majority of the cell wall polysaccharides in the quadrant in which mouthfeel attributes were located. The mechanism by which potassium contributes to wine mouthfeel is unclear. Potassium may not have a direct impact on wine sensations. Harbertson and Harwood (2009) reported the pH dependence of potassium extraction and suggested that the carboxylic functional groups of acidic

polysaccharides such as RG I and HG could potentially behave like an ion exchange resin to exchange a proton for potassium at low pH. In addition, cross linking of a monovalent ion to a polymer commonly occurs during food processing (McFeeters, 1985). By contrast, TA, which influences the sensory perception of acidity or sourness (Fischer et al., 1994), has no correlation with sour or any other taste or mouthfeel attribute because relatively small differences in the reported values would not be expected to lead to perceivable sensory differences.

PC 2 captures the remaining variance in the normalized data set. It is arguable that the separation observed on PC 2, which accounts for 5.3 % of the variation, might not effectively explain the resulting correlations between variables. However, the information obtained from PC 2 is of significant value. All sensory attributes were significantly different in their intensity ratings ($p < 0.1$) (Supplementary Table S3.4) despite their proximity on PC 2. Therefore, the separation on PC 2 suggests meaningful correlations between variables. Although the total SCWC content was positively correlated with the overall mouthfeel attributes as a group, the detailed composition of the SCWC provided greater in-depth insights to differentiate the relationships between individual polysaccharides and their respective effects on wine mouthfeel and the identification of specific key drivers to predict targeted mouthfeel attributes.

PC 2 revealed that all polysaccharides and their derived oligosaccharides, except HG and HX, are highly associated with wine mouthfeel attributes and sweet taste. Arabinan and HM had a stronger correlation with mouth coating and chalkiness. Studies of the sensory and textural effect of galactomannan, a plant polysaccharide in the HM family, in probiotic beverages have revealed that galactomannan enhances the texture and sensory quality of products (Buriti et al., 2014). The present study now indicates that plant-derived HM also affects wine mouthfeel and that yeast-derived mannan is not an important determinant of mouthfeel. XG and AG II were shown to have stronger positive correlations with viscosity, astringency, mouth roughness and sweet taste (Figure 3.2). form complexes with astringent compounds. In general, mixtures of the polysaccharides AG I, AG II, RG I/II and XG enhanced overall mouthfeel in the present study.

Compared with the grape SCWCs, the correlation of yeast mannan and yeast glucan with wine mouthfeel was much weaker (Figure 3.2). Both of these polysaccharides, particularly yeast mannan, were highly associated with burn. Although polysaccharides are known to modulate the sensory properties of food, there is a lack of scientific evidence to explain their mode of action and whether yeast polysaccharides can directly or indirectly attribute to burn.

In addition to the polysaccharide/oligosaccharide composition, polysaccharide size may also relate to wine mouthfeel (Figure 3.2). It has been reported that red wine oligosaccharides could influence the perception of astringency through their ability to chelate cations (Apolinar-Valiente et al., 2015). This aspect in wine needs further investigation. The correlation between phenolic compounds and wine mouthfeel and taste was relatively weak on PC 2.

3.4. Conclusions

Chemical analyses of the SCWCs from the red wine used in this study presented unusual challenges that are not usually experienced in linkage analyses of well-characterized polysaccharides found in grape and yeast cell walls. In particular, the SCWCs were highly complex mixtures that appeared to consist predominantly of low molecular weight oligosaccharides, which represented extensively degraded products of pectic polysaccharides from the grapes, yeast mannans/mannoproteins and type II AGPs from the grapes. Nevertheless, their compositions could be determined and their likely polysaccharides or origin could be deduced from the data. This enabled the statistical analysis of the effects of these SCWC constituents on sensory attributes of the wine.

Despite popular belief in the importance of phenolic compounds and their mouthfeel contributions in wine, in this study both SCWCs and phenolic compounds have been shown to play important and approximately similar roles in affecting wine mouthfeel. Thus, it can be concluded that SCWCs, particularly those derived from grapes, play an important role in affecting wine mouthfeel. The differentiation of wine mouthfeel on the basis of polysaccharide families was demonstrated. All polysaccharide families, except HG which was related to sour taste, were positively correlated with

overall wine mouthfeel attributes. Our study shows that arabinan, XG and AG II are the key drivers of mouthfeel attributes such as viscosity and mouth coating. While one might assume that the link between SCWCs and mouthfeel would be related to the propensity of cell wall polysaccharides to form aqueous solutions of high viscosity, the present data suggests that this is unlikely. The SCWCs are in relatively low concentrations and most are of relatively low molecular weight. For these reasons their contribution to viscosity could be disputed. As mentioned in the discussion above, it is perhaps more likely that the SCWCs exert their influence on mouthfeel attributes via specialized taste receptors in the mouth.

The correlations of specific SCWC components with mouthfeel characteristics raises the possibility that these data could be used to develop simple and rapid assays for mouthfeel in fermenting or mature wines. Thus, the ability to identify and select for key polysaccharides and their specific mouthfeel attributes through simple marker assays of specific monosaccharides in wine will enable the wine industry to select and grow grapes that contain more desirable polysaccharides or to adjust the winemaking process itself, depending on the levels of the marker molecules. If a simple, rapid marker assay could be developed, this could ultimately enable the delivery of superior wine-like mouthfeel than using non-grape commercial mouthfeel additives such as gums or wood tannins. Conversely, wine makers could select against undesirable mouthfeel sensations such as sour taste through simple marker assays of HG or GalA residues.

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3.7. Supplementary Material

Supplementary Table S3.1. Mouthfeel (M) and taste (T) attribute list with definitions and reference standards

Attribute	Definition	Reference Standard
M-Astringency	Drying sensation on tongue and pulling in and tightening of cheeks and mouth surfaces, sensed without moving tongue over surfaces	Grape seed extract ^a
M-Burn	Burning/warming sensation on surfaces of mouth; heat	Grain neutral spirits ^b
M-Chalky	Subcategory of mouth coating, chalky, powdery coating perceived after expectoration.	Milk of Magnesia
M-Coating	Any sensation that a coating has been left on the surfaces of the mouth after expectoration, especially sensed by moving tongue over surfaces of the mouth, roof, teeth, and gums	
M-Roughness	Roughness of mouth surfaces, sensed by running tongue over surfaces, especially roof of mouth, includes roughening of tongue's surface	Concentrated juice from Zinfandel ^a
M-Viscosity	Thickness, weight of wine, increase or decrease in force necessary to move tongue through liquid, must be evaluated while wine is in mouth. Includes syrupy mouthfeel	Gum Arabic ^b
M-Overall Sensation in Mouth	Includes the mouth feel sensations of chemical feeling factors (alcohol burn, astringency and spritz) and texture (mouth roughness, viscosity and mouth coating); may also include basic taste sensations but not the actual taste; what you feel when wine is in your mouth, what sensations you have on the surfaces of your mouth after expectoration; an overall perception of what is felt in the mouth	
T-Sweet	Sweet taste	Sucrose ^b
T-Sour	Sour/acidic taste	Tartaric acid ^b
T-Bitter	Bitter taste	Caffeine ^b

^aStandards are presented in a commercial white wine

^bStandards are presented in a commercial red wine

Supplementary Table S3.2. Phenolic composition (mg/L) of Cabernet Sauvignon wines^a

WAV	Caffeic Acid	Gallic Acid	Catechin	EP ^b	Gallic Acid	GRP ^c	Malvidin	PP ^d	Polymeric Tannin	QC ^e	Quercetin Glycosides
9	5 ± 1ab	59 ± 4a	41 ± 2ab	19 ± 2	14 ± 3a	4 ± 1abc	151 ± 16	26 ± 6a	351 ± 80a	4 ± 1ab	18 ± 4abc
10	7 ± 1ab	65 ± 2ab	45 ± 10ab	19 ± 4	17 ± 3ab	6 ± 1c	183 ± 20	46 ± 8b	669 ± 119b	5 ± 1bc	23 ± 5c
11	5 ± 1ab	52 ± 3bc	45 ± 10ab	17 ± 3	17 ± 2ab	4 ± 1ab	178 ± 22	43 ± 8b	644 ± 165b	5 ± 2bc	22 ± 3bc
12	5 ± 1ab	48 ± 4cd	34 ± 4ab	19 ± 3	14 ± 2a	5 ± 0bc	168 ± 15	42 ± 6ab	576 ± 49ab	4 ± 1abc	19 ± 3abc
13	6 ± 1ab	49 ± 3cd	35 ± 4ab	14 ± 4	16 ± 2ab	4 ± 1ab	182 ± 24	43 ± 7b	627 ± 118b	5 ± 2bc	22 ± 2bc
14	5 ± 1ab	48 ± 3cd	34 ± 4ab	15 ± 3	17 ± 1b	5 ± 1ab	177 ± 18	46 ± 4b	639 ± 83b	5 ± 1bc	23 ± 4c
15	8 ± 2b	44 ± 2d	45 ± 3ab	13 ± 4	21 ± 1 bc	5 ± 1ab	194 ± 14	50 ± 7b	746 ± 56b	6 ± 1c	23 ± 3c
16	8 ± 2b	38 ± 2e	52 ± 11b	20 ± 8	24 ± 5c	4 ± 0ab	191 ± 14	41 ± 6ab	660 ± 93b	2 ± 0a	14 ± 3ab
17	12 ± 2c	31 ± 3e	34 ± 5a	14 ± 1	20 ± 3abc	3 ±1 a	181 ± 23	35 ± 8ab	512 ± 145ab	2 ± 0a	11 ± 5a

^aMean within a row followed by different letters indicate are significantly different (P = 0.1, one-way ANOVA, Tukey's post hoc)

^bEP, Epicatechin; GRP, Grape Reaction Product; PP, Pigmented Polymer; QC, Quercetin

Supplementary Table S3.3.Unassigned glycosyl residues

Glycosyl Residue	Deduced Glycosidic Linkage	Linkage Composition (mol %) / WAV								
		9	10	11	12	13	14	15	16	17
Galactopyranosyl (Galp)	2-a	0	0.1	0.1	0.2	0	0	0.1	0.1	0
Glucopyranosyl (GlcP)	2-c	0	0.1	0.1	0	0	0	0.1	0.2	0.4
	2,3-c	0	0	0	0	0	0	0	0	0.1
	3,4-c	0	0	0	0	0	0	0	0.4	0.2
Rhamnopyranosyl (Rhap)	4-c	0	0.3	0.4	1.2	0.5	0.2	0.3	0.6	0.8
Xylopyranosyl (Xylp)	Terminal ^b	0	0	0	0	0	0	0.2	0	0
Arabinopyranosyl (Arap)/	Terminal(p) ^b	0	0	0	0	10.6	3.5	0.5	0	0
Mannopyranosyl (Manp)	2,3-c	0	0	0.3	0	0	0	0.2	0	0.4
Galacturonopyranosyl ^a (GalAp)	2-c	0	0.2	0.2	0.6	0.3	0	0.1	0.2	0
	3-c	0	0.8	0.8	1.0	0	0	0.3	0.7	0.3
Glucuronopyranosyl ^a (GlcAp)	3-c	0	0	0	0	0	0	0	0.2	0
	4-c	0.2	0.2	0.2	0.3	0	0.1	0.2	0.5	0.5
	Terminal ^b	0	0	0	0	1.4	1.4	1.6	0	0
Fucopyranosyl (Fucp)	Terminal ^b	0	0	0	0	0	0	0.6	0	0.1
Galactofuranosyl (GalF)	Terminal ^c	0	0	0.1	0.4	0.2	0.3	0.3	0.4	0.3
Galacturonofuranosyl (GalAf)	Terminal ^c	1.3	1.5	0.8	1.8	1.2	1.4	2.4	0.8	1.7

^aLinkage type that can be assigned to well-characterized SCWCs from plant and yeast, but it is unassigned in this study because it does not meet the criteria laid out in the Materials and Methods section. For example, according to Pettolino et al. (2012), 2-Galp must be ≤ 2-Xylp in xyloglucan. In this study, 2-Galp is present but it cannot be assigned to xyloglucan because of the absence of 2-Xylp

^bRemaining terminal linkage types after successive assignments to SCWCs in Table 3.4

Linkage types that cannot be assigned to well-characterized SCWCs from plants and yeast

Supplementary Table S3.4. Mean intensity ratings for sensory attributes of Cabernet Sauvignon wines during ripening in 2012^a

Attributes ^b	WAV						
	9	11	13	14	15	16	17
M-Astringency	4.9a	5.2b	5.5c	5.7d	6.1e	6.2ef	6.3f
M-Burn	5.5abc	5.4a	5.5 bcd	5.4ab	5.5cd	5.5bcd	5.5d
M-Chalky	0.2a	0.3a	0.9b	1.2c	1.5d	1.5d	1.5d
M-Coating	0.2a	0.3a	0.9b	1.3c	1.5cd	1.5d	1.5d
M-Roughness	1.2a	1.3a	1.6b	1.8c	2.0d	2.1de	2.2e
M-Viscosity	3.0a	3.0a	3.0a	3.3b	3.4c	3.4c	3.5c
M-Overall Sensation in Mouth	6.1a	6.0a	6.1a	6.3b	6.5c	6.7d	6.9e
T-Sweet	2.3a	2.5b	2.7c	3.0d	3.4e	3.4e	3.6f
T-Sour	6.0c	5.9c	5.9c	5.8b	5.5a	5.5a	5.5a
T-Bitter	4.8d	4.5c	4.5c	4.2b	4.0a	4.0a	4.0a

^aMean within a row followed by different letters indicate are significantly different (P = 0.1, one-way ANOVA, Tukey's post hoc)

^bM, mouthfeel attribute; T, taste attribute

Chapter 4

Soluble Cell Wall Carbohydrates and Their Relationship with Sensory Characteristics in Chardonnay Wine

Preface

In accordance with University of Adelaide thesis guidelines, this Chapter is presented in manuscript format. Here, the Materials and Methods section, together with calculations associated with linkage analyses, are described at the level expected in common plant and food journals. Full details of the materials and precise descriptions of the methods used are provided in Chapter 2 of the thesis (Development and Adaptation of Methods). Some minor repetition was therefore necessary between Chapters 2, 3 and 4 in these Materials and Methods sections. In addition, repetition would be likely in the Results and Discussion sections of Chapters 3 and 4. Formats for the text, referencing, figures and tables have been standardized throughout the thesis.

Soluble Cell Wall Carbohydrates and Their Relationship with Sensory Characteristics in Chardonnay Wine

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yeast

Abstract

Wines made from Chardonnay grapes at different ripening stages exhibit significantly different chemical and sensory profiles. The soluble cell wall carbohydrate (SCWC), aroma and phenolic compositions of wines were analyzed and compared with their sensory characteristics, namely aroma, mouthfeel and taste. Ten classes of grape- and yeast-derived SCWCs were identified in the Chardonnay wines, and the SCWCs from grapes, especially neutral SCWCs, were shown to have a significant influence on wine sensory perceptions. A perceived sour taste was associated with homogalacturonan content, whereas xyloglucan and arabinogalactan type I was associated with burn and viscosity mouthfeel. The data also suggested that a multimodal sensory effect can be used to explain the correlation between sweet taste and fruity aroma perception. SCWCs can influence wine sensory perceptions directly through their chemical properties and indirectly as a result of their interactions with other classes of compounds in wines. This knowledge enables the production of a Chardonnay wine with optimized, consumer-preferred sensory characteristics from naturally occurring compounds in grapes using viticultural practices and winemaking techniques, particularly when there are limitations on the addition of adjuncts.

4.1. Introduction

Strong growth in white wine consumption, which began in the 1980s, significantly increased the commercial value of Chardonnay grapes produced globally (Jackson, 2008). Chardonnay is now the most planted wine grape variety in California, occupying over 38,000 ha (United States Department of Agriculture Statistics Service, 2015), and is the most important white wine grape in Australia, accounting for almost 50 % of total national white wine production (Australian Bureau of Statistics, 2015).

Typical US Chardonnay wine is primarily made to pair with specific culinary contexts and is often described as “acidic, floral, fruity, oak-aged and full mouthfeel” (Robinson, 1999; Lee and Noble, 2006; Asimov, 2013). According to Bernadette (2010), mouthfeel is a key attribute that drives the popularity of white wines. Similarly, beers are routinely categorized by mouthfeel, which has a strong effect on consumer preferences; for example, lager-style beer has relatively lower “viscosity” and lower “oily mouthcoat” characteristics than heavier ales, such as stout (Langstaff et al., 1991). The influence of mouthfeel has also been reported in soft drinks (Redondo et al., 2014). Despite its commercial importance, there is little consensus within the wine industry as to the chemical compounds that contribute to mouthfeel. Thus, mouthfeel is one of the least understood aspects of white wine sensory characteristics and hence quality.

The mouthfeel of white wines is often evaluated on the basis of being light, medium or full-bodied. However, most of these characteristics are qualitatively different from those exhibited in red wines (Runnebaum et al., 2011). One major difference between white and red wines lies in the winemaking technique. White wine is made by fermenting just the grape juice, whereas red wine is made by fermenting juice and skins together for an extended period of time. Consequently, white wines contain significantly lower amounts of tannins, which are mouthfeel constituents, when compared with red wines (Gawel et al., 2014). The characterization of mouthfeel of Chardonnay wines becomes more challenging as the wine evolves stylistically. The traditional, more oxidative winemaking technique for Chardonnay, which principally involves aging the wine in oak barrels, has

been gradually replaced by aging in stainless steel tanks in which greater control of oxygen level is possible. In the past decade, “unwooded” or “unoaked” Chardonnay has become highly appealing to Millennial wine drinkers, who have different expectations of wine style compared with previous generations (Berger, 2014). This phenomenon is not surprising, because consumers with diverse backgrounds would not be expected to have homogenous wine style preferences. The change from wooden barrels to stainless steel tanks leads to differences in wine chemical composition in which oak tannins, compounds known to impact mouthfeel (Versari et al., 2013), are missing. The inherently low grape tannin levels because of the winemaking technique and the lack of exogenous tannin additions in Chardonnay wines thus brings forth the importance of other naturally occurring mouthfeel compounds in the fruit.

Soluble cell wall carbohydrates (SCWCs) in wine, derived from the cell walls of grapes and yeast and resistant to fermentation conditions, are of great interest because of their organoleptic properties (Vidal et al., 2000; Vicens et al., 2009). The composition and structure of these carbohydrates are believed to correlate with wine mouthfeel (Vidal et al., 2004b). Their characteristics are similar to those of commercial polysaccharides, such as pectins isolated from plants, which display hydrocolloid properties that improve the mouthfeel of a range of beverages (Haderspeck, 2014). The abundance of SCWCs in white wine has been reported to increase the “fullness” mouthfeel sensation (Vidal et al., 2004a).

Aside from mouthfeel, SCWCs are widely known for their influence on the rate and intensity of aroma release in food matrices, but this knowledge has been hitherto overlooked in the study of wine quality. Polysaccharides can affect the volatility of aroma molecules through covalent chemical interactions and the non-covalent, physical entrapment of these molecules within the food matrix (Hansson et al., 2001; Mitropoulou et al., 2011).

Furthermore, these interactions can be perceived at both the physiochemical level and the neurobiological level. Different sensory modalities interact in non-linear ways to form a principal perception (Simons et al., 1999; Bult et al., 2007). Previous studies demonstrated this concept by

showing that the perception of aroma can be influenced by varying the SCWC-dependent viscosity levels of food, while the in-mouth volatile concentrations remained unaffected (Verhagen and Engelen, 2006; Bult et al., 2007). The multimodal nature of sensory integration has been studied in humans and non-human primates by neuroscientists to identify the neurons that respond to oral stimuli and sensory outputs resulting from recognition of a particular mouthfeel characteristic of food (Rolls, 2005; Verhagen and Engelen, 2006). To formulate successful products for the wine industry, these factors should be considered when assessing the sensory mechanisms of wine mouthfeel.

Wine composition is very complex and several components may contribute to the important sensory property of mouthfeel. Therefore, it is essential not only to consider the composition of the wine, but also to define the impact of interactions between different wine components on the sensory properties of the final product. Elucidating the role of SCWCs in Chardonnay wine mouthfeel has been limited by a lack of rigorous studies linking polysaccharide chemistry and wine sensory profiles, together with the difficulty associated with isolating and characterizing wine carbohydrates. The goal of this study was to investigate possible relationships between Chardonnay grape SCWCs and wine aroma, mouthfeel and taste, and to determine the effects of interactions of these chemical compounds as determinants of wine sensory perceptions. The study was performed using wines made from Chardonnay grapes picked at different ripening stages.

4.2. Materials and Methods

4.2.1. Winemaking

The location, field site management, picking and general methods for production of the grapes were largely as described in Chapter 3. The Chardonnay grapevines (*Vitis vinifera* L.) were grown in a commercial vineyard located in the Central Valley of California (38° 10' 36.00" N, 121° 14' 28.00" W). The vines were planted in 1991 on Freedom rootstock, with 2.1 m x 3.4 m vine row and row spacing, and trellised to a horizontally divided training system. Standard viticulture practices for the variety and growing region were used for grape production. The field trial was conducted in 2012 with

the use of a randomized complete block design. Seven different harvest dates (treatments) were replicated four times using seven-vine plots. Randomly selected 20 middle grape clusters from the middle five vines in each plot of each replicate were collected weekly, beginning at six weeks after veraison (WAV) to two weeks post-harvest. Fruit was stored in zip tight bags and transported to the research laboratory in insulated ice coolers for chemical analyses. Following the sampling of grape clusters from each replicate plot within a treatment, 50 KG of fruit from each replicate plot was collected and stored overnight at 4 °C for micro-vinification the next day.

Fruits were de-stemmed and crushed in a Magitec A15DC destemmer-crusher (Helmut Amos, Paarl, South Africa) into a 100 L plastic bin. Sulfur dioxide was added to the must at a rate of 40 mg/L immediately after destemming, followed by the addition of a 0.3 mL/L commercial Rohavin MX pectolytic enzyme mixture (AB Enzymes, Darmstadt, Germany) into the must at about 10 °C, to increase juice yield. After 2 h at 10 °C, the must was pressed using a Diemme GB00C299181 membrane press (Diemme Enologia, Emilia-Romagna, Italy), and the resulting juice was transferred into a 50 L jacketed fermentation tank for cold settling at 2 °C. Skins and seeds were discarded following pressing. After two days, the juice was racked into another 50 L jacketed fermentation tank and the juice sugar level was either increased to 22.5 °Brix with the addition of a 2 parts glucose: 1 part fructose mixture (approximately 333 g of glucose and 168 g of fructose dissolved in 1 L of water) or diluted to 22.5 °Brix with water, in order to standardize the alcohol potential for wines made from grapes of all ripening levels. Similarly, the juice tartaric acid level was either increased to 6.7 g/L with the addition of tartaric acid or decreased to 6.7 g/L with the addition of calcium carbonate, in order to standardize the acidity potential of wines. Diammonium phosphate (DAP) was added to supplement the yeast assimilable nitrogen (YAN) to 300 mg/L before inoculation with Vin13 commercial yeast (Scott Laboratories, CA, USA) at a rate of 0.12 g/L. Fermentation was conducted at 18 °C. The jacketed tank was set to 2 °C when the reducing sugar decreased below 2 g/L, in order to settle out solids before racking the wine into a 30 L stainless steel canister for cold storage at 4 °C. The resulting wine was clarified within a week of cold storage by passing through a 1.0 µm pad filter, a Meissner

0.8 μm cartridge filter and a 0.45 μm cartridge filter in series, and bottled into 750 mL screw cap glass bottles, which were stored at 13 °C.

4.2.2. Chemical Analysis

As described in Chapter 3, the percent alcohol, titratable acidity (TA), pH and concentration of malic acid of each wine were analyzed using a WineScan FT-120 Fourier Transform Infrared Spectroscopy (FTIR) (FOSS North America, MN, USA). Similarly, potassium (K) levels were determined using a PerkinElmer Optima 7000 DV inductively coupled plasma with optical emission spectroscopy (ICP-OES) (PerkinElmer Inc., MA, USA). The method used to quantify the phenolic composition of white wine was adapted from Somers and Ziemelis (1985) and the absorbances at 280 nm (index for phenols) and 320 nm (index for hydroxycinnamates) of wine samples were measured with an Agilent 8453 spectrophotometer (Agilent, CA, USA), as also described in Chapter 3.

4.2.3. Wine Aroma Composition Analysis

The wine aroma method was adapted from Siebert et al. (2005), with some modifications. Wine aroma was analyzed using an Agilent 6890 series gas chromatograph (GC) with a 5975 series mass selective (MS) detector and a 25 m x 0.25 mm x 0.25 μm DB-5ms Ultra Inert capillary column (Agilent, CA, USA). Each wine sample was diluted with Milli Q water to achieve a 10 mL final sample volume at 8.5 % (v/v) ethanol, before dispensing into a 20 mL headspace vial (Wheaton, NJ, USA). After the addition of 100 μL internal standards solution (ethyl nonanoate, β -damascone, propyl propionate, ethyl heptanoate and d_{13} -hexanol), the vial was capped and mixed before analysis. During the solid phase microextraction (SPME), a Supelco DVB/CAR/PDMS fiber (Sigma-Aldrich Corp., MO, USA) was inserted into the headspace of the vial, heated to 50 °C and held for 20 min to adsorb volatiles. After that, the fiber was transferred into the GC inlet port for the thermal desorption of volatiles, with helium gas acting as the carrier gas. The oven temperature was initially 40 °C and was increased linearly to 275 °C within the 52 min run time. For data acquisition, selective ion mode (SIM)

was used and the ions monitored were based on characteristic m/z values of each aroma compound (Table 4.1).

Table 4.1. The characteristic m/z values and the chemical standards of aroma compounds

Aroma Compounds	m/z values
β -damascenone	190, 175, 121
β -ionone	177, 135, 192
Ethyl decanoate	88, 101, 155
Ethyl dihydrocinnamate	178, 91, 104
Ethyl butanoate	71, 88, 43
Ethyl isobutyrate	43, 71, 116
Ethyl isovalerate	88, 85
Ethyl hexanoate	88, 99, 60
Ethyl octanoate	88, 101, 127
Isoamy acetate	43, 70, 55
Hexanol	56, 43, 55

4.2.4. SCWC Preparation

Precisely as described in Chapter 3, an aliquot of 200 mL wine from each of the four replicates vinified from each treatment was mixed with 1 L of ethanol and stored at 4 °C overnight. The alcohol-insoluble residue (AIR) was recovered by centrifugation at 6577 x g for 10 min, washed twice with absolute ethanol and dried under a stream of nitrogen. The dried AIR was re-dissolved in water and dialyzed against water in 100 - 500D MWCO tubing (Spectrum Laboratories, CA, USA) for 24 h at 22 °C before being freeze dried. The weight of the freeze dried AIR was recorded and the SCWC content was reported in mg/L. As described in Chapter 2, a composite sample was prepared from 10 mg AIR materials from each of the four replicates. The sample was suspended in phenol saturated with 500 mM Tris-HCl buffer, pH 7, placed on a shaker for 60 min before centrifuged at 6863 x g for 10 min to recover the pellets. This procedure was repeated twice and the pellets were treated stepwise with ethanol, acetone and chloroform:methanol (1:1, v/v) with continuous stirring for 60 min each. A sintered glass funnel (Ace Glass Inc., NJ, USA) was used to separate the solvents from the pellets. The treated pellets were finally washed with acetone, dried under nitrogen and kept in a desiccator for glycosyl linkage analysis.

4.2.5. Glycosyl Linkage Analysis

Uronic acids in AIR material were reduced to 6,6-dideuterio glycosyl residues in 0.5 M methanolic HCl at 80 °C for 20 min and treated with sodium borodeuteride at room temperature for 2 h. The samples were neutralized with glacial acetic acid and dried under a stream of nitrogen. Methylation and linkage analyses were performed by the Complex Carbohydrate Research Center (Georgia, USA) essentially as described by Ciucanu and Kerek (1984), York et al. (1985) and Heiss et al. (2012), and in Chapters 2 and 3. Quantitated peak areas were corrected to mol % as described by Pettolino et al. (2012).

As described in detail in Chapter 2, estimations of specific classes of SCWCs were based on the structures of well-characterized SCWCs from grapes (Nunan et al., 1998), wines (Pellerin et al., 1996) and yeast (Kwiatkowski et al., 2009; Cuskin et al., 2015), together with information in Pettolino et al. (2012).

4.2.6. Size Exclusion Chromatography

As described in Chapter 3, high-performance size exclusion chromatography (HPSEC) was performed on an Agilent 1200 liquid chromatograph that was equipped with a refractive index detector. Separation was achieved using a 7.8 mm x 300 mm Ultrahydrogel 250 column (Waters Corp., MA, USA) at a flow rate of 0.4 mL/min and an eluent of 50 mM ammonium formate, pH 5.5, at room temperature. The column was calibrated using dextran standards (Sigma-Aldrich, St. Louis, MO, USA) with molecular weights ranging from 410,000 to 5,000.

4.2.7. Wine Sensory Evaluation

The wine sensory analyses performed were similar to those described in Chapter 3. The panelists were trained to identify white wine sensory characteristic comprising mouthfeel, taste and aroma (Supplementary Table S4.1). Representative wine samples from this study were presented to the panelists for calibration using unoaked Chardonnay wine style.

Wines were evaluated in order of increasing grape ripening levels by five experienced wine tasters from E & J Gallo Winery. After the evaluation, 24 wines from six subsets of different ripening

levels (each subset had four technical replicates) with distinguishable sensory differences were selected for wine descriptive analysis. These wines were from WAV 6, 7, 8, 9, 11 and 12. The subset of wines from WAV 10 were excluded from the wine descriptive analysis because none of the tasters were able to describe any detectable differences compared with wines of ripening levels a week shorter or longer. Approximately 60 mL of each wine sample was served in a black glass labeled with a random three-digit code. All 24 wines were evaluated in two repetitions. Intensity scores were given by the panelists to the sensory characteristics of each sample based on the universal wine ballot 15-point universal scale (anchored) and were recorded using SIMS software (Solana Beach, CA, USA).

4.2.8. Statistical Analysis

A one-way analysis of variance (ANOVA) was performed on the chemical and sensory data, using a post hoc Tukey's test (SPSS version 21; IBM, 2012). The XLSTAT version 2015.1.03.16174 program (Addinsoft, 2015) was used for principal component analysis (PCA).

4.3. Results and Discussion

4.3.1. Wine Chemistry

Standard chemical analyses of the wines are presented in Table 4.2. The alcohol levels and TA values did not differ significantly between wines during berry development. The alcohol levels were essentially constant for all wines because their must sugar levels had been adjusted to the same level before fermentation. This step was included to minimize the influence of the alcohol concentration during sensory evaluations (Fischer et al., 1994). Similarly, the TA of the juice was adjusted to the same level before fermentation to prevent overwhelming sour taste that could potentially affect wine mouthfeel sensory characteristics such as viscosity. As anticipated, malic acid steadily decreased from 3.5 g/L on WAV 6 to 1.5 g/L on WAV 12 (Hrazdina et al., 1984). The potassium concentration was highest and lowest in wines from WAV 6 and WAV 9, respectively, and values were generally comparable with the range reported for commercial California white wines (Berg et al., 1979).

Both hydroxycinnamates (HDCs) and phenolics exhibited similar profiles, and their concentrations increased over time. Thus, values remained low for the first three weeks and subsequently increased consistently until a peak was reached on WAV 12. HDCs are the most abundant phenolic group found in white wines and predominantly accumulate in the flesh tissues of grape berries (Teixeira et al., 2013). The increase of HDCs observed after three weeks in the current study is likely due to the softening of the grape berries, which enhances solubilization of HDCs from the skin and pulp into juice during the mechanical crushing and pressing of whole berries.

Table 4.2. Wine chemistry

WAV	Alcohol (% v/v)	TA (g/L)	pH	Malic Acid (g/L)	K (g/L)	HDCs (AU)	Phenolics (AU)	SCWC (mg/L)
6	12.7 ± 0.1	6.7 ± 0.4	3.27 ± 0.13b	3.5 ± 0.2c	0.69 ± 0.12b	1.6 ± 0.4a	0.3 ± 0.6a	1234 ± 181a
7	12.7 ± 0.1	6.5 ± 0.2	3.22 ± 0.11ab	2.6 ± 0.3b	0.66 ± 0.16ab	1.9 ± 0.3ab	0.5 ± 0.2a	1743 ± 132d
8	12.8 ± 0	6.5 ± 0.1	3.12 ± 0.04ab	2.0 ± 0.2a	0.52 ± 0.05ab	1.9 ± 0.6ab	0.5 ± 0.4a	1601 ± 42bcd
9	12.8 ± 0.1	6.4 ± 0.3	3.09 ± 0.06a	1.8 ± 0.2a	0.50 ± 0.06a	2.4 ± 0.5abc	0.9 ± 0.2ab	1283 ± 128ab
10	12.9 ± 0.1	6.6 ± 0.1	3.17 ± 0.06ab	1.9 ± 0.2a	0.58 ± 0.05ab	2.7 ± 0.4bc	1.4 ± 0.4bc	1383 ± 205abc
11	12.8 ± 0.2	6.3 ± 0.1	3.15 ± 0.03ab	1.5 ± 0.1a	0.56 ± 0.01ab	3.0 ± 0.1c	1.5 ± 0.3bc	1672 ± 63cd
12	12.9 ± 0.1	6.3 ± 0.2	3.14 ± 0.05ab	1.5 ± 0.3a	0.54 ± 0.04ab	3.2 ± 0.2c	1.9 ± 0.1c	1385 ± 200abc

Values are the mean of 4 technical replicates. Values followed by different letters indicate a significant difference ($p=0.05$, one-way ANOVA, Tukey's posthoc)

In contrast, the SCWC contents of the wines exhibited greater variations during ripening compared with the other characteristics listed in Table 4.2. The SCWC levels in the wines rapidly increased from WAV 6 to WAV 7, dropped to nearly the lowest level at WAV 9, peaked at WAV 11, and reached a final value of 1385 mg/L on WAV 12. According to Resende et al. (2013) and Gawel et al. (2014) the chemical and physiological differences between grape varieties, in conjunction with winemaking techniques, can affect SCWC availability. The SCWC content dropped dramatically in the last wine sample to a level similar to that of the first wine sample, suggesting that the ripening of the grapes was not directly proportional to the SCWC availability. This finding is in agreement with previous studies where the molecular mass, solubility and structure of various polysaccharides vary

continuously without a significant change in overall cell wall polysaccharide content during fruit ripening (Fischer and Bennett, 1991; Nunan et al., 1998) 4.3.2. Aroma Composition

Based on their distinct odor characteristics, detected aroma compounds were primarily grouped into three categories, namely alcohol, norisoprenoids and esters (Table 4.3).

Table 4.3. Concentration ($\mu\text{g/L}$) of aroma compounds in wines

Compound	Odor	Thres- hold ($\mu\text{g/L}$) ^{5,6}	6	7	8	9	10	11	12
WAV									
<i>C6 compound</i>									
1-Hexanol	Green grass, toasted, dry ^{1,2}	8000	1415 $\pm$ 177c	1285 $\pm$ 145c	1159 $\pm$ 187bc	912 $\pm$ 75ab	678 $\pm$ 40a	653 $\pm$ 7a	629 $\pm$ 120a
<i>Norisoprenoids</i>									
β -Damascenone	Honey, cooked apple ¹	0.05	1.03 $\pm$ 0.87	1.48 $\pm$ 0.10	2.80 $\pm$ 2.05	0.85 $\pm$ 1.33	1.05 $\pm$ 0.66	3.08 $\pm$ 1.26	3.90 $\pm$ 1.97
β -Ionone	Floral, apricot ^{3,7}	0.09	0.18 $\pm$ 0.10ab	0.20 $\pm$ 0ab	0.33 $\pm$ 0.15ab	0.28 $\pm$ 0.10ab	0.10 $\pm$ 0.08a	0.38 $\pm$ 0.13b	0.33 $\pm$ 0.10ab
<i>Esters</i>									
Ethyl butanoate	Fruity, banana ¹	20	428 $\pm$ 153	450 $\pm$ 93	506 $\pm$ 78	472 $\pm$ 85	460 $\pm$ 43	433 $\pm$ 59	498 $\pm$ 35
Ethyl decanoate	Fruity ¹	200	543 $\pm$ 133	731 $\pm$ 298	575 $\pm$ 201	398 $\pm$ 7	570 $\pm$ 121	413 $\pm$ 212	590 $\pm$ 51
Ethyl dihydrocinnamate	Floral ²	1.6	1.9 $\pm$ 0.1a	2.0 $\pm$ 0.2a	2.1 $\pm$ 0.3a	2.0 $\pm$ 0.1a	2.7 $\pm$ 0.3b	2.3 $\pm$ 0.2ab	2.5 $\pm$ 0.1b
Ethyl hexanoate	Fruity, apple peel, tropical ^{1,3,4}	14	818 $\pm$ 237	784 $\pm$ 182	849 $\pm$ 207	732 $\pm$ 120	702 $\pm$ 30	736 $\pm$ 122	852 $\pm$ 17
Ethyl isobutyrate	Fruity, strawberry ^{1,2}	15	35 $\pm$ 8	38 $\pm$ 6	41 $\pm$ 9	32 $\pm$ 2	34 $\pm$ 2	33 $\pm$ 3	32 $\pm$ 3
Ethyl isovalerate	Fruity, sweet ²	3	3.4 $\pm$ 0.8	3.4 $\pm$ 0.8	8.0 $\pm$ 7.1	3.9 $\pm$ 0.3	5.2 $\pm$ 0.3	5.4 $\pm$ 0.8	5.4 $\pm$ 1.2
Ethyl octanoate	Fruity, fresh, citrus ^{1,2,3}	5	1508 $\pm$ 403	1433 $\pm$ 510	1643 $\pm$ 332	1195 $\pm$ 177	1215 $\pm$ 77	1460 $\pm$ 315	1620 $\pm$ 85
Isoamyl acetate	Banana ¹	30	3373 $\pm$ 838	3558 $\pm$ 1633	4098 $\pm$ 1032	3268 $\pm$ 628	4365 $\pm$ 637	3248 $\pm$ 260	3730 $\pm$ 934

Values are the mean of 4 technical replicates. Values followed by different letters indicate a significant difference ($p < 0.05$, one-way ANOVA, Tukey's posthoc)

¹Lee and Noble (2003); ²Aznar et al. (2001); ³Bowen and Reynolds (2012); ⁴Lorrain et al. (2006); ⁵Ferreira et al. (2000);

⁶Guth (1997); ⁷Solis-Solis et al. (2007)

Although wines can have hundreds of aroma compounds, only a small number of these significantly contribute to the sensory profile (Lee and Noble, 2006). Furthermore, all of the compounds identified in this study have been reported previously in white wines of different

varieties, such as Chardonnay and Gewürztraminer, as the determinants of the base aroma that define the wine style (Guth, 1997; Lee and Noble, 2003). In agreement with these studies, in the present work, there were only a few aroma compounds that varied significantly between the wines. Thus, the majority of detected aroma compounds constituted the base aroma of the Chardonnay. Notably, the concentrations of individual esters and norisoprenoids were above their published threshold levels, indicating that they are impact odorants (Table 4.3). In contrast, 1-hexanol, which is known to be present in Chardonnay wines, was present below its published threshold level. However, 1-hexanol can have an important effect on wine sensory perceptions when present with other aroma compounds via synergistic or additive effects (San-Juan et al., 2011).

4.3.3. Monosaccharide Composition and Linkage Analysis

The monosaccharide compositions of the wine samples were calculated from the methylation data (Pettolino et al., 2012). The results showed that GalA, Man, Glc and Gal were the most abundant monosaccharide residues (Table 4.4). These results are similar to those previously reported for white wines (Del Barrio-Galán et al., 2011; Gawel et al., 2016).

Table 4.4. Glycosyl residue composition (mol %) of the wine SCWCs

Glycosyl residue	WAV						
	6	7	8	9	10	11	12
Gal	7.9	4.9	11.3	2.0	8.0	6.5	16.7
Glc	4.2	7.9	4.2	7.6	12.7	20.8	11.9
Rha	2.3	1.4	0.4	2.0	1.5	3.5	4.6
Xyl	0	0	0.1	0.9	1.0	0	0
Ara	0	6.4	0	1.5	3.6	11.5	0.9
Man	17.9	20.9	60.6	18.9	26.7	16.3	37.8
GalA	66.4	57.1	23.2	65.7	45.2	40.2	26.5
GlcA	1.3	1.4	0.2	1.4	1.3	1.2	1.6

0, not detected

Table 4.5. Glycosyl residue linkage analysis of SCWCs from Chardonnay wines^a

Glycosyl Residue	Deduced Glycosidic Linkage	Linkage Composition (mol %) / WAV						
		6	7	8	9	10	11	12
Galactopyranosyl (Galp)	2-	0	0	0.1	0	0	0	0
	3-	0	0	1.1	0	0.4	0	0.9
	4-	0	0	0	0	0	0	1.2
	6-	0.7	0	1.4	0.3	0.7	0	1.7
	2,4-	0	0.1	0.1	0.1	0.1	0	0.2
	3,4-	0	0	0	0	0.2	0	0
	3,6-	1.0	0	5.3	0	0	0	2.0
	4,6-	0	0	0	0	0	0	0.4
	Terminal	6.0	4.2	3.3	1.1	5.8	5.5	9.7
Glucopyranosyl (Glc p)	2-	0	0	0.1	0.4	0.3	0.6	0.4
	3-	0.4	1.1	0.5	0.8	1.0	3.6	0.3
	4-	1.4	2.8	2.4	2.3	2.1	8.8	3.1
	6-	0.4	0	0.3	0.9	1.3	0	0.6
	3,4-	0	0	0	0	0.6	0	1.7
	4,6-	0	0	0	0	0.6	1.6	1.5
	Terminal	2.0	4.0	0.9	3.2	6.8	6.2	4.3
Rhamnopyranosyl (Rhap)	2-	2.1	1.4	0.4	2	1.5	3.5	2.1
	2,3-	0	0	0	0	0	0	0.4
	2,4-	0.2	0	0	0	0	0	0.2
	Terminal	0	0	0	0	0	0	1.9
Xylopyranosyl (Xylp)	4-	0	0	0.1	0	1	0	0
	2,3,4-	0	0	0	0.9	0	0	0
Arabinopyranosyl (Arap)/ Arabinofuranosyl (Araf) ^a	4- / 5-	0	0	0	0	0	1.4	0
	Terminal(p)	0	2.8	0	0	1.4	0	0
	Terminal(f)	0	3.6	0	1.5	2.2	10.1	0.9
Mannopyranosyl (Manp)	2- / 3-	6.6	5.3	33.4	5.8	9.0	5.4	14.3
	4-	0	0	1.2	0	0	0	1.8
	6-	0.4	0	0.6	0.4	0.5	0	1.3
	2,3-	0.4	0.7	0.6	1	0.8	0	0.5
	2,4-	0	0	0.3	0	0.2	0	0.4
	2,6-	1.2	0.2	4.1	0.5	0.5	0	0
	3,4-	0	0	0	0	0	0	0.4
	3,6-	0	0	0.4	0.3	0.3	0	0
	4,6-	0	0	0	0	0.3	0	0.5
	Terminal	9.3	14.7	20	10.9	15.1	10.9	18.6

^a 4-Arap and 5-Araf peaks co-eluted. The value here was represented by 5-Araf^b Uronic acids were reduced to 6,6'-dideuterio neutral sugars before methylation
0, not detected

Table 4.5. Glycosyl residue linkage analysis of SCWCs from Chardonnay wines^a (continued)

Glycosyl Residue	Deduced Glycosidic Linkage	Linkage Composition (mol %) / WAV						
		6	7	8	9	10	11	12
Galacturonopyranosyl ^b (GalAp)	2-	0	0	0.1	0	0	0	0
	3-	1.7	0.4	0	1.2	0.4	0	0
	4-	40.2	30.2	15.4	29.5	21.6	20.9	12.7
	2,4-	0.5	0.3	0.1	0.6	0.1	0	0.2
	3,4-	3.3	3.6	0.5	5.5	1.2	0	2.2
	Terminal	17.7	14.2	6.8	20.2	13.6	10.3	10
Glucuronopyranosyl ^b (GlcAp)	4-	0.2	0	0.2	0.3	0.2	0	0.3
	Terminal	1.1	1.4	0	1.1	1.1	1.2	1.3
Galactofuranosyl (GalF)	Terminal	0.2	0.6	0	0.5	0.8	1	0.6
Galacturonofuranosyl ^b (GalAf)	Terminal	3	8.4	0.3	8.7	8.3	9	1.4

^a 4-Arap and 5-Araf peaks co-eluted. The value here was represented by 5-Araf

^b Uronic acids were reduced to 6,6'-dideuterio neutral sugars before methylation
0, not detected

The 4-GalAp and t-GalAp residues appear to have originated from homogalacturonan (HG), because the abundance of Rha residues is relatively low (Table 4.5). In this study, 4-Arap and 5-Araf residues co-eluted in the chromatogram of the linkage analysis. Here, the value is attributed to 5-Araf because it is known that arabinan containing 5-Araf residues is found in the side chains of pectic polysaccharides (Mohnen, 2008). Examination of the Man residues shows that the major contributors to mannans in the wine are yeast (1,2/1,3;1,6)-linked mannans, although some grape (1,4)-mannans are likely to be present (Table 4.5). Besides yeast mannan, 4-Glcp residues, which are present in relatively low abundance (Table 4.5), suggests that some yeast α -(1,4)-glucans are also present. This polysaccharide is present in the outermost regions of fungal cell walls and is more soluble than the β -glucans that form the rigid core of cell walls (Barreto-Bergter and Figueiredo, 2014). Kwiatkowski et al. (2009) indicated that α -(1,4)-glucan is covalently bound to β -(1,6)-glucan in *Saccharomyces cerevisiae* cell walls. Here, 4-Glcp residues are assigned to yeast glucan (Table 4.5), together with 3-Glcp, 6-Glcp and 3,6-Glcp residues. It is acknowledged that 4-Glcp residues might also have come from either degraded XG or cellulose. Analysis of the Gal residues indicates that 3-Galp, 6-Galp and 3,6-Galp residues are relatively abundant. Thus, this suggests that type II arabinogalactans (AG) of

the AGP type are abundant in the wine samples. The high levels of t-Araf residues indicates the abundance of pectic polysaccharides and type II AGs.

The methylation analyses in Table 4.5 indicate that terminal residues of many monosaccharides are relatively high. As noted in Chapter 3, this suggests that the SCWCs of the wine are of low molecular weight and/or highly branched. This is discussed in more detail in the section “Size Exclusion Chromatography” below (section 4.3.5.).

4.3.4. Estimation of Polysaccharide Composition

The linkage analyses performed here enable the abundance of various classes of polysaccharides to be estimated (Table 4.6). These estimations are based on methods that use known structures of commonly occurring plant and yeast cell wall polysaccharides (Nunan et al., 1998; Caffall and Mohnen, 2009; Burton et al., 2010; Pettolino et al., 2012).

Table 4.6. Probable sources of SCWCs in Chardonnay wines

Polysaccharide ^a	Relative Amount (mol %) / WAV						
	6	7	8	9	10	11	12
G-Arabinan	0	0	0	0	0	1.4	0
G-Type I Arabinogalactan (AG I)	0	0	0	0	0.4	0	2.0
G-Type II Arabinogalactan (AG II)	8.7	4.2	11.1	1.4	6.9	5.5	15.6
G-Homogalacturonan (HG)	57.9	44.4	22.2	49.7	35.2	31.2	22.7
G-Rhamnogalacturonan I/II (RG I/II)	6.2	9.4	1.1	10.8	4.3	3.5	7.7
G-Heteroxylan (HX)	0	0	0.1	0.9	1.0	0	0
G-Heteromannan (HM)	0	0	2.4	0	0.6	0	4.6
G-Xyloglucan (XG)	0	0	0	0	0.6	3.2	2.0
Y-Glucan	4.2	7.9	2.9	7.2	10.9	17.0	5.5
Y-Mannan/Mannoprotein	17.5	20.2	58.5	17.9	25.4	16.3	34.2
Unassigned ^b	5.5	13.9	1.7	12.1	14.7	21.9	5.7

^a G - denotes grape derived SCWCs; Y- denotes yeast derived SCWCs

^b Sum of linkage types that cannot be assigned to well-characterized SCWCs from plants and yeast
0, not detected

Thus, estimates of SCWCs of Chardonnay wines were calculated from the linkage analyses and were based on the summation of the mol % of individual glycosyl residues and linkage types that have been described in the literature for well-characterized or pure polysaccharides (Doco and Brillouet, 1993; Nunan et al., 1998; Kwiatkowski et al., 2009; Pettolino et al., 2012).

The results are shown in Table 4.6. The complexity and heterogeneity of cell wall carbohydrates complicated the unequivocal identification of the types and proportions of the various carbohydrates in the test samples. The complexity also prevented the accurate, independent estimation of the RG I and RG II polysaccharides, so the abundances of these were expressed in a single value for rhamnogalacturonans (Table 4.6).

Of the total of ten different types of carbohydrates (Table 4.6), it is clear that the pectic polysaccharides, HG and RG I/II, derived from the grape berries are the major components. On a mol % basis, derivatives from pectic polysaccharides constituted between 23.3 % and 64.1 % of the total SCWCs in Chardonnay wines in this study. In all cases, the proportion of HG was greater than that of RG I/II. The amount of RG I/II remained stable during ripening, except for a drop to 1.1 % on WAV 8. The RG I/II content observed in this study was lower than that in a previous study of a Verdejo wine (Vidal et al., 2003). This discrepancy is likely because the wines were from different grape varieties and analytical methods employed to deduce the polysaccharide composition were slightly different. Wine RG II is structurally similar to the RG II found in the primary cell wall of sycamore, suggesting that it is highly resistant to fermentation conditions (O'Neill et al., 2004) and is stable at the wine pH range that favors borate cross-linked dimers (Pellerin et al., 1996).

As mentioned above, type II AGs of the AGP class were readily detectable in the Chardonnay wines (Table 4.6). Relatively low levels of the hemicelluloses HM, HX and XG were present in the wines during the ripening period (Table 4.6). While Apolinar-Valiente et al. (2015) reported the presence of trace amounts of hemicellulose in red wines, there are no reported findings of hemicelluloses in white wines. Other major constituents of the SCWC fractions originated from yeast during the fermentation stage of the winemaking process (Table 4.6).

Yeast cell wall polysaccharides constituted 21.7 – 61.4 mol % of the total polysaccharides in the wines (Table 4.6). Here, yeast mannan was the main factor driving the wide range of mol %. The changes in yeast mannan might be associated with the yeast cell numbers (not recorded in this study) during fermentation. According to Del Barrio-Galán et al. (2011), mannoprotein comprised 25 % of the

total polysaccharides in red wines (Vidal et al., 2003) and 58 % in Verdejo white wine. Comparatively, yeast glucan remained relatively constant in the wines until WAV 9, temporarily increased to 17 mol % on WAV 11 and then decreased to a final concentration of 5.5 mol %. A somewhat surprising observation is that the levels of yeast glucan in the white Chardonnay wines (Table 4.6) are much higher than those in the red Cabernet Sauvignon wines analyzed in Chapter 3 (see Table 3.4 of Chapter 3). It is clear that the overall yeast SCWC contents vary greatly, both between and within varieties, and this is most likely attributable to the fact that polysaccharides released from yeast are dependent on specific winemaking techniques and yeast strains.

In this study, AG II was relatively more abundant than arabinan and AG I across all wine samples (Table 4.6). Both Ara and Gal residues are the predominant neutral glycosyl residues of the side chains of RG I, which are composed of arabinan and AG I. It has been suggested that AG II might also be covalently linked with pectic polysaccharides (Mohnen, 2008; Tan et al., 2013). The side chain ratio of RG I may vary with the physiological state in plants (Mohnen, 2008) and RG I with fewer side chains is suggested to be more soluble (Vidal et al., 2001).

Many of the glycosyl linkage types could not be readily assigned to well-characterized cell wall polysaccharides from plants and yeast, and are therefore listed as “Unassigned” in Table 4.6. The specific residues of the “Unassigned” component shown in Table 4.6 are listed in Supplementary Table S4.2. Unassignable residues are commonly observed when glycosyl linkage analyses are used to estimate polysaccharide composition, but as described in detail in Chapters 2 and 3, values of about 10 % or less are usually found Pettolino et al. (2012). The higher values listed in Table 4.6 could result from under-methylation, from aberrant residues generated during the analyses, or from bacterial extracellular polysaccharides. As noted in Chapter 3, the unusual 5-ring glycosyl linkage types observed here, t-GalAf and t-Galf, have not been reported previously and are likely artifacts of the methylation method. The 2,3-, 2,4- and 3,4-Manp residues have not been previously reported in plant HM or yeast mannan and hence cannot be assigned to known polysaccharide types in Table 4.6. It is also possible that these Man residues might result from undermethylation.

The high levels of terminal residues observed in the linkage analyses (Table 4.5) once again suggest that the SCWCs in white wine are of low molecular weight, presumably because the cell wall polysaccharides are extensively degraded during the winemaking process, as described in detail in Chapter 3. As a result, the methods for estimating 'parent polysaccharide' composition presented by Pettolino et al. (2012) and outlined in Chapter 2 required significant modification here (see section 3.3.5. of Chapter 3). To check the apparent low molecular weight of the SCWCs, they were subjected to size exclusion chromatography.

4.3.5. Size Exclusion Chromatography

As mentioned in Chapter 3, the high levels of terminal residues observed in the linkage analyses (Table 4.5), probably indicate that the SCWCs found in the Chardonnay wines were derived from oligosaccharides of low molecular weight or from highly branched and substituted polysaccharides such as RG II. The molecular sizes of the SCWCs from the Chardonnay wines were therefore examined by high-performance size exclusion chromatography (HPSEC). The elution profiles revealed that there were low levels of high molecular weight polysaccharides in the samples (Figure 4.1) but that the major peak eluted much later than the elution time of the smallest dextran standard of 5,000 MW. This suggests that the SCWCs in the Chardonnay wine are mostly low molecular weight oligosaccharides, which is consistent with the linkage analyses shown in Table 4.5. As noted above, these data should not be over-interpreted, but they are nevertheless in general agreement with the results of Apolinar-Valiente et al. (2015), who reported that wine oligosaccharides contain between three and 15 monosaccharide residues. Thus, the SCWCs in all wines had similar size distribution profiles, with relatively high levels of oligosaccharides. This observation is in agreement with the high proportion of t-GalAp in relation to 4-GalAp, which suggests the presence of short HG oligosaccharides (Table 4.4).

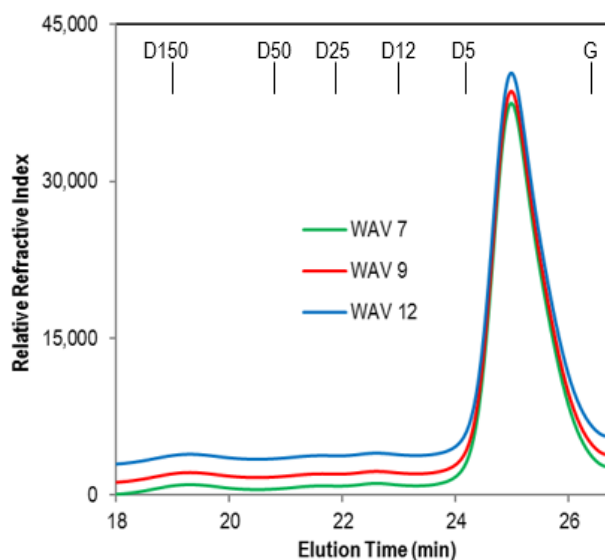


Figure 4.1. The elution profiles of SCWCs from WAV 7, 9 and 12 (other data not shown). Inserts show elution times of dextran standards (D x 1000 MW); G, glucose

Although it is expected that the addition of the commercial pectolytic enzyme preparation mixture into the mechanical crushed berries, which is standard procedure for the production of white wines, would lead to the degradation of pectic polysaccharides, extensive degradation of pectins was also observed in red wine (Chapter 3), where exogenous enzyme was not added. To characterize the oligosaccharide structures detected here in the SCWCs from Chardonnay wines, more advanced techniques such as matrix assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI) in conjunction with mass spectrometry would be required.

4.3.6. Relationship between The Sensory and Chemical Properties of Wine

The estimated polysaccharide and oligosaccharide contents in the SCWC samples (Table 4.6) were used to investigate any statistically significant correlations between SCWC composition and sensory characteristics. The mean intensity ratings of the aroma, mouthfeel and taste sensory characteristics for the wines are presented in Supplementary Table S4.3. Analysis of variance (ANOVA) of the DA data indicated that all sensory characteristics, except citrus aroma, were significantly different ($p < 0.1$). The PCA was performed on the mean intensity ratings of the key

sensory characteristics of all wines from different ripening stages. Principal Component (PC) 1 and PC 2 accounted for 88.1 % and 9.1 % of the variation, respectively (Figure 4.2).

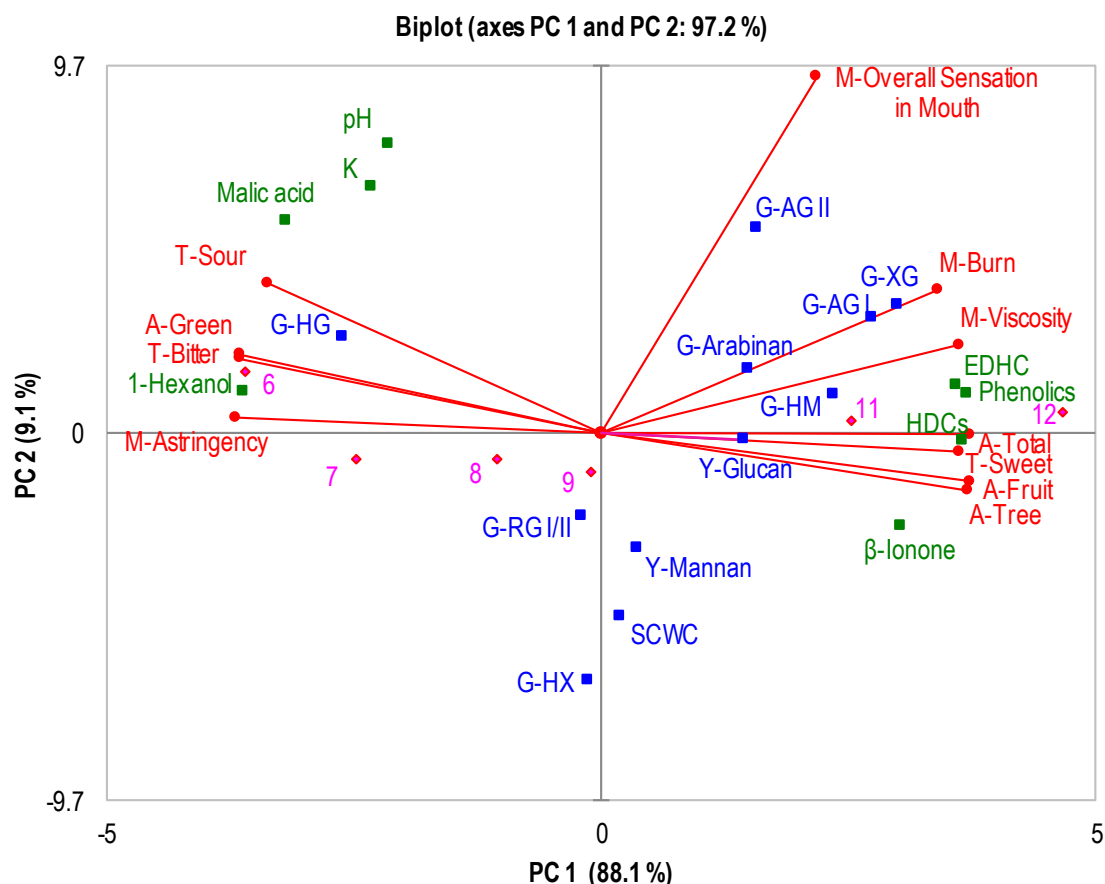


Figure 4.2. Principal component analysis of taste, aroma and mouthfeel sensory, and chemistry attribute ratings of Chardonnay wines. Only variables that showed a significant difference between wine samples across treatments, represented by WAV (indicated in pink diamonds) are shown ($p \leq 0.05$; $p \leq 0.1$) with the exception for polysaccharide composition. Sensory metrics (indicated in red circles); polysaccharides (indicated in blue squares); and other chemical metrics (indicated in green squares). G- denotes grape derived polysaccharide; Y- denotes yeast derived polysaccharide. Sensory characteristics (T- denotes taste; A- denotes aroma; M- denotes mouthfeel; A-Total, total fruit aroma; A-Tree, tree/stone fruit aroma). HDCs, hydroxycinnamates; EDHC, ethyl dihydrocinnamate

The PC 1 mainly separated the wines on the basis of taste, aroma and mouthfeel. The variation in the wines was almost entirely explained by PC 1. Wines from the later ripening stage were located in the right quadrants, whereas wines from the earlier harvest were located in the left quadrants (Figure 4.2). A distinct separation of the two major clusters of sensory characteristics was observed. Sour and bitter taste, green aroma, and astringent mouthfeel were clustered together in the left quadrants, and they are usually perceived as negative characteristics that adversely affect wine quality and consumer liking. These characteristics were correlated with wines from the earlier harvest.

As the ripening level of the fruit increased, the wines were progressively perceived to have a sweeter taste, higher fruit aroma, and greater burn and viscosity (used here as a sensory descriptor) mouthfeel, as indicated by the shift of the WAV from the left quadrants towards the right quadrants. Previous studies have reported that mouthfeel and aroma intensity were higher and that acidity, astringency and bitter taste were lower in Chardonnay, Cabernet Sauvignon and Tempranillo wines made from riper grapes (Gil et al., 2012; Gambetta et al., 2014).

The chemical characteristics that significantly differed were overlaid on the PC plot for matrix analysis. PC 1 suggests that the separation is primarily driven by the polysaccharide type, aroma and phenolic compounds. It is clear that HG, which was in the upper left quadrant, was isolated from the majority of the polysaccharides, which were predominantly located in the right quadrants. The acid parameters of pH and malic acid, together with potassium, were closely correlated with sour taste in the upper left quadrant. Wine pH is influenced by berry potassium and the total amount of acid (Conde, 2007,). The presence of potassium has been linked with acids and pH, and musts with high potassium contents tend to have high pH values and malate contents (Jackson and Lombard, 1993). Fruits at the early ripening stage tend to result in wines with excess acidity and a sugar deficiency (Kennedy, 2008). Green aroma, bitter and sour taste, and astringent mouthfeel were present in the same quadrant. This is not surprising because wine pH is known to contribute to sensory acidity and astringency (Gawel et al., 2014), whereas malic acid relates to a green note in the wine (Conde, 2007,).

In this study, the correlation between green aroma with 1-hexanol, which has odor descriptions of “green grass”, “toasted”, “herbaceous” and “dry”, is not surprising (Aznar et al., 2001; Lee and Noble, 2003; Gómez-Míguez et al., 2007; González-Barreiro et al., 2015). Furthermore, 1-hexanol, a major C6 compound responsible for the green aroma in wines, decreases during grape ripening due to changes in the lipoxygenase pathway (Kalua and Boss, 2009). White wines produced from grapes in early ripening display more “herbaceous” notes than those obtained from riper grapes (Gómez-Míguez et al., 2007; González-Barreiro et al., 2015). Our results on the correlation between

green aroma and 1-hexanol, which infers their chemical and sensory relationship, agree with previous reports. Although 1-hexanol has never been described as a taste or mouthfeel compound in wine, its correlation with bitter taste and astringent mouthfeel is clear (Figure 4.2). Similarly, HG, which was separated from all other SCWCs in the right quadrants, was in close proximity with sour and bitter taste, green aroma and astringent mouthfeel. Thus, HG exhibited a strong correlation with these sensory characteristics. The correlation of HG to sour taste in the sampled wines is not unexpected given the acidic nature of this polysaccharide. However, the reason for the apparent association of HG with bitter taste, green aroma and astringent mouthfeel is unclear. During the modification of the physical properties of a food matrix, physiochemical reactions can occur between existing aroma compounds and the food matrix. In a model food matrix containing pectin, cis-3-hexenol, which is a C₆ volatile compound similar to 1-hexanol, was 'repulsed' from the food matrix. This effect was attributed to the increase of cis-3-hexenol diffusion from the food matrix to the vapor phase (Seuvre et al., 2006).

Multimodal interactions may influence the correlations between chemical compounds that have no clear functions on taste, mouthfeel or aroma, as observed in the correlation between HG with bitter taste. Verhagen and Engelen (2006) illustrated that the decrease of perceived sensory intensity was not attributable to a decrease in the actual concentration of the stimulant measured in human subjects, but rather a change in texture, such as viscosity of the food matrix. This suggests that the integration of signals from different sensory modalities modulate sensory perception. Another explanation for these correlations involves sensory interactions at the cognitive level (Sáenz-Navajas et al., 2012). In a yogurt study, Kora et al. (2003) found that the presence of aroma notes, such as green apple, caused tasters to associate it with their experience of the astringency of unripe apples and give higher astringency scores to the test sample. The correlation between 1-hexanol, which has a dominant green grassy note, and a bitter taste and astringent mouthfeel supports this hypothesis.

In the right quadrants, XG and AG I correlated strongly with viscosity and burn mouthfeel, despite the low levels of this polysaccharide in the wines (Table 4.6). While not including viscosity as

a wine sensory attribute in their evaluation, Quijada-Morín et al. (2014) reported that in Tempranillo red wines, the oligosaccharide fraction containing Xyl residues negatively correlated with astringency. We found the same correlation in our current study. Comparatively, a slightly weaker correlation was observed here between HM, AG II, arabinan and yeast glucan with viscosity and burn mouthfeel (Figure 4.2). The correlation between XG and AG I with burn mouthfeel is contradictory to the assumption that a burn mouthfeel is mainly affected by the ethanol concentrations in wines (Figure 4.2). In the current study, XG and AG I might not have contributed directly to a burn mouthfeel but instead might have modulated the burn sensation at the receptor levels. In this study, AG II, arabinan, HM and yeast glucan showed a weaker correlation with these sensory characteristics compared with XG and AG I. Although the overall sensation in mouth did not strongly correlate with any specific chemical compounds, the appearance of most SCWCs in the same quadrant suggests that these SCWCs collectively affect the overall sensation in the mouth.

The clustering of SCWC content, RG I/II, HX and yeast mannan near the middle of the PCA plot indicated they have minimal to no relationship with the sensory characteristics, as explained by PC 1 (Figure 4.2). Aside from SCWCs, phenolics and HDCs also showed a strong correlation with sensory viscosity (Figure 4.2). Although phenolics have been linked to a bitter taste and astringent mouthfeel in many red wines, their effects in white wines appear to be very different. Test et al. (1986) reported that a significant increase in the phenolic content of Chardonnay wines did not translate into a proportionate increase in bitterness on a duo-trio difference test. Similarly, Gawel et al. (2014) reported that a higher phenolic content is associated with an increased perceived viscosity, but not astringency, and subsequently suggested that this is likely the result of insufficient hydrogen bonding sites for salivary proteins in white wine phenolics. Furthermore, Smith and Waters (2012) demonstrated that the addition of phenolic extracts from a Gewürztraminer wine into a Chardonnay base wine increased the sensory viscosity of the Chardonnay base wine. The correlation between phenolics and viscosity observed in the current study is in agreement with previous reports. Hydroxycinnamates (HDCs), which are the most dominant class of phenolics in white wines, displayed

a slightly different correlation pattern with wine sensory characteristics (Figure 4.2). Vèrette et al. (1988) reported that HDCs did not play a direct role in the bitter taste of white wine using a duo-trio difference test. In agreement with this finding, the PCA clearly confirms that phenolics and HDCs had no effect on bitterness (Figure 4.2). HDCs did not show any positive correlations with bitter taste. In contrast, HDCs grouped closely to a distinct combination of fruit, total fruit and tree/stone fruit aroma, and sweet taste in the lower right quadrant of the PCA (Figure 4.2). While HDCs may not have a direct relationship with wine sensory perceptions, the presence of HDCs in the right quadrant (Figure 4.2) could be a result of the proportion of esterified HDCs to grape SCWCs in the late fruit ripening stages of WAV 11 and WAV 12. It is known that the esterification of HDCs to polysaccharides mediates cross-linking between polysaccharides in plant cell walls to strengthen the walls (Bunzel, 2009).

Based on their respective positions on the PCA plot, ethyl dihydrocinnamate (EDHC) and β -ionone were key drivers of the wine aroma of total fruit, fruit and tree/stone fruit (Figure 4.2). These compounds have been reported to impart fruity and floral aroma characters in wines (Aznar et al., 2001; Bowen and Reynolds, 2012). Notably, β -ionone was most strongly correlated with tree/stone fruit (Solís-Solís et al., 2007). This observation is well supported by previous studies that identified β -ionone as a common contributor to the fruity notes of apricot cultivars (Erdogan-Orhan and Kartal, 2011).

PC 2 contains the remaining variance in the normalized data set (Figure 4.2). It can be argued that the separation observed on PC 2, which accounted for 9.1 % of the variation, might not fully explain the correlations between variables. However, the information obtained from PC 2 suggests meaningful correlations between variables. All sensory characteristics were significantly different in their intensity ratings ($p < 0.1$) (Supplementary Table S4.3) despite their proximity on PC 2.

Here, the linkage data provided greater in-depth insights to differentiate the relationships between individual polysaccharides and their respective effects on wine sensory characteristics and on the identification of specific key drivers that might be used to predict targeted sensory characteristics. PC 2 revealed that HG was highly associated with sour taste, whereas XG and AG I

appeared to have stronger positive correlations with burn and viscosity mouthfeel (Figure 4.2). Notably, yeast mannan and glucan did not correlate strongly with taste, aroma and mouthfeel. The correlations between phenolics, EDHC, HDCs, and β -ionone with fruity aroma characteristics were more pronounced on PC 2.

In addition to the SCWC composition, the molecular size of polysaccharide may influence wine mouthfeel characteristics. According to Apolinar-Valiente et al. (2015), red wine oligosaccharides could influence the perception of astringency. However, this aspect needs further research.

Attempts at linking chemical analyses with unimodal sensory perception do not always provide conclusive results because of the complexities of wine and human sensory perception at the receptor and cognitive levels. Few studies have focused on the association of specific wine chemical compounds with multimodal sensory responses. In the current study, sweet taste was obviously clustered with fruity aroma sensory characteristics on the PC plot (Figure 4.2), suggesting the potential influence of multimodal interactions. A highly trained sensory panel reported an increased sweet taste and decreased bitter taste and astringent mouthfeel when fruity aroma extracts from Chardonnay wines were added into model wines (Sáenz-Navajas et al., 2010). Subsequent investigations found that aroma perception plays a significant role on in-mouth perception and could change the sweetness perception of red wines (Sáenz-Navajas et al., 2012). Interactions can also occur at the cognitive level. Gawel et al. (2007) reported an association of sweetness with fruit flavor in Riesling wines that may be derived from the sensory experience when consuming ripe fruits. A multimodal interaction approach of evaluating data must be used to fully understand the sensory response to wines because wine is a complex matrix that requires integration of multiple diverse factors.

In general, the average SCWC content in Cabernet Sauvignon wines that were fermented with grape skins was twofold higher, on a weight basis, than the average content in Chardonnay wines, which were made from pressed juice, without skins (Table 4.7). The neutral polysaccharides collectively affect the overall sensation in the mouth, irrespective of wine type. In Cabernet Sauvignon arabinan, AG II and XG were closely related to astringency, chalky, coating, roughness, viscosity and

burn (Table 4.7), whereas in Chardonnay wines, AG I and XG were strongly related to burn and viscosity. It is obvious that HG consistently correlated with sour and bitter taste in both Cabernet Sauvignon and Chardonnay wines (Table 4.7). In both studies, HX and yeast polysaccharides showed minimum effect on mouthfeel (Table 4.7).

Table 4.7. Comparison between Cabernet Sauvignon and Chardonnay wine SCWCs and their correlations with wine sensory attributes

Polysaccharide	Cabernet Sauvignon ^a		Chardonnay	
	% mol ^b	Sensory Attributes ^c	% mol	Sensory Attributes ^c
G-Arabinan	0 - 0.3	OS, astringency, chalky, coating, roughness, viscosity, burn	0 - 1.4	OS
G-AG I	0 - 1.7	OS	0 - 2.0	OS, burn, viscosity
G-AG II	4.6 - 19.4	OS, astringency, chalky, coating, roughness, viscosity, burn	1.4 - 15.6	OS
G-HG	27.4 - 72.0	sour, bitter	22.2 - 57.9	sour, bitter, astringency
G-RG I/II	8.5 - 28.6	OS	1.1 - 10.8	m
G-HX	0 - 0.6	m ^d	0 - 1.0	m
G-HM	0 - 0.8	OS, chalky, coating	0 - 4.6	OS, viscosity
G-XG	0 - 1.6	OS, astringency, chalky, coating, roughness, viscosity, burn	0 - 3.2	OS, burn, viscosity
Y-Glucan	2.9 - 7.4	OS, burn	2.9 - 17.0	OS
Y-Mannan/Mannoprotein	2.3 - 10.0	OS, burn	16.3 - 58.5	m
SCWCs (mg/L)	3098 ^d		1486 ^d	

^aresults reported in Chapter 3 of this thesis

^brange of polysaccharide present across wine samples

^csensory attributes used here are specific to wine type; OS, overall sensation in the mouth; m, minimum to no sensory effect

^daverage SCWCs content of all wine samples

4.4. Conclusions

Here, we have fully characterized the compositions of grape and yeast SCWCs from Chardonnay wine. Both SCWCs and phenolic compounds have been shown to play important and approximately similar roles in affecting wine aroma, taste and mouthfeel characteristics. Previously, it was believed that phenolics and related compounds from yeast, such as mannoprotein, were largely responsible for the mouthfeel sensory characteristics perceived in white wine. The results from this

study provide new insights from a comprehensive assessment of the relationships between chemical composition and the sensory characteristics of the studied wines. It is obvious that wine sensory characteristics can be differentiated on the basis of SCWC families. HG was related to bitter and sour tastes, and astringency, whereas HX, RG I/II and yeast mannan had no significant effect on mouthfeel. Neutral grape polysaccharides such as XG, AG I and HM were positively correlated with viscosity and burn mouthfeel. It is important to point out that the overall sensation in the mouth was almost entirely influenced by neutral SCWCs, including AG II, arabinan and yeast glucan. The major findings here for the Chardonnay white wine are similar to the findings reported in Chapter 3, which featured the study of Cabernet Sauvignon wines. However, it should be noted that sensory characteristics such as chalky, coating and roughness are measured in red wine but not in white wine.

The wine industry strategically applies viticulture practices and winemaking techniques to successfully produce wines that meet consumer expectations of sensory experience. In this study, the important SCWC contributors to aroma, taste and mouthfeel have been identified. The low molecular weights of the SCWCs indicate that any contribution to mouthfeel is likely to result from the chemical attributes of the SCWCs, rather than their physical viscosity. The chemical attributes will be detected by specific epithelial membrane receptors in the mouth and it will be possible in the future to explore the specificity of the receptors for SCWCs, such as those that link HG and sour taste. In addition, it will be possible to devise rapid, simple assays for individual monosaccharides during winemaking that will predict selected sensory characteristics in the final wine, as also mentioned in Chapter 3.

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4.7. Supplementary Material

Supplementary Table S4.1. Mouthfeel (M), taste (T) and aroma (A) attribute list with definitions and reference standards

Attribute	Definition	Reference Standard*
M-Astringency	Drying sensation on tongue and pulling in and tightening of cheeks and mouth surfaces, sensed without moving tongue over surfaces	Grape seed extract
M-Burn	Burning/warming sensation on surfaces of mouth; heat	Grain neutral spirits
M-Chalky	Subcategory of mouth coating, chalky, powdery coating perceived after expectoration.	Milk of Magnesia
M-Coating	Any sensation that a coating has been left on the surfaces of the mouth after expectoration, especially sensed by moving tongue over surfaces of the mouth, roof, teeth, and gums	
M-Roughness	Roughness of mouth surfaces, sensed by running tongue over surfaces, especially roof of mouth, includes roughening of tongue's surface	Concentrated juice from Zinfandel ^a
M-Viscosity	Thickness, weight of wine, increase or decrease in force necessary to move tongue through liquid, must be evaluated while wine is in mouth. Includes syrupy mouthfeel	Gum Arabic ^b
M- Overall Sensation in Mouth	Includes the mouth feel sensations of chemical feeling factors (alcohol burn, astringency and spritz) and texture (mouth roughness, viscosity and mouth coating); may also include basic taste sensations but not the actual taste; what you feel when wine is in your mouth, what sensations you have on the surfaces of your mouth after expectoration; an overall perception of what is felt in the mouth	
T-Sweet	Sweet taste	Sucrose
T-Sour	Sour/acidic taste	Tartaric acid
T-Bitter	Bitter taste	Caffeine
A-Total Fruit	Fruit	
A-Fruit	Fruit	
A-Citrus	Lemon, lime, orange	Lemon juice, lime juice, orange juice
A-Tree/Stone Fruit	Pear, apple, peach, apricot	Pear nectar, apple juice, peach nectar, apricot nectar
A-Green	Fresh green, stemmy, cut grass, bell pepper	wheatgrass, tomato stems, green bell pepper

*Standards are presented in E & J Gallo standard commercial white wine

Supplementary Table S4.2. Unassigned glycosyl residues

Unassigned Glycosyl Residue	Deduced Glycosidic Linkage	Linkage Composition (mol %) / WAV						
		6	7	8	9	10	11	12
Galactopyranosyl (Galp)	2-	0	0	0.1	0	0	0	0
Glucopyranosyl (Glc p)	2-	0	0	0.1	0.4	0.3	0.6	0.4
	3,4-	0	0	0	0	0.6	0	1.7
Arabinopyranosyl (Arap)/	Terminal(p)	0	2.4	0	0	1.4	0	0
Arabinofuranosyl (Araf)	Terminal(f)	0	0	0	0	0.6	10.1	0
Mannopyranosyl (Manp)	2,3-	0.4	0.7	0.6	1.0	0.8	0	0.5
	2,4-	0	0	0.3	0	0.2	0	0.4
	3,4-	0	0	0	0	0	0	0.4
Galacturonopyranosyl ^a (GalAp)	2-	0	0	0.1	0	0	0	0
	3-	1.7	0.4	0	1.2	0.4	0	0
Glucuronopyranosyl ^a (GlcAp)	4-	0.2	0	0.2	0.3	0.2	0	0.3
	Terminal	0	1.4	0	0	1.1	1.2	0
Galactofuranosyl (Gal f)	Terminal	0.2	0.6	0	0.5	0.8	1.0	0.6
Galacturonofuranosyl (GalAf)	Terminal	3.0	8.4	0.3	8.7	8.3	9.0	1.4

0, not detected

Supplementary Table S4.3. Mean intensity ratings for sensory attributes of Chardonnay wines during ripening 2012^a

Attributes ^b	WAV					
	6	7	8	9	11	12
M-Burn	5.1ab	5a	5.1ab	5.1b	5.3c	5.4c
M-Astringency	1.3d	1.2cd	1.0bc	1.0bc	0.8ab	0.6a
M-Viscosity	3.0a	3a	3.1a	3.1a	3.3b	3.4b
M-Overall Sensation in Mouth	5.8ab	5.7a	5.7a	5.7a	5.8b	5.9b
T-Sweet	2.4a	2.5a	2.6a	2.9b	2.9b	3.2c
T-Sour	6.3c	6.1b	6.1b	6.0ab	5.9a	5.9a
T-Bitter	4.4d	4.1c	4.0c	4.0c	3.7b	3.5a
A-Total	4.0a	4.1ab	4.2bc	4.4c	4.6d	4.8e
A-Fruit	3.2a	3.4b	3.6b	3.8c	4.0d	4.3e
A-Citrus	2.0	1.9	1.9	2.0	1.9	1.9
A-Tree/Stone Fruit	2.6a	2.9b	3.1bc	3.3c	3.5d	3.8e
A-Green	2.3d	2.1c	2.0bc	2.0bc	1.9b	1.7a

^aMean within a row followed by different letters indicate are significantly different (P = 0.1, one-way ANOVA, Tukey's posthoc).^bM, mouthfeel attributes; T, taste attributes; A, aroma attributes

Chapter 5

Changes in Soluble Cell Wall Carbohydrates During Grape Berry Maturation

Preface

In accordance with University of Adelaide thesis guidelines, this Chapter is presented in manuscript format. Here, the Materials and Methods section, together with calculations associated with linkage analyses, are described at the level expected in common plant and food journals. Full details of the materials and precise descriptions of the methods used are provided in Chapter 2 of the thesis (Development and Adaptation of Methods). Some minor repetition was therefore necessary between Chapters 2, 3, 4 and 5 in these Materials and Methods sections. In addition, repetition would be likely in the Results and Discussion sections of Chapters 3, 4 and 5. Formats for the text, referencing, figures and tables have been standardized throughout the thesis.

Changes in Soluble Cell Wall Carbohydrates During Grape Berry Maturation

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Keywords: Cabernet Sauvignon, Chardonnay, fungal, mouthfeel; polysaccharides; taste; wine

Abstract

The soluble cell wall carbohydrate (SCWC) contents and compositions were examined in Chardonnay and Cabernet Sauvignon grape (*Vitis vinifera* L.) berries harvested at different stages of maturity. Ten types of grape- and fungal-derived SCWCs were identified by linkage analysis in both cultivars. Homogalacturonan was present in a larger proportion than rhamnogalacturonan I/II. The trends of arabinogalactan I and II were similar but opposite those of rhamnogalacturonan I/II, and heteromannan was the most abundant hemicellulose. The overall results indicate that no major changes occurred in total grape-derived SCWC content during ripening of Chardonnay and Cabernet Sauvignon grape berries, but the large changes in SCWC composition suggest modifications of specific polysaccharides. The occurrence in grape berries of homogalacturonan, rhamnogalacturonan I/II, arabinan, arabinogalactan I, arabinogalactan II, heteromannan, and xyloglucan, which are related to wine mouthfeel sensations, correlated with their presence in wines, suggesting that these SCWCs are important sources of mouthfeel. The grape-derived SCWCs could therefore be important wine quality markers, through which winemakers could manage grape harvest timing to optimize the levels of key polysaccharide determinants of mouthfeel and their precursors. In addition, viticultural practices and fermentation conditions could be altered to enhance selected SCWCs and their precursors. All of these viticultural and enological techniques could enable the delivery of consumer-preferred wines.

5.1. Introduction

Plant cell walls consist of a network of cellulose microfibrils embedded in a gel-like matrix of hemicelluloses and pectic polysaccharides. Hemicelluloses of the matrix phase are predominantly represented by xyloglucan (XG), whereas complex pectic polysaccharides are generally categorized as homogalacturonan (HG), rhamnogalacturonan (RG) I and RG II; side chains of arabinan, arabinogalactan (AG) I and AG II might also be present, although the AG II is not always covalently linked with the pectic polysaccharides (Caffall and Mohnen, 2009). According to Nunan et al. (1998), cell wall polysaccharides isolated from the mesocarp of grape berries consist of 30 - 35 mol % cellulose, approximately 10 mol % hemicelluloses and 48 - 53 mol % pectic polysaccharides, with proportions varying slightly at different ripening stages.

Grape berries, which are non-climacteric fleshy fruits, exhibit a double sigmoidal growth curve with three characteristic developmental phases (Coombe and Hale, 1973). The first phase is a period of rapid berry growth through cell division and cell enlargement, the second is a lag phase with minimal or no berry growth, and rapid berry growth resumes in the third phase. At the beginning of the third phase, berry ripening coincides with berry softening, which is also termed “veraison”, and growth is limited to cell enlargement (Coombe and McCarthy, 2000). Cosgrove (2005) noted that fruit softening with cell expansion or loosening is a complex physiological event in which cell wall synthesis, deposition and degradation are highly coordinated. It is widely believed that fruit softening is a consequence of cell wall degradation, associated with the disassembly of the walls and middle lamella and the depolymerization of hemicelluloses and pectic polysaccharides (Saladié et al., 2007; Paniagua et al., 2014). Yakushiji et al. (2001) reported that during the early stages of Kyoho berry softening, the depolymerization of pectic polysaccharides and XG and an overall decrease in hemicellulose and cellulose occurred in the mesocarp tissues. On the other hand, Nunan et al. (1998) indicated that modifications of specific cell wall polysaccharides, such as a decrease in AG I, occurs when ripening Muscat Gordo Blanc grape berries soften. Similarly, Vicens et al. (2009) reported that, during ripening, the cell wall polysaccharide content remained constant but that wall polysaccharides

underwent modifications, such as a slight decrease in galactosyl residues in insoluble polysaccharides and a decrease in the degree of esterification of pectic polysaccharides, from 51 % to 36 % in the skins of Shiraz grape berries.

The majority of grape cell wall studies have focused on understanding the relationship between berry softening and cell wall polysaccharide chemistry during the berry development period, especially between pre- and post-veraison berries and, in a few cases, between pre-veraison and ripened berries (Yakushiji et al., 2001; Huang et al., 2005; Thomas et al., 2008). However, berry softening occurs substantially earlier than the point at which fruit is normally harvested for commercial winemaking. Grapes are normally harvested once the berries achieve the optimal quality, as determined by the accumulation of sugar, color, aroma, flavor and mouthfeel compounds, and minimal amounts of negative organoleptic compounds (Dokoozian and Kliewer, 1996). Limited knowledge exists about the changes and compositions of soluble cell wall carbohydrates (SCWCs) of grape berries during the late stages of maturity, when the commercial harvest for winemaking occurs.

Grape SCWCs are of great enological interest because of their mouthfeel sensory properties in wines. Eight classes of grape-derived SCWCs, namely HG, RG I/II, XG, arabinan, AG I, AG II, heteromannan (HM) and heteroxylan (HX), have been identified in Chardonnay and Cabernet Sauvignon wines made from grapes at different stages of maturity (see Chapters 3 and 4). When analyses were expanded to compare the detailed composition of these SCWCs with specific mouthfeel sensory attributes, it was shown that, irrespective of wine type, the overall mouthfeel sensation was mainly correlated with neutral SCWCs, whereas perceived sour and bitter tastes were strongly correlated with HG, which is an acidic SCWC (see Chapters 3 and 4). In the hemicellulose family, HX had minimal to no effect on wine mouthfeel, but XG and HM were related to various mouthfeel attributes, such as viscosity in both wines, and roughness, astringency and coating in Cabernet Sauvignon wine (see Chapters 3 and 4). These grape SCWCs, which survive fermentation conditions and are soluble in the wine matrix, constitute the precursors of wine polysaccharides and

their oligosaccharide degradation products (Chapters 3 and 4) (Barnavon et al., 2000; O'Neill et al., 2004).

As described in Chapters 3 and 4, SCWCs derived from yeast comprising (1,2/1,3; 1,6)- α -mannans and (1,3;1,6)- β -glucans were abundant in both wines, and measurable levels of bacterial exopolysaccharides were also found. These SCWCs collectively affected the overall wine mouthfeel sensation but their sensory impact was generally lower than the impact exhibited by grape SCWCs (see Chapters 3 and 4). The microbial SCWCs associated with grapes are derived from endogenous sources, whereas those that are present in wines can not only originate from these surface contaminants, but also from inoculated yeast strains or from contaminated manufacturing equipment. The SCWCs of bacterial origin have been disregarded in the present study, because their concentrations in wine are very low (see Chapters 3 and 4).

Commercial grape harvest timing can be optimized to ensure that the compounds that contribute positively to mouthfeel are present in abundance, and/or that negative mouthfeel components are minimized. In this study, the SCWCs of Chardonnay and Cabernet Sauvignon were examined because of their commercial importance in wine sensory quality. The study was aimed at understanding the yields of different grape SCWCs in the juice used for the production of white and red wines. Thus, Chardonnay wine is fermented on juice, whereas Cabernet Sauvignon wine is fermented on both skin and juice. The Chardonnay and Cabernet Sauvignon grapes were collected from approximately four weeks after veraison (WAV) and seven WAV, respectively, until two weeks after the commercial harvest, in order to capture the primary period of harvest, harvest logistics and subsequent winemaking. The study of grape SCWCs from the juice supernatant of the grape homogenate is relevant to the winemaking process and can indicate the total amount of wine carbohydrate mouthfeel precursors. The results found here from grape extracts were linked with the SCWC chemistry data reported previously for the wines themselves (see Chapters 3 and 4), and were expected to facilitate a thorough understanding of the changes and transformations of the compounds from grapes to wine.

5.2. Materials and Methods

5.2.1. Grape Samples

The *Vitis vinifera* L. cultivars Chardonnay and Cabernet Sauvignon were used in this study during the 2012 grape growing season. The planted locations, viticulture practices, and field trial designs were similar to those described in Chapters 2, 3 and 4. Briefly, field experiments, which employed a randomized complete block design, were conducted in a commercial vineyard near Lodi, California. Chardonnay and Cabernet Sauvignon grapevines were planted in close proximity, sharing the same growing environment. Standard viticultural practices, which included fertilization, irrigation, pruning and pest control, were followed. The Chardonnay grapevines were planted in 1991 and grown on Freedom rootstock using a horizontal divided trellis system with 2.1 m x 3.4 m within row and between row spacing. The Cabernet Sauvignon grapevines were planted in 1995 and grown on 5C rootstock using a horizontal divided trellis system with 1.8 m x 3.3 m within row and between row spacing. Nine and 11 different harvest dates (treatments) were each replicated four times for Chardonnay and Cabernet Sauvignon throughout berry development, respectively. Twenty randomly selected clusters from one seven-vine plot within the experimental block were collected weekly, beginning at four weeks after veraison (WAV) and seven WAV for Chardonnay and Cabernet Sauvignon, respectively. The fruit samples were collected at weekly intervals from WAV 4 to WAV 12 (Chardonnay) and from WAV 7 to WAV 17 (Cabernet Sauvignon). The fruit samples were placed in sealed plastic bags and transported in insulated ice coolers to a cold storage unit (4 °C) in the laboratory for processing the next day. All twenty clusters from each fruit sample were de-stemmed using a mechanical destemmer, and the whole berries obtained were divided into two equal portions. One portion was transferred into a sealed plastic bag where the berries were uniformly hand-pressed to prepare a well-mixed juice sample, while the other portion was machine pulverized using a Knife Mill Grindomix GM 200 (Retsch, Haan, Germany) into a berry homogenate sample for laboratory analysis. The juice and homogenate samples of each cultivar were aliquoted according to the requirements of the methods described in Sections 5.2.2 to 5.2.6.

5.2.2. Standard Grape Analysis

As described in Chapter 2, approximately 45 mL juice sample was centrifuged to remove solids, and the clear juice (about 30 mL) was analyzed by WineScan FT-120 Fourier Transform Infrared Spectroscopy (FTIR) (FOSS North America, MN, USA) for °Brix, pH, titratable acidity (TA), tartaric acid and malic acid.

5.2.3. Color and Phenolic Composition

Chardonnay

A total of 0.5 mL 9 g/L sulfur dioxide was added to each 45 mL juice to prevent juice browning until analyzed. The mixture was clarified by centrifugation and filtration (0.45 µm) before analysis. The absorbances at 280 nm (for phenols), 320 nm (for hydroxycinnamates) and 420 nm (for 'brown color') were measured using an Agilent 8453 spectrophotometer equipped with a photodiode array detector (Agilent, CA, USA). The method was adapted from Somers and Ziemelis (1985) as described in Chapter 2

Cabernet Sauvignon

One gram of berry homogenate was extracted with 10 mL 50 % v/v ethanol (adjusted to pH 2 using 12 M HCl for complete extraction of phenolic compounds from whole berries) for 1 h. After centrifugation, 1 mL of extract was diluted with 10 mL 1 M HCl. Following dilution, aliquots of 8 mL were used for the anthocyanin analysis as described in Iland (2004), while the remaining volume was used for the phenolic composition analysis as described in Waterhouse et al. (1999). Anthocyanin was analyzed using an Agilent 8453 spectrophotometer with a photodiode array detector at an absorbance of 520 nm and a cuvette with a path length of 5 mm (Agilent, CA, USA). The reading at 520 nm was used to calculate the concentration of anthocyanin per gram of berry. The phenolic compounds were analyzed using an Agilent 1200 reversed phase high-performance liquid chromatography (HPLC) with a 4.6 X 50 mm Zorbax Eclipse Plus C18 column (Agilent, CA, USA). The elution times were monitored at absorbances of 280 nm, 320 nm, 360 nm, 420 nm and 520 nm, using a diode array detector. The separation was performed using a gradient mobile phase system

consisting of acidified water (0.2 % v/v phosphoric acid) and acetonitrile at a flow rate of 1 mL/min. The phenolic compounds were identified and quantitated by comparison with the absorbance, elution order and peak area of chemical standards, namely gallic acid, (+)-catechin, (-)-epicatechin, caffeic acid, quercetrin, quercetin and malvidin-3-O-glucoside chloride. These methods were described in detail in Chapter 2.

5.2.4. Total Six-carbon (C6) Volatile Aroma Compounds

For each Chardonnaysample, 0.5 mL 20 % sodium dodecyl sulfate (SDS) solution was added to 45 mL of juice until analyzed. For each Cabernet Sauvignon sample, 0.5 mL 20 % SDS solution was added to 35 mL of homogenate, followed by the addition of 14.5 mL of saturated NaCl solution. Each sample of the treated Chardonnay juice (10 mL) and Cabernet Sauvignon homogenate (10 g) was transferred into a gas chromatograph (GC) headspace vial (Microliter, GA, USA), spiked with 10 μ L internal standard solution (hexanal-d₁₂, hexanol-d₁₃ and 2-heptanol) and capped immediately. The GC vials used for Chardonnay juice samples were prefilled with 3 g of NaCl. The method used here was adapted from Siebert et al. (2005). The volatile compounds were analyzed using an Agilent 7890B Series GC that was equipped with a 5973 Series Mass Selective (MS) Detector (Agilent, CA, USA) and a Gerstel multipurpose sampler (Gerstel Inc., MD, USA). The separation was achieved using a 30 m x 0.25 mm x 0.5 μ m DB-wax Ultra Inert capillary column. A Supelco DVB/CAR/PDMS fiber (Sigma-Aldrich Corp., MO, USA) was used to adsorb headspace volatiles in the GC vial during solid phase microextraction (SPME) at 70 °C for 20 min, followed by thermal desorption of volatiles at the GC inlet port with helium carrier gas. The oven temperature was programmed to start at 35 °C and was increased linearly to 275 °C within the 24 min run time. Mass detection was performed using a selective ion mode (SIM) and the qualifying ions monitored, the identification and quantification of peaks were based on the characteristic *m/z* values, the retention time and peak area of chemical standards (see Chapter 2, Sections 2.6). The reporting value is as follows:

$$\text{Total C6 aroma compounds (ppb)} = \text{hexanol (ppb)} + \text{hexanal (ppb)} + \text{trans-2-hexenol (ppb)} + \text{trans-3-hexenol (ppb)} + \text{cis-3-hexenol (ppb)} + \text{trans-2-hexenal (ppb)}.$$

5.2.5. Linalool Precursor Content

As described in Chapter 2, the method used to determine the linalool precursor level in grapes was adapted from Singh et al. (2011) and Chong and Cleary (2012) with slight modifications. The homogenate sample (45 mL) was centrifuged and 10 mL supernatant was collected for the analysis of linalool precursor. The pH of the supernatant was adjusted to pH 12 by the addition of 1.5 mL 10 M NaOH prior to precursor extraction using an Oasis HLB plate. Following extraction, 5 mL of 0.2 M citric acid, pH 3.2 solution was added to 1.2 mL of extract, and hydrolyzed at 100 °C for 45 min. The analysis was performed using an Agilent 6890 GC equipped with a 5973 series MS detector (Agilent, CA, USA). The separation was achieved using a 25 m x 0.25 mm x 0.25 µm DB-5ms Ultra Inert capillary column and linalool d₃ was used as reference standard. The SPME and the data acquisition procedures were the same as for the grape total C6 aroma content method described above. The oven temperature was started at 40 °C and was increased linearly to 250 °C within the 45 min run time.

5.2.6. SCWC Preparation

The method used to determine the SCWC content was outlined in detail in Chapter 2. Supernatant (20 mL) obtained after centrifugation of berry homogenate was treated with five volumes of cold ethanol. The precipitate, designated as the “alcohol-insoluble residue (AIR)”, was recovered by centrifugation, washed twice with ethanol and dried under nitrogen. The AIR was redissolved in water, dialyzed exhaustively against water, freeze dried and weighed. The AIR material was suspended in phenol solution saturated with 500 mM Tris-HCl, centrifuged and the recovered pellet was washed twice with the same phenol solution. The pellet was sequentially treated with ethanol, acetone and chloroform:methanol (1:1 v/v). Before drying, the pellet was washed with acetone and a sintered glass filter was used to separate the acetone from the pellet. The treated pellet was used as the starting material for glycosyl linkage analysis.

5.2.7. Glycosyl Linkage Analysis

The method is described in detail in Chapter 2. Briefly, the uronic acids of the cell wall preparations were reduced to their corresponding 6,6-dideuterio glycosyl residues before methylation. Samples were esterified in 0.5 M methanolic HCl at 80 °C for 20 min, reduced with sodium borodeuteride at room temperature for 2 h, neutralized with acetic acid and dried under nitrogen. Methylation analysis was performed by the Complex Carbohydrate Research Center at (CCRC) Georgia (USA) using the methods described by Ciucanu and Kerek (1984), York et al. (1985) and Heiss et al. (2012). The quantitated peak areas were converted to mol % using the calculations described by Pettolino et al. (2012).

Polysaccharide assignment was based on the linkage structures of well-characterized SCWCs from grapes (Nunan et al., 1998), wines (Pellerin et al., 1996) and fungal (Kwiatkowski et al., 2009; Cuskin et al., 2015), and information in Pettolino et al. (2012)

5.2.8. Size Exclusion Chromatography

The SCWCs were separated using an Ultrahydrogel 250 column (Waters Corp., Milford, MA, USA). The column was equilibrated with 50 mM ammonium formate, pH 5.5. The peaks were monitored by comparison of their elution order with commercial dextran standards. The size exclusion chromatography was performed using an Agilent 1200 liquid chromatography with a refractive index detector (Agilent, CA, USA). The operating parameters were outlined in Chapter 2.

5.2.9. Statistical Analysis

Each replicated set of chemical and sensory data was subjected to analysis of variance (ANOVA) in conjunction with a post hoc Tukey's test using SPSS version 21 (IBM, 2012). These are described in general terms in Chapter 2 and in relation to SCWCs from red and white wines in Chapters 3 and 4.

5.3. Results and Discussion

5.3.1. Grape Chemistry

In the present study, the grapes used for SCWC analysis of juice (white wine) and homogenate (red wine) were the same as those used for the preparation of wines described in Chapters 3 and 4.

Table 5.1. Chardonnay grape chemistry

WAV	Brix	pH	TA (g/L)	Tartaric Acid (g/L)	Malic Acid (g/L)	C6 μg/L	Linalool Precursor μg/L	HDCs (AU)	Phenolics (AU)	Brown Color (AU)
4	16.6 ± 0.5a	2.99 ± 0.02a	12.7 ± 0.1d	5.5 ± 0.9ab	7.9 ± 1.2c	2851 ± 108b	1.75 ± 0.14a	3.5 ± 0.3abc	1.3 ± 0.2a	0.1 ± 0 ab
5	17.7 ± 1.2a	3.00 ± 0.03a	11.4 ± 0.1d	6.0 ± 0.2b	6.6 ± 1.0c	2124 ± 416b	2.04 ± 0.30a	3.6 ± 0.3abc	0.9 ± 0.2a	0.1 ± 0 a
6	20 ± 0.3b	3.14 ± 0.02b	8.3 ± 0.4c	5.1 ± 0.2a	4.4 ± 0.3b	2639 ± 633b	2.45 ± 0.33ab	3.0 ± 0.4a	1.6 ± 0.2a	0.1 ± 0 ab
7	21.8 ± 0.6c	3.30 ± 0.02c	6.9 ± 0.4bc	5.3 ± 0.2a	3.5 ± 0.4ab	1174 ± 157a	4.36 ± 0.34bc	4.0 ± 0.1bcd	2.5 ± 0.3b	0.2 ± 0 ab
8	22.7 ± 0.2cd	3.37 ± 0.02c	6.1 ± 0.3ab	5.5 ± 0.1ab	2.8 ± 0.2a	1364 ± 345a	5.93 ± 0.50c	3.4 ± 0.6ab	2.5 ± 0.3b	0.2 ± 0.1 abc
9	23.9 ± 0.7de	3.46 ± 0.06d	5.8 ± 0.3ab	5.1 ± 0.2a	2.6 ± 0.2a	1324 ± 179a	8.44 ± 1.12d	3.5 ± 0.3abc	3.1 ± 0.4b	0.3 ± 0.1 dc
10	25 ± 0.4ef	3.59 ± 0.03e	5.5 ± 0.3ab	5.0 ± 0.3a	2.8 ± 0.1a	1018 ± 214a	11.14 ± 0.71e	4.3 ± 0.4cd	4.2 ± 0.4c	0.3 ± 0.1 dc
11	26.2 ± 0.5f	3.63 ± 0.04ef	4.8 ± 0.2a	5.1 ± 0.3a	2.2 ± 0.2a	1129 ± 153a	10.79 ± 1.85e	4.7 ± 0.3de	4.8 ± 0.3c	0.3 ± 0 dc
12	26.2 ± 0.3f	3.70 ± 0.04f	5.1 ± 0.2a	5.4 ± 0.3a	2.5 ± 0.5a	925 ± 113a	11.68 ± 1.04e	5.4 ± 0.3e	5.8 ± 0.4d	0.3 ± 0.1 c

Values are the mean of 4 technical replicates. Values followed by different letters indicate a significant difference ($P=0.05$, one-way ANOVA, Tukey's posthoc)

The °Brix, pH, TA, tartaric acid and malic acid were as generally expected of white and red grapes during maturation (Tables 5.1 and 5.2). The linalool aroma precursor, which contributes to the fruit and floral characters of Chardonnay wine (Lee and Noble, 2003), steadily accumulated at WAV 4 - 12 and reached its highest level between WAV 10 and 12 (Table 5.1). In Cabernet Sauvignon berries, polymeric tannins, which are responsible for red wine astringency, increased with maturity (Table 5.2). Many aroma and phenolic compounds that are critical for wine style definitions are produced at a later stage of berry ripening (Gómez-Míguez et al., 2007; Gil et al., 2012). For information only, Chardonnay and Cabernet Sauvignon fruit for commercial wines released by the E & J Gallo Winery were harvested at WAV 10 and WAV 15, respectively. At this time, the abundance

of the favorable compounds for wine quality such as linalool precursor and phenolics were maximized, and the presence of C6 compounds, which have an unwanted “green grass” aroma, was minimized (Tables 5.1 and 5.2).

Table 5.2. Cabernet Sauvignon grape chemistry

WAV	Brix	pH	TA (g/L)	Tartaric Acid (g/L)	Malic Acid (g/L)	C6 (µg/L)	Linalool Precursor (µg/L)	Polymeric tannins (mg/L)	Anthocyanin (mg/g)
7	20.6 ± 0.5a	3.21 ± 0.04a	7.5 ± 0.6e	7.1 ± 0.3e	3.3 ± 0.6d	5812 ± 701cd	0.7 ± 0.1ab	1476 ± 97ab	0.87 ± 0.08a
8	21.5 ± 0.4ab	3.30 ± 0.03ab	6.2 ± 0.6d	6.0 ± 0.4bcd	2.8 ± 0.5cd	6596 ± 689bcd	1.4 ± 0.9b	1889 ± 207bc	1.02 ± 0.18a
9	22.3 ± 0.4bc	3.38 ± 0.02b	5.8 ± 0.2d	6.5 ± 0.1de	2.5 ± 0.3bc	5465 ± 728bcd	0.6 ± 0.1ab	1460 ± 133ab	0.82 ± 0.08a
10	23.7 ± 0.8cd	3.53 ± 0.06c	4.4 ± 0.5c	5.9 ± 0.4bcd	1.7 ± 0.3ab	5471 ± 357ab	0.8 ± 0.2ab	1775 ± 170bc	1.03 ± 0.16a
11	24 ± 0.5cd	3.60 ± 0.05cde	4.6 ± 0.4c	6.4 ± 0.2cde	1.5 ± 0.3a	5275 ± 615ab	0.6 ± 0 ab	1734 ± 125bc	0.97 ± 0.17a
12	23.6 ± 0.7cd	3.60 ± 0.05cde	4.2 ± 0.3bc	5.7 ± 0.3bc	1.4 ± 0.3a	5983 ± 429cd	0.6 ± 0.1a	1710 ± 170bc	0.90 ± 0.16a
13	23.5 ± 0.5cd	3.54 ± 0.04cd	4.2 ± 0.2bc	5.9 ± 0.3bcd	1.3 ± 0.2a	7313 ± 1970d	1.0 ± 0.3ab	1883 ± 236bc	1.02 ± 0.31a
14	24.2 ± 0.4d	3.62 ± 0.05cde	3.9 ± 0.3abc	5.9 ± 0.3bcd	1.1 ± 0.3a	4682 ± 865bcd	0.7 ± 0.1ab	1874 ± 296bc	1.03 ± 0.19a
15	24.3 ± 0.4d	3.70 ± 0.06e	3.1 ± 0.2a	4.9 ± 0.3a	0.9 ± 0.07a	5582 ± 987bcd	0.8 ± 0ab	2019 ± 122c	1.08 ± 0.16a
16	23.6 ± 1.6cd	3.65 ± 0.05de	3.4 ± 0.3ab	5.6 ± 0.3b	1.0 ± 0.3a	3864 ± 142bcd	0.8 ± 0.2ab	2131 ± 199c	1.01 ± 0.21a
17	24.4 ± 0.3d	3.67 ± 0.05e	3.6 ± 0.2abc	5.9 ± 0.2bcd	1.1 ± 158a	3055 ± 427a	0.8 ± 0.2ab	1116 ± 163a	0.91 ± 0.19a

Values are the mean of 4 technical replicates. Values followed by different letters indicate a significant difference ($P=0.05$, one-way ANOVA, Tukey's posthoc)

5.3.2. SCWC content

The study shows quite different profiles for SCWC development in Chardonnay and Cabernet Sauvignon grapes (Figure 5.1A). In the Chardonnay grapes, a clear peak of SCWC content was observed at WAV 6 - 8, but this was not observed in the Cabernet Sauvignon grapes. Thus, the Chardonnay SCWC content rapidly increased early in development, exhibiting an approximately 3-fold increase from WAV 4 to 7, but decreased at WAV 8 and thereafter remained relatively constant until the end of development (Figure 5.1A).

In contrast, relatively small changes in the SCWC content were seen in Cabernet Sauvignon from WAV 7 to 17 despite significant metabolic activity in older red wine berries (Fasoli et al., 2012).

At this stage we are unable to explain this difference between the red and white wine grapes. Nunan et al. (1998) also noted that the water-soluble cell wall materials in grapes from the white wine variety, Muscat Gordo Blanco were approximately constant from WAV 6 – 8 but had shown a twofold increase from WAV 2 – 6. In another study, Silacci and Morrison (1990) showed that the total concentration of water-soluble acidic polysaccharides in Cabernet Sauvignon grapes did not change significantly from WAV 7 to WAV 10.

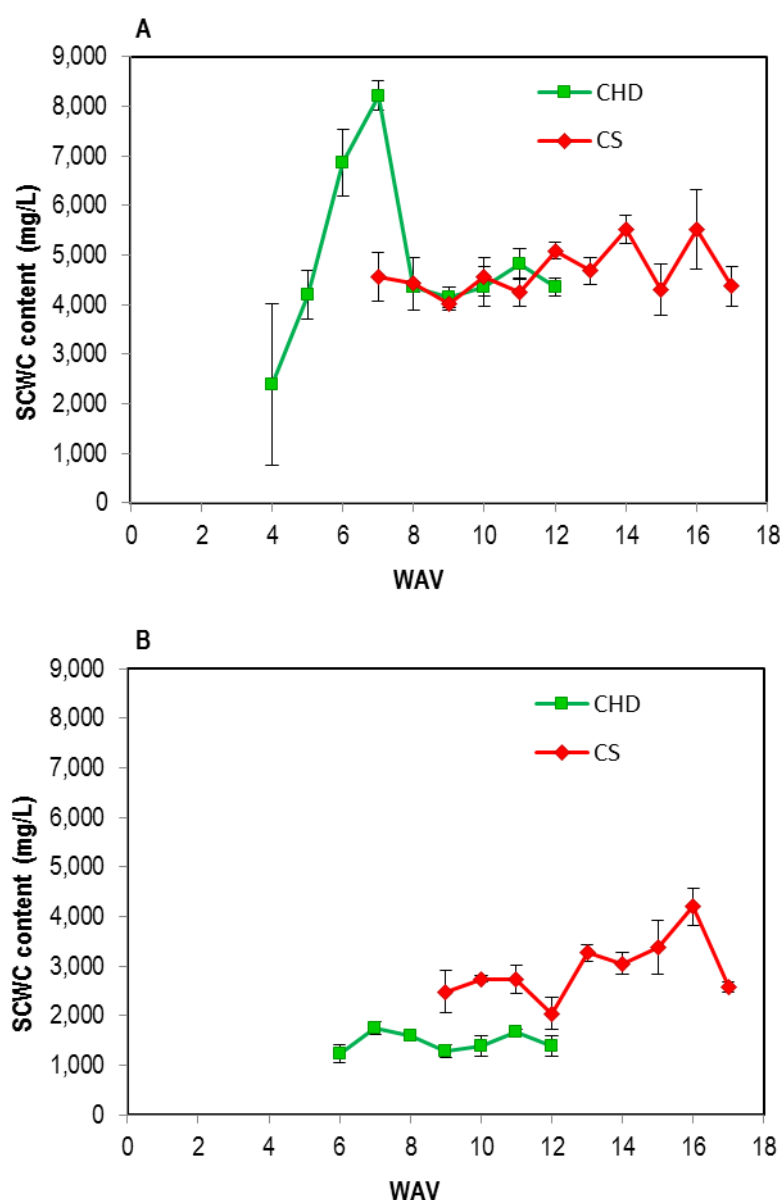


Figure 5.1. Changes of soluble cell wall polysaccharide content during ripening. A) Comparison between Chardonnay (CHD) and Cabernet Sauvignon (CS) grape berries; B) Comparison between CHD and CS wines. Wines that were made starting on WAV 6 and WAV 9 for CHD and CS, respectively. Wine SCWCs data reported in Chapter 3 and 4.

These developmental patterns of SCWCs in grapes may be contrasted with those in wine (Figure 5.1B), where SCWC contents remained approximately constant over the ripening period. However, the levels of SCWCs in Cabernet Sauvignon wines are generally higher than those in Chardonnay wines.

5.3.3. Size Exclusion Chromatography

The elution profiles from Chardonnay grape berries from WAV 6, 8 and 11 revealed three molecular size populations of SCWCs (Figure 5.2). The third peak was the largest and its elution time was later than the elution time of the smallest dextran standard of 5,000 MW, but before glucose, suggesting that oligosaccharides were present in relatively high abundance. This may be attributable to the presence of various oligomers of different polysaccharides or to the high substitution of the SCWCs in the berries (see Chapters 3 and 4). In particular, the observation indicates that extensive degradation of cell wall polysaccharides from grapes occurred during ripening. Small amounts of higher molecular weight material were observed at 50,000 MW – 150,000 MW (Figure 5.2). The elution profile of SCWCs from Cabernet Sauvignon grapes also showed a major low molecular weight peak, with small amounts of higher molecular weight material (Figure 5.3).

It is widely known that depolymerization is one of the main modifications of cell wall polysaccharides during fruit ripening (Saladié et al., 2007; Shiga et al., 2009). Ishimaru and Kobayashi (2002) showed that the molecular weight of hemicelluloses decreased during grape berry softening. In another study, Yakushiji et al. (2001) reported that hot-water-soluble pectic polysaccharides from the mesocarp of Kyoho grape berries were depolymerized from veraison to four WAV, as evidenced by a significant shift in the mean molecular mass of the pectic polysaccharides. Guillaumie et al. (2011) reported a differential expression profile of the Chardonnay grapevine *VvPL1* gene, which encodes a pectate lyase, during ripening; this is consistent with the depolymerization of pectic polysaccharides. In another study, Nunan et al. (2001) showed that transcripts for β -galactosidase, pectin methylesterase, polygalacturonase, pectate lyase (EC 4.2.2.2) and xyloglucan endotransglycosylase

(EC 2.4.1.207) were present during grape berry ripening, which is again consistent with the depolymerization of pectic polysaccharides.

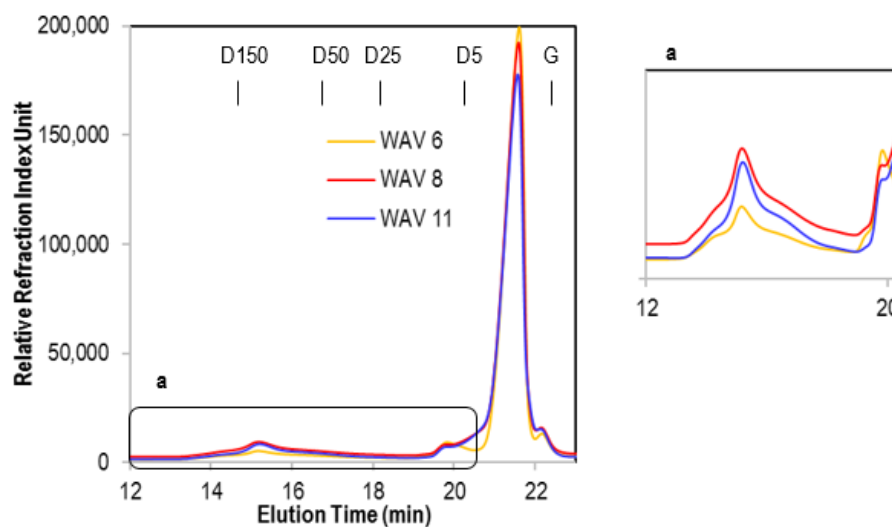


Figure 5.2. The elution profiles of SCWCs of Chardonnay grape berries, WAV 6, 8, 11 (other data not shown). Inserts show elution times of dextran standards (D x 1000 MW); G, glucose. A) Increased resolution between elution time 12 to 20 min.

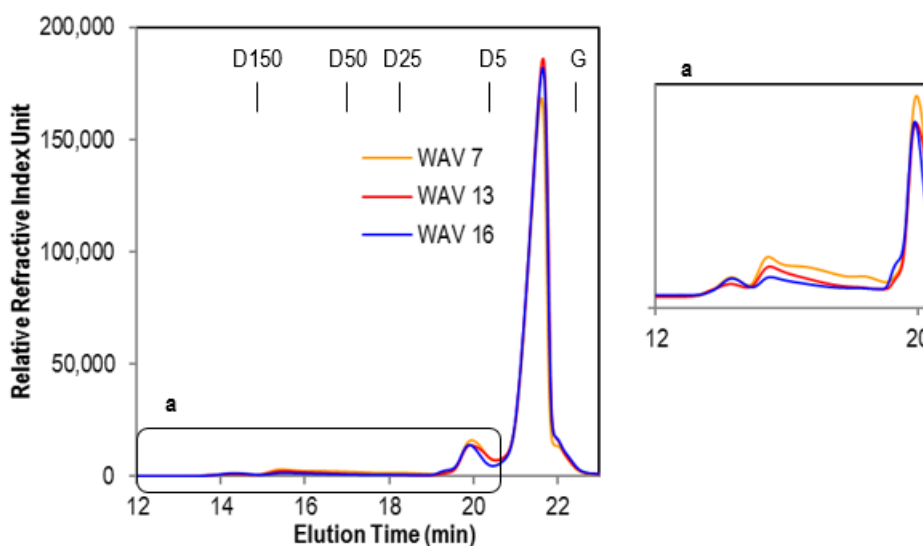


Figure 5.3. The elution profiles of SCWCs of Cabernet Sauvignon grape berries, WAV 7, 13, 16 (other data not shown). Inserts show elution times of dextran standards (D x 1000 MW); G, glucose. A) Increased resolution between elution time 12 to 20 min.

5.3.4. Monosaccharide Composition and Linkage Analysis

The monosaccharide compositions of the grape polysaccharides were calculated from the methylation data (Tables 5.3 and 5.4) and showed that Gal, Rha, Ara, Man and GalA were the most abundant monosaccharide residues

Table 5.3. Glycosyl residue composition (mol %) of the Chardonnay grapes

Glycosyl Residue	WAV								
	4	5	6	7	8	9	10	11	12
Gal	14.5	11.8	8.9	12.8	14.4	9.2	(23.9)	15.2	18.5
Glc	1.8	(14.1)	5.5	12.1	3.7	3.0	4.4	5.3	1.3
Rha	4.8	6.5	(1.1)	(0.6)	5.7	6.0	2.2	5.3	8.3
Xyl	8.2	4.7	(0.5)	(0.6)	2.9	7.8	1.9	1.6	(0.6)
Ara	13.0	(30.2)	(0.5)	(0.3)	7.8	22.5	2.8	3.5	15.9
Man	10.7	8.9	(51.7)	33.7	12.0	4.3	21.6	25.9	(1.9)
GalA	34.3	(20.0)	30.7	37.9	49.3	41.7	37.9	39.2	49.8
GlcA	12.5	3.2	1.1	2.0	4.2	5.1	5.1	3.6	3.3
Fuc	0.2	0.6	0	0	0	0.4	0.2	0.4	0.4

0, not detected; Brackets, obvious outliers

Table 5.4. Glycosyl residue composition (mol %) of the Cabernet Sauvignon grapes

Glycosyl Residue	WAV										
	7	8	9	10	11	12	13	14	15	16	17
Gal	18.5	15.5	19.9	10.9	10.8	13.8	16.7	16.4	14.4	21.0	19.7
Glc	1.3	2.6	(11.9)	3.8	4.0	6.8	4.0	2.6	4.0	5.2	6.5
Rha	8.4	6.8	4.9	11.5	13.7	8.9	8.8	12.9	10.1	14.3	14.1
Xyl	0.6	0.4	0.8	0.3	0.1	0.7	0.2	0.1	0.1	0.2	0.4
Ara	(15.9)	(13.5)	0.6	6.6	1.1	1.2	0.9	2.4	1.2	1.6	0.7
Man	(1.9)	(1.7)	15.1	3.5	11.5	11.4	11.4	13.9	7.8	13.4	(21.1)
GalA	49.7	52.0	45.7	60.1	57.5	55.4	56.5	50.0	60.6	42.2	35.5
GlcA	3.3	7.3	1.1	2.6	1.3	1.8	1.5	1.7	1.8	2.1	2.0
Fuc	0.4	0.2	0	0.7	0	0	0	0	0	0	0

0, not detected; Brackets, obvious outliers

It is clear that there are a number of anomalously high and low outliers in the datasets, especially for the SCWCs from Chardonnay grapes. However, from Figure 5.1, it is also apparent that the amount of SCWCs from Chardonnay grapes varies more than those from Cabernet Sauvignon grapes. The most obvious outliers have been placed in brackets in Tables 5.3 and 5.4. The outliers have been included in all calculations, but are indicated here because they might distort trends in the values and could also cause fluctuations in the estimations of cell wall polysaccharide types from

which the SCWCs were derived (Tables 5.5 and 5.6). It is also worth noting that the CCRC re-analyzed some of the outliers and confirmed the values presented here. As a result, the absolute values in Tables 5.3 and 5.4 should be treated with some caution.

5.3.5. Estimation of Polysaccharide Composition

A total of ten different types of possible parental polysaccharides of the SCWCs were identified in the grape berries. These are predominantly acidic pectic polysaccharides and include grape-derived HG, RG I, RG II and smaller amounts of a mixture of grape- and fungal-derived neutral polysaccharides, such as arabinan, AG I, AG II, HX, HM, XG, fungal glucan and fungal mannan (Tables 5.5 and 5.6).

Table 5.5. Glycosyl residue linkage analysis of SCWCs from Chardonnay grapes

Glycosyl Residue	Deduced Glycosidic Linkage	Linkage Composition (mol %) / WAV								
		4	5	6	7	8	9	10	11	12
Galactopyranosyl (Galp)	2-	0	0.3	0.2	0.1	0	0.1	0.2	0.1	0.2
	3-	0.9	0.4	0.9	1.4	0.9	0.4	3	1.8	0.9
	6-	3.8	2.1	2.0	3.2	2.6	1.3	6.6	3.1	2.4
	2,4-	0	0.2	0.2	0.2	0.3	0.2	0.1	0.2	0.1
	3,6-	2.9	2.3	2.2	4.0	4.1	1.7	5.3	2.8	2
	4,6-	0.6	0.3	0.1	0	0.6	0	0.5	0.6	0
	2,3,4-	0.5	0	0	0	0.6	0.7	0	0	0
	2,3,6-/2,4,6-	0	0	0	0	0	0	0.3	0	0
	3,4,6-	0.9	0.4	0	0	0.6	0.5	0.6	0.4	0
	2,3,4,6-	0	0.8	0	0	0	0	0.4	0	0
	Terminal	4.7	4.6	3.2	3.8	4.3	4	6.8	6	12.1
Glucopyranosyl (Glc p)	2-	0.1	0.6	0.2	0	0.1	0.1	0	0.1	0
	3-	0.1	0.8	0.1	3.6	0.4	0.3	0.4	0.5	0.1
	4-	0.9	4.5	3.8	5.6	1.4	1.4	2.3	0.9	0.4
	6-	0	1.5	0.2	0.3	0.3	0	0.3	0.9	0
	2,3-	0	0	0	0.2	0	0	0	0	0
	3,4-	0	0.7	0.1	0.6	0.4	0	0	0	0
	3,6-	0	0	0	0.4	0	0	0.3	0.5	0
	4,6-	0.2	0.9	0.3	0.6	0	0.5	0.3	0.4	0
	2,3,4,6-	0	1.6	0	0	0	0	0	0	0
	Terminal	0.5	3.5	0.8	0.8	1.1	0.7	0.8	2.0	0.8

^a 4-Arap and 5-Araf peaks co-eluted. The value here was represented by 5-Araf

^b Uronic acids were reduced to 6,6-dideuterio neutral sugars before methylation

0, not detected

Table 5.5. Glycosyl residue linkage analysis of SCWCs from Chardonnay grapes (continued)

Glycosyl Residue	Deduced Glycosidic Linkage	Linkage Composition (mol %) / WAV								
		4	5	6	7	8	9	10	11	12
Rhamnopyranosyl (Rhap)	2-	2.2	3.5	0.7	0.6	3.9	3.7	1.1	2.4	5.2
	3-	0.5	0.4	0	0	0	0.1	0	0.7	0
	2,3-	0.3	0.3	0.1	0	0.3	0.3	0.1	0.2	0.1
	2,4-	0.6	0.5	0.2	0	0.5	0.4	0.3	0.7	0.1
	Terminal	1.2	1.8	0.1	0	1.0	1.5	0.7	1.3	2.9
Xylopyranosyl (Xylp)	3-	0.4	0.6	0	0	0	0.2	0	0	0
	4-	5.6	2.4	0.4	0.3	1.8	4.9	1.3	1.0	0.2
	2,4-	1.4	0.8	0	0.3	0.7	1.1	0.4	0.3	0
	Terminal	0.8	0.9	0.1	0	0.4	1.6	0.2	0.3	0.4
Arabinopyranosyl (Arap)	3-	0	0	0	0	0	0	0	0	0.2
Arabinofuranosyl (Araf)	3-	0.3	1.8	0	0	0	0.3	0.1	0.1	0.1
(Arap)/(Araf)	4- / 5- ^a	2.1	5.3	0	0	1.4	1.8	0.4	0.5	0.8
(Arap)/(Araf)	3,4-/3,5-	0.3	0	0	0	0	0.3	0	0	0
	Terminal (p)	6.3	9.5	0	0.3	2	7.6	1.3	1.5	12.5
	Terminal (f)	4.0	13.6	0.5	0	4.4	12.5	1.0	1.4	2.3
Mannopyranosyl (Manp)	2-	7.4	0	0	0	0	2.0	0	0	0
	2- / 3-	0	2.2	28.6	17.2	6.0	0	10.1	12.8	0.2
	4-	0.5	3.0	1.5	3.1	0.5	0.5	1.5	0.7	0.2
	6-	0.1	0.1	0.3	0.5	0	0	0.4	0.4	0.1
	2,3-	0.8	0.3	0.7	0.8	0.6	0.6	0.5	0.8	0
	2,4-	0.5	0.1	0.4	0.2	0.2	0.1	0.1	0.3	0
	2,6-	0.7	0.3	3.5	1.9	0.6	0.2	1.7	1.5	0
	3,6-	0.2	0.3	0.6	0.3	0.2	0	0.5	0.7	0
	4,6-	0	0.5	0.1	0.6	0	0	0.3	0.2	0
	2,3,6-/2,4,6	0	0	0.4	0	0	0	0.3	0	0
	2,3,4,6-	0	0.3	0	0	0	0	0	0	0
	Terminal	0.5	1.8	15.6	9.1	3.9	0.9	6.2	8.5	1.4
Galacturonopyranosyl ^b (GalAp)	2-	0	0	0.1	0.1	0	0.1	0	0.1	0.1
	3-	0	0	0	0	0	0	0	0	0.1
	4-	28.8	16.2	17.7	30.2	41.5	35.2	30.7	29.1	43.8
	2,4-	1.2	0.6	0.1	0.4	1.3	1.5	0.4	0.6	0.9
	3,4-	3.2	1.6	0.7	0.9	2.5	3.2	1.7	2.0	1.9
	Terminal	1.1	1.5	10.6	5.8	3.8	1.6	4.9	6.9	2.9

^a 4-Arap and 5-Araf peaks co-eluted. The value here was represented by 5-Araf

^b Uronic acids were reduced to 6,6-dideuterio neutral sugars before methylation

0, not detected

Table 5.5. Glycosyl residue linkage analysis of SCWCs from Chardonnay grapes (continued)

Glycosyl Residue	Deduced Glycosidic Linkage	Linkage Composition (mol %) / WAV								
		4	5	6	7	8	9	10	11	12
Glucuronopyranosyl ^b (GlcAp)	2-	0.2	0	0.1	0	0.3	0.3	0	0.3	0
	4-	8.2	1.3	0.5	1.3	2.1	1.9	2.8	1.4	0.3
	3,4-	0.8	0	0	0	0	0.3	0.3	0.3	0
	Terminal	3.3	1.9	0.5	0.7	1.8	2.6	2.0	1.6	3.0
Fucopyranosyl (Fucp)	Terminal	0.2	0.6	0	0	0	0.4	0.2	0.4	0.4
Galactofuranosyl (Galf)	Terminal	0.2	0.4	0.1	0.1	0.4	0.3	0.1	0.2	0.8
Galacturonofuranosyl (GalAf)	Terminal	0	0.1	1.5	0.5	0.2	0.1	0.2	0.5	0.1

^a 4-Arap and 5-Araf peaks co-eluted. The value here was represented by 5-Araf

^b Uronic acids were reduced to 6,6-dideuterio neutral sugars before methylation
0, not detected

Table 5.6. Glycosyl residue linkage analysis of SCWCs from Cabernet Sauvignon grapes

Glycosyl Residue	Deduced glycosidic linkage	Linkage Composition (mol %) / WAV										
		7	8	9	10	11	12	13	14	15	16	17
Galactopyranosyl (Galp)	2-	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0	0	0.2	0.1
	3-	0.9	2.9	1.8	0.7	1.0	0.8	1.1	1.2	0.6	1.2	1.8
	6-	2.4	2.3	3.0	2.3	2.3	1.9	3.1	2.1	2.2	3.3	3.6
	2,4-	0.1	0	0.3	0	0.1	0.1	0	0.1	0.1	0.4	0.1
	3,6-	2.0	4.6	5.3	0.7	0	2.5	2.4	0	2.6	2.7	0
	4,6-	0	0.2	0.3	0	0.2	0.3	0.3	0.2	0.3	0.8	0.1
	Terminal	12.1	4.7	9.1	6.7	6.9	8.0	9.5	12.7	8.5	12.3	13.7
Glucopyranosyl (GlcP)	2-	0	0	0.2	0.1	0.2	0.1	0.1	0	0.2	0.2	0
	3-	0.1	0.1	0.2	0.2	0.1	0.3	0.1	0	0.1	0.3	0.6
	4-	0.4	1.5	8.6	0.4	1.2	3.6	1.2	0.9	0.4	1.7	2.5
	6-	0	0	0.3	0.5	0.5	0.3	0.5	0	0.4	0.6	0.6
	2,4-	0	0	0	0.1	0	0	0	0	0	0	0
	3,4-	0	0	0.3	0	0	0	0	0	0	0	0
	4,6-	0	0	0.4	0	0	0.5	0	0	0	0.1	0
	Terminal	0.8	1.0	1.9	2.5	2.0	2.0	2.1	1.7	2.9	2.3	2.8
Rhamnopyranosyl (Rhap)	2-	5.2	3.6	3.8	5.6	11.6	6.8	7.4	9.4	7.6	12.2	12.5
	4-	0	0	0	0.2	0	0	0	0	0	0	0
	2,3-	0.1	0.1	0.3	0.5	0.4	0.3	0.3	0.3	0.3	0.4	0.5
	2,4-	0.1	0.2	0.3	0.5	0.3	0.3	0.3	0.5	0.8	0.5	0.5
	Terminal	3.0	2.9	0.5	4.7	1.4	1.5	0.8	2.7	1.4	1.2	0.6
Xylopyranosyl (Xylp)	4-	0.2	0.1	0.8	0.0	0.1	0.7	0.2	0.1	0.1	0.2	0.4
	Terminal	0.4	0.3	0	0.3	0	0	0	0	0	0	0
Arabinopyranosyl (Arap)	3-	0.2	0.1	0	0	0	0	0	0	0	0	0
Arabinofuranosyl (Araf)	3-	0.1	0.1	0	0	0	0	0	0	0	0	0
	2- / 3-	0.2	0	5.2	1.1	4.5	4.1	4.0	4.6	2.3	4.4	7.2

^a 4-Arap and 5-Araf peaks co-eluted. The value here was represented by 5-Araf

^b Uronic acids were reduced to 6,6-dideuterio neutral sugars before methylation
0, not detected

Table 5.6. Glycosyl residue linkage analysis of SCWCs from Cabernet Sauvignon grapes (continued)

Glycosyl Residue	Deduced Glycosidic Linkage	Linkage Composition (mol %) / WAV										
		7	8	9	10	11	12	13	14	15	16	17
(Arap)/(Araf)	4- / 5- ^a	0.8	0.5	0	0.3	0	0	0	0	0	0	0
	Terminal (p)	12.5	9.0	0.6	3.5	1.1	1.2	0.9	2.4	1.2	1.6	0.7
	Terminal (f)	2.3	3.8	0	2.8	0	0	0	0	0	0	0
Mannopyranosyl (Manp)	2-	0	0.2	0	0	0	0	0	0	0	0	0
	2- / 3-	0.2	0	5.2	1.1	4.5	4.1	4.0	4.6	2.3	4.4	7.2
	4-	0.2	0.1	1.8	0	0.4	0.4	0.6	0.2	0.2	1.4	1.1
	6-	0.1	0.1	0.3	0.1	0.3	0.2	0.2	0	0	0.2	0.8
	2,3-	0	0	0.2	0	0	0	0.2	0	0.1	0.2	0.1
	2,6-	0	0	1.1	0.2	1.1	0.8	0.9	0.6	0.5	0.7	1.2
	Terminal	1.4	1.3	6.5	2.1	5.2	5.9	5.5	8.5	4.7	6.5	10.7
	2-	0.1	0	0.2	0.2	0.4	0.2	0.2	0.0	0.1	0.3	0.2
	3-	0.1	0	0.5	0.4	0.5	0.8	0.3	0.8	0.8	0.4	0
Galacturonopyranosyl ^b (GalAp)	4-	43.8	38.4	30.5	32.8	39.1	34.9	36.4	31.6	39.9	29.1	22.6
	2,4-	0.9	0.5	0.3	-	0.4	0.2	0.2	0.3	0.4	0.2	0.1
	3,4-	1.9	1.5	1.8	1.4	2.5	1.7	1.5	1.6	2.1	2.2	1.1
	Terminal	2.9	11.5	12.4	23.2	14.3	17.5	17.7	15.5	17.1	10.0	11.4
	2-	0	0	0	0.1	0	0	0	0	0	0	0
	4-	0.3	3.1	0	0.2	0.3	0.3	0.2	0.2	0.2	0.4	0.4
Glucuronopyranosyl ^b (GlcAp)	2,4-	0	0	0	0.3	0	0	0	0	0	0	0
	Terminal	3.0	4.2	1.1	2.0	1.0	1.5	1.3	1.5	1.6	1.7	1.6
	Terminal	0.4	0.2	0	0.7	0	0	0	0	0	0	0
	Terminal	0.8	0.7	0	0.4	0.2	0.1	0.2	0.1	0.1	0.1	0.3
Fucopyranosyl (Fucp)	Terminal	0.0	0.1	0	2.1	0.3	0.1	0.2	0.2	0.2	0	0.1
Galactofuranosyl (Galf)	Terminal	0.0	0.1	0	2.1	0.3	0.1	0.2	0.2	0.2	0	0.1
Galacturonofuranosyl (GalAf)	Terminal	0.0	0.1	0	2.1	0.3	0.1	0.2	0.2	0.2	0	0.1

^a 4-Arap and 5-Araf peaks co-eluted. The value here was represented by 5-Araf

^b Uronic acids were reduced to 6,6-dideuterio neutral sugars before methylation
0, not detected

Pectic polysaccharides

The changes in the SCWC contents of both Chardonnay and Cabernet Sauvignon during development are largely driven by changing HG levels at each ripening stage (Tables 5.7 and 5.8), which is not surprising because HG was the most abundant parent polysaccharides of SCWCs in the studied samples. Chardonnay grapes contained 17.7 – 46.7 mol % HG, whereas Cabernet Sauvignon grapes contained 34 - 57 mol % HG over the ripening period. As mentioned above, the absolute values calculated here should be viewed with caution. However, the types of parental polysaccharides can be predicted with confidence and distinct trends can still be observed during berry development. At face value, HG in Chardonnay exhibited greater variation during ripening, initially increasing, but

remaining relatively constant at WAV 9 - 11 and finally peaking at WAV 12. In contrast, the HG in Cabernet Sauvignon grapes remained relatively constant at 42.9 - 57 mol % at WAV 7 - 15 and decreased to below 40 mol % at WAV 16-17 (Tables 5.7 and 5.8).

It has been reported that galacturonans change during fruit ripening mainly because cell wall-modifying enzymes cause the cell walls to disassemble and the middle lamella to dissolve, leading to the solubilization, de-esterification and depolymerization of galacturonans (Saladié et al., 2007; Shiga et al., 2009; Guillaumie et al., 2011; Paniagua et al., 2014)

In the pectic polysaccharide family, HG was present in higher proportions than RG I/II in all cases. Temporary decreases in RG I/II in Chardonnay at WAV 6, 7 and 10 and in Cabernet Sauvignon at WAV 8 and 9 were observed. Otherwise, the amounts remained at 10 - 20 mol % during ripening (Tables 5.7 and 5.8). In this study, AG II was consistently more abundant than AG I during ripening of both cultivars. Mohnen (2008) reported that the side chains of RG I are composed of arabinan, AG I and AG II, although AG II structures of the AGP class are not necessarily covalently linked with the pectic polysaccharide complexes. Willats et al. (2001) suggested that no single RG I structure exists in all plant cell walls and that the variation of its side chains appears to be carefully regulated during cell development, as observed in the carrot root apex and pea cotyledon. Our findings suggest that in grapes, RG I may exhibit development-dependent expression and that AG I and AG II may modify the RG I structure and solubility. As mentioned above, it has been reported that RG I is highly variable in its structure and occurrence within the cell walls. This phenomenon is not well understood, although some studies have suggested that the variation may contribute to the structure-function relationship of the mechanical properties of cell walls (Mohnen, 2008).

A small amount of arabinan was present in Chardonnay and Cabernet Sauvignon during ripening, although this was highly variable (Tables 5.7 and 5.8). This result is consistent with that of Nunan et al. (1998), who reported that arabinan was a minor polysaccharide in grape cell walls. Villettaz et al. (1981) reported that a low-molecular weight and highly branched arabinan with a

backbone composed of (1,5)- α -linked *Araf* residues substituted with α -*Araf* residues at the C3 positions was present in the supernatant after the ethanol precipitation of Pinot Noir juice.

Table 5.7. Probable sources of SCWCs in Chardonnay grapes

Polysaccharide ^a	Relative Amount (mol %) / WAV									
	4	5	6	7	8	9	10	11	12	
G-Arabinan	2.7	5.3	0	0	1.4	2.4	0.4	0.5	0.8	
G-Type I Arabinogalactan (AG I)	1.2	0.6	0.2	0	1.2	0	1.0	1.2	0	
G-Type II Arabinogalactan (AG II)	17.9	12.9	9.2	13.1	17.8	10.6	24.8	16.5	19.4	
G-Homogalacturonan (HG)	29.9	17.7	28.3	36	45.3	36.8	35.6	36.0	46.7	
G-Rhamnogalacturonan I/II (RG I/II)	14.7	12.1	2.3	2.4	12.8	16.8	6.1	10.6	14.3	
G-Heteroxylan (HX)	8.4	4.0	0.4	0.6	2.5	7.1	1.7	1.3	0.2	
G-Heteromannan (HM)	1.0	7.0	3.2	7.4	1.0	1.0	3.6	1.8	0.4	
G-Xyloglucan (XG)	0.6	1.2	0.4	0	0	1.5	0	0.4	0	
F-Glucan	0.8	6.9	3.2	7.6	2.7	1.4	2.6	3.9	1.1	
F-Mannan	8.9	4.7	48.6	29.0	10.7	3.1	18.9	23.9	1.7	
Unassigned ^b	13.9	27.6	4.2	3.9	4.6	19.3	5.3	3.9	15.4	

^a G- denotes grape derived polysaccharide; F- denotes fungal derived polysaccharide

^b Sum of linkage types that cannot be assigned to well-characterized soluble cell wall polysaccharide from plant and fungal

0, not detected

Table 5.8. Probable sources of SCWCs in Cabernet Sauvignon grapes

Polysaccharide ^a	Relative Amount (mol %) / WAV										
	7	8	9	10	11	12	13	14	15	16	17
G-Arabinan	0.8	0.5	0	0.3	0	0	0	0	0	0	0
G-Type I Arabinogalactan (AG I)	0	0.4	0.3	0	0.2	0.3	0.3	0.2	0.3	0.8	0.1
G-Type II Arabinogalactan (AG II)	19.4	19.1	20.3	11.1	10.2	14.7	17.4	16	15.5	21.2	19.1
G-Homogalacturonan (HG)	46.7	49.9	42.9	56	53.4	52.4	54.1	47.1	57.0	39.1	34.0
G-Rhamnogalacturonan I/II (RG I/II)	11.4	8.8	7.9	12.8	18.8	12	11.4	15	13.9	18.7	17.1
G-Heteroxylan (HX)	0.2	0.1	0.8	0	0.1	0.7	0.2	0.1	0.1	0.2	0.4
G-Heteromannan (HM)	0.4	0.2	3.6	0	0.8	0.8	1.2	0.4	0.4	2.8	2.2
G-Xyloglucan (XG)	0	0	0.8	0	0	1	0	0	0	0.2	0
F-Glucan	1.1	2.5	8.8	3.6	3.4	5.3	3.3	2.4	3.6	3.4	5.4
F-Mannan	1.7	1.6	13.1	3.5	11.1	11	10.6	13.7	7.5	11.8	19.9
Unassigned ^b	18.3	16.9	1.5	12.7	2	1.8	1.5	5.1	1.7	1.8	1.8

^a G- denotes grape derived polysaccharide; F- denotes fungal derived polysaccharide

^b Sum of linkage types that cannot be assigned to well-characterized soluble cell wall polysaccharide from plant and fungal

0, not detected

Hemicelluloses

The hemicelluloses XG, HX and HM appeared to vary greatly across samples in both cultivars.

The amounts of hemicellulose in Cabernet Sauvignon were much lower than those in Chardonnay,

and their overall changes were smaller. HX and HM were more abundant than XG in Chardonnay, whereas HM was more abundant than HX and XG, which were present in relatively low proportions in Cabernet Sauvignon. This result contrasts with other studies, in which XG was reported to be the most dominant hemicellulose in the primary walls of dicotyledonous plants and grape cultivars, constituting 8 - 10 mol % of the walls of Grenache Blanc grapes and Muscat Gordo Blanco, respectively (Nunan et al., 1998; Doco et al., 2003; Johnston et al., 2012). This difference could be attributable to the fact that our study investigated the soluble fraction of cell wall polysaccharides in the juice of berries, whereas the aforementioned studies analyzed whole berries, which would include juice-soluble and insoluble cell wall polysaccharides. This might also indicate that xyloglucanases are more active than other hydrolases during the final stages of ripening. According to Scheller and Ulvskov (2010), the diverse hemicellulose compositions depend on the species, tissue types and developmental stages, and usually consist of XG, HX and HM in various proportions.

Xyloglucan

In this study, XG in Chardonnay and Cabernet Sauvignon remained relatively low during ripening. Guillaumie et al. (2011) reported the down-regulation around harvest of the transcription of four isoforms of xyloglucan endotransglucosylase (XTH) genes. These genes are thought to encode enzymes involved in the alteration of XG size distribution associated with berry softening, while Brummell et al. (1999) suggested that XTH activity is limited in over-ripe fruit. This latter result is consistent with that of Nunan et al. (1998), who reported that the solubility of XG hardly changed during ripening. These considerations may explain why the amount of soluble XG was relatively low in our samples.

Heteroxylan

In Chardonnay grapes, higher amounts of HX were found than in Cabernet Sauvignon grapes (Tables 5.7 and 5.8), but they nevertheless remained relatively low throughout ripening. In contrast, in Cabernet Sauvignon grapes, HX remained relatively constant at levels below 1 mol % during ripening. Thus, HX was more abundant in Chardonnay than in Cabernet Sauvignon grapes in terms

of the relative proportion (mol % of total polysaccharides) of each polysaccharide in the cultivar, and the changes of HX in Chardonnay were larger than those in Cabernet Sauvignon during ripening (Tables 5.7 and 5.8).

According to Nunan et al. (1998), HX becomes slightly more soluble as the grape ripens. The activities of endoxylanase and xylan endotransglycosylase increase significantly in papaya fruit during ripening (*Carica papaya*), suggesting that modifications of HX, including depolymerization, may contribute to the availability of soluble oligosaccharides that are not bound to the walls (Paull and Chen, 1983; Shiga et al., 2009). Carpita and Gibeaut (1993) reported that the chemical structure of HX can also determine its solubility in juice. Highly substituted HX is more soluble and does not interact with cellulose as strongly as the less-substituted HX, which can interact more strongly with the wall-bound xylan and cellulose in the mature wall (Carpita and Gibeaut, 1993).

Heteromannan

Little is known about the role of HM in the cell wall matrix compared with other wall polysaccharides. Based on the relative proportion of each carbohydrate in each cultivar's SCWCs, Chardonnay grapes consistently had more HM than Cabernet Sauvignon during ripening. Notably, in both cultivars, HM was more abundant and exhibited larger changes during ripening than HX and XG.

Based on a genome-wide transcriptomic atlas of grapevine, 20 mannanase genes with various expression levels were detected across all tissues during ripening (Fasoli et al., 2012). This suggests that the HM may be released from the cell wall into the soluble fraction and that low molecular weight products are likely to be present. HM with a lower degree of polymerization and substituted HMs, such as galactomannan and galactoglucomannan, were found to be more water soluble than unsubstituted mannan (Whitney et al., 1998; Capek et al., 2000; Ishrud et al., 2001; Capek, 2009; Schröder et al., 2009). In general, these findings suggest that HMs are present in the juice-soluble fraction of grapes because they are more water soluble and less tightly bound to the walls than XG and HX.

Fungal SCWCs

Fungal SCWCs were found at all stages of Chardonnay and Cabernet Sauvignon grape berry ripening (Tables 5.7 and 5.8). This finding was somewhat unexpected because the grape berry extracts were not inoculated with wine yeast in these experiments. However, in the vineyard, surfaces of grapes provide a physical environment on which a complex microbial ecosystem of diverse species and strains of wild yeasts, bacteria and fungi can grow (Setati et al., 2012). Fungal SCWCs constituted 2.8 - 25.3 mol % of the total SCWCs in Cabernet Sauvignon, whereas a significantly larger amount of fungal SCWCs 2.8 - 51.8 mol % of total SCWCs was found in Chardonnay. These results are clearly spurious if these polysaccharides were derived from contaminating fungi. It is possible that some of the samples were contaminated with *Saccharomyces cerevisiae*, although the experiments were performed in a laboratory that was distant from wine yeast propagation laboratories.

According to Vail et al. (1998), Chardonnay grapes are highly susceptible to rot in the vineyard because of the compactness of the cluster, which can lead to damage to the compressed berries and decreased evaporation of free water. In addition, Sabate et al. (2002) reported that fungal microbiota could differ significantly among cultivars. Despite these reports of the widespread contamination of grapes with field fungi, it is unlikely that the high proportions of fungal mannan and glucan calculated here (Tables 5.7 and 5.8) can be attributed to contamination in the vineyard or during harvesting.

5.3.5. Comparison of Grape-derived SCWCs in Grapes and Wine

This study shows that the SCWC contents (mg/L) in Cabernet Sauvignon wines were twice those in Chardonnay wines across samples (Figure 5.1B). The average retention rates of the fermentation-resistant SCWC contents in Cabernet Sauvignon and Chardonnay wines with respect to the initial levels of their precursors in grapes throughout ripening were approximately 64 % and 33 %, respectively (Figures 5.1A and 5.1B). This difference was mainly attributed to the different winemaking techniques. Cabernet Sauvignon wines are made by fermenting juice and skin, whereas Chardonnay wines are made by fermenting juice. Nonetheless, the wines made from both cultivars contained appreciable amounts of SCWCs, and the SCWC abundance was similar to that of tannins (see

Chapter 3), which are widely believed to be important wine mouthfeel constituents (Ducasse et al., 2010; Quijada-Morín et al., 2014).

In this study, HG and RG I/II, which are acidic grape SCWCs, were consistently the most abundant polysaccharide type found in Chardonnay and Cabernet Sauvignon grapes and wines (Tables 5.7, 5.8 and 5.9). As described in Chapters 3 and 4, HG was positively correlated with bitter and sour tastes in both Chardonnay and Cabernet Sauvignon wines. Even though the neutral grape SCWCs were less abundant, they are important wine mouthfeel drivers (see Chapter 3 and 4). For example, arabinan, AG II, HM and XG contributed to “mouth coating” in Cabernet Sauvignon wines, whereas AG I and XG correlated with “viscosity” in Chardonnay wines. As shown in Table 5.9, the ratio of acidic SCWCs to neutral SCWCs found in wines is twice the ratio found in grapes, suggesting that neutral SCWCs in grapes might have been lost during the winemaking processes. Since neutral grape SCWCs are important mouthfeel contributors, innovative grape growing and winemaking techniques could be applied to enhance the level of these SCWCs, not only in the grape source, but also throughout the winemaking process.

Table 5.9. Comparison of acidic and neutral SCWCs in grapes and wine

Grape SCWC Type	Chardonnay		Cabernet Sauvignon	
	Grapes	Wine ^c	Grapes	Wine ^d
Acidic SCWCs (% mol) ^a	47	44	63	73
Neutral SCWCs (% mol) ^b	22	10	18	12
Ratio (acidic SCWCs / neutral SCWCs)	2	4	3	6

^a average of HG and RG I/II present in samples from WAV 6 – 12 of Chardonnay and WAV 9 – 17 of Cabernet Sauvignon

^b average of arabinan, AG I, AG II, HM, HX and XG present in samples from WAV 6 – 12 for Chardonnay and WAV 9 – 17 of Cabernet Sauvignon

^c wine data reported in Chapter 4

^d wine data reported in Chapter 3

RG II is a complex pectic polysaccharide component with at least 12 different glycosyl residues, which are linked together by more than 20 different glycosidic linkages, and has been reported to be resistant to microbial enzymes (O'Neill et al., 2004). RG I consists of repeating units of 2-Rhap and 4-GalAp and is highly substituted with significantly heterogeneous side chains; the

conservation of RG I in plant extracts suggests that RG I is also resistant to modifications (Caffall and Mohnen, 2009). The abundance of wine hemicelluloses decreased substantially relative to their initial levels in grapes, suggesting that hemicelluloses may be more readily hydrolyzed during fermentation or may bind to the precipitated juice solids.

5.4. Conclusions

The present work highlights the changes in SCWCs from grapes to wine during ripening in two cultivars with unique traits. Overall, the results indicate that during ripening, while no major change in grape-derived SCWC contents occurred in Chardonnay and Cabernet Sauvignon grape berries, significant variations in structure and composition were observed. This suggested potential modifications of specific polysaccharides during ripening and during the winemaking process. The observed differences between Chardonnay and Cabernet Sauvignon berries during ripening can be attributed to many factors, including genotypic effects, viticultural practices and climate conditions (van Leeuwen et al., 2004; Myles et al., 2011; Mendez-Costabel et al., 2014).

In general, both cultivars share some common grape-derived SCWC composition profiles. Indeed, HG was present in higher proportions than RG I/II, AG I and AG II, whereas HM was the most abundant hemicellulose. Cell wall metabolism is highly complex and dynamic. Limited investigations have been performed to fully understand the modifications of grape cell walls during berry development in the late ripening stages, when cell wall expansion, elasticity and degradation may occur via a highly orchestrated and finely regulated process. Transcript data may be useful in the future to elucidate the process and to identify the key enzymes or hormones involved in this process.

The linkage analyses also show that fungal SCWCs are detectable in grapes, with levels varying greatly at different ripening stages. There are a number of anomalously high and low outliers, especially for the Man residues, in the linkage datasets. The information related to these SCWCs of apparent fungal origin observed here should therefore be treated with some caution.

As described in Chapters 3 and 4, grape-derived SCWCs significantly affect the wine mouthfeel. The appearance of abundant grape SCWCs in wine suggests that grape polysaccharides and their oligosaccharides can be precursors for wine mouthfeel. The knowledge obtained in the present study should be of considerable value for the wine industry because if the key polysaccharide precursors can be measured and monitored during grape development, and if these are positively correlated with desirable wine mouthfeel sensations, the wine industry will have rational new methods to optimize grape harvest timing so that the key polysaccharide precursors are present in high abundance. In addition, viticultural practices and fermentation conditions might be adjusted to maximize the positive mouthfeel characteristics.

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5.7 Supplementary Material

Supplementary Table S5.1. Unassigned glycosyl residues of Chardonnay grapes

Glycosyl Residue	Deduced Glycosidic Linkage	Linkage Composition (mol %) / WAV								
		4	5	6	7	8	9	10	11	12
Galactopyranosyl (Galp)	2- ^a	0	0.3	0.2	0.1	0	0.1	0.2	0.1	0.2
	2,3,4- ^c	0.5	0	0	0	0.6	0.7	0	0	0
	2,3,4,6- ^c	0	0.8	0	0	0	0	0.4	0	0
	2,3,6-/2,4,6- ^c	0	0	0	0	0	0	0.3	0	0
Glucopyranosyl (GlcP)	2- ^c	0.1	0.6	0.2	0	0.1	0.1	0	0.1	0
	2,3- ^c	0	0	0	0.2	0	0	0	0	0
	2,3,4,6- ^c	0	1.6	0	0	0	0	0	0	0
	3,4- ^c	0	0.7	0.1	0.6	0.4	0	0	0	0
Xylopyranosyl (Xylp)	3- ^c	0.4	0.6	0	0	0	0.2	0	0	0
	Terminal ^b	0.6	0.5	0	0	0	1.1	0	0	0.4
Arabinopyranosyl (Arap)	3- ^c	0	0	0	0	0	0	0	0	0.2
	Terminal ^b	1	9.5	0	0	0	7.6	0	0	9.7
Arabinopyranosyl (Araf)	3- ^c	0.3	1.8	0	0	0	0.3	0.1	0.1	0.1
	Terminal ^b	0	7	0	0	0	3.9	0	0	0
Mannopyranosyl (Manp)	2,3- ^c	0.8	0.3	0.7	0.8	0.6	0.6	0.5	0.8	0
	2,4- ^c	0.5	0.1	0.4	0.2	0.2	0.1	0.1	0.3	0
	2,3,4,6- ^c	0	0.3	0	0	0	0	0	0	0
	2,3,6-/2,4,6- ^c	0	0	0.4	0	0	0	0.3	0	0
Galacturonopyranosyl ^a (GalAp)	2- ^c	0	0	0.1	0.1	0	0.1	0	0.1	0.1
	3- ^c	0	0	0	0	0	0	0	0	0.1
Glucuronopyranosyl ^a (GlcAp)	3,4- ^c	0.8	0	0	0	0	0.3	0.3	0.3	0
	4- ^c	8.2	1.3	0.5	1.3	2.1	1.9	2.8	1.4	0.3
	Terminal ^b	0.3	1.1	0	0	0	1.5	0	0	3
Fucopyranosyl (Fucp)	Terminal ^b	0.2	0.6	0	0	0	0.4	0	0	0.4
Galactofuranosyl (GalF)	Terminal ^b	0.2	0.4	0.1	0.1	0.4	0.3	0.1	0.2	0.8
Galacturonofuranosyl (GalAf)	Terminal ^b	0	0.1	1.5	0.5	0.2	0.1	0.2	0.5	0.1

^aLinkage type that can be assigned to well-characterized SCWCs from plant and yeast, but it is unassigned in this study because it does not meet the criteria laid out in the Materials and Methods section. For example, according to Pettolino et al. (2012), 2-Galp must be $\leq$ 2-Xyl in xyloglucan. In this study, 2-Galp is present but it cannot be assigned to xyloglucan because of the absence of 2-Xylp.

^bRemaining terminal linkage types after successive assignments to SCWCs in Table 5.7.

^cLinkage types that cannot be assigned to well-characterized SCWCs from plants and yeast.

Supplementary Table S5.2. Unassigned glycosyl residues of Cabernet Sauvignon grapes

Glycosyl Residue	Deduced Glycosidic Linkage	Linkage Composition (mol %) / WAV										
		7	8	9	10	11	12	13	14	15	16	17
Galactopyranosyl (Galp)	2- ^a	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0	0	0.2	0.1
Glucopyranosyl (Glc p)	2- ^c	0	0	0.2	0.1	0.2	0.1	0.1	0	0.2	0.2	0
	2,4- ^c	0	0	0	0.1	0	0	0	0	0	0	0
	3,4- ^c	0	0	0.3	0	0	0	0	0	0	0	0
Rhamnopyranosyl (Rhap)	4- ^c	0	0	0	0.2	0	0	0	0	0	0	0
Xylopyranosyl (Xylp)	Terminal ^b	0.4	0.3	0	0.3	0	0	0	0	0	0	0
Arabinopyranosyl (Arap)	3- ^c	0.2	0.1	0	0	0	0	0	0	0	0	0
	Terminal(p) ^b	12.5	9.0	0	3.5	0	0.1	0	2.3	0	0	0
Arabinofuranosyl (Araf)	3- ^c	0.1	0.1	0	0	0	0	0	0	0	0	0
	Terminal(f) ^b	0.2	0	0	2.1	0	0	0	0	0	0	0
Mannopyranosyl (Manp)	2,3- ^c	0	0	0.2	0	0	0	0.2	0	0.1	0.2	0.1
Galacturonopyranosyl (GalAp)	2- ^c	0.1	0	0.2	0.2	0.4	0.2	0.2	0	0.1	0.3	0.2
	3- ^c	0.1	0	0.5	0.4	0.5	0.8	0.3	0.8	0.8	0.4	0
Glucuronopyranosyl (GlcAp)	2,4- ^c	0	0	0	0.3	0	0	0	0	0	0	0
	4- ^c	0.3	3.1	0	0.2	0.3	0.3	0.2	0.2	0.2	0.4	0.4
	Terminal ^b	3.0	3.2	0	2.0	0	0	0	1.5	0	0	0.6
Fucopyranosyl (Fucp)	Terminal ^b	0.4	0.2	0	0.7	0	0	0	0	0	0	0
Galactofuranosyl (Gal f)	Terminal ^c	0.8	0.7	0	0.4	0.2	0.1	0.2	0.1	0.1	0.1	0.3
Galacturonofuranosyl (GalAf)	Terminal ^c	0	0.1	0	2.1	0.3	0.1	0.2	0.2	0.2	0	0.1

^aLinkage type that can be assigned to well-characterized SCWCs from plant and yeast, but it is unassigned in this study because it does not meet the criteria laid out in the Materials and Methods section. For example, according to Pettolino et al. (2012), 2-Galp must be $\leq$ 2-Xyl in xyloglucan. In this study, 2-Galp is present but it cannot be assigned to xyloglucan because of the absence of 2-Xylp.

^bRemaining terminal linkage types after successive assignments to SCWCs in Table 5.8.

^cLinkage types that cannot be assigned to well-characterized SCWCs from plants and yeast.

Chapter 6

Summary and Future Directions

The grape- and yeast-derived soluble cell wall carbohydrates (SCWCs) of Chardonnay and Cabernet Sauvignon grapes and wines were fully characterized in this study. Changes in the SCWC composition of grapes harvested at different ripening stages and their corresponding wines were evaluated. The chemical and sensory profiles of Chardonnay and Cabernet Sauvignon wines made from grapes at different ripening stages vary greatly. Here, the chemical profiles, including the SCWC composition, of wines were analyzed in parallel with the sensory evaluation. The present work reports the changes in SCWCs from grapes to wines during the ripening of two cultivars of global commercial importance, namely Chardonnay and Cabernet Sauvignon.

6.1. Summary of Results

6.1.1. SCWCs and Mouthfeel

The principle objective of this study was to determine whether or not mouthfeel characteristics of red and white wines are related to constituents in the wine that were derived from cell walls, either of plant or yeast origin. Indeed, the SCWCs from grapes and yeast affected mouthfeel.

It is now clear that the SCWCs in grapes and wine are highly complex and heterogenous in nature. They consisted predominantly of low molecular weight oligosaccharides of pectic polysaccharides and type II AGPs from the grapes, and yeast mannans/mannoproteins. Eight types of grape-derived SCWCs and two types of yeast-derived SCWCs were identified by linkage analysis, and the sensory data indicated that mouthfeel could be discriminated based on SCWC type.

It was generally believed that yeast mannoprotein is largely responsible for the mouthfeel sensory attributes perceived in white wine, whereas grape phenolic compounds are widely considered the predominant driver for the mouthfeel sensory attributes perceived in red wines. In this study, it was found that both SCWCs and phenolic compounds were correlated with mouthfeel, and play important and possibly similar roles in influencing this characteristic in both Chardonnay and Cabernet Sauvignon wine. The results provide new insights, insofar as yeast mannoprotein and grape phenolic compounds are not the only mouthfeel drivers; the contribution of SCWCs, especially those derived

from grapes, cannot be neglected. In some cases, the statistical analysis implied that the differentiation of wine mouthfeel sensory attributes on the basis of grape derived SCWCs was slightly greater than that of phenolic compounds, and this effect was more obvious in Chardonnay wines.

Most polysaccharide families, except HG, which was correlated with bitter and sour tastes, were positively correlated with overall wine mouthfeel attributes. In Cabernet Sauvignon XG and AG II were highly correlated with viscosity, mouth roughness and astringency, whereas HM and arabinan had a slightly greater correlation with mouth coating and chalkiness. Similarly, in Chardonnay wines, almost all polysaccharide families were positively correlated with overall wine mouthfeel attributes. Neutral grape-derived SCWCs such as XG, AG I and HM were positively correlated with viscosity and burn mouthfeel, whereas HG was correlated with sour and bitter tastes and astringency. In Chardonnay wines, HX showed no significant effect on mouthfeel. Even though each neutral SCWC type exhibits varying relationships with mouthfeel attributes, they collectively affect the overall sensation in the mouth, irrespective of wine type.

In wines, yeast-derived SCWCs are derived from two sources: endogenous yeast populations on grape berries and inoculated yeast (*Saccharomyces cerevisiae*) used for fermentation. Yeast-derived SCWCs contributed to the total SCWCs in the grapes and were present at varying levels during berry maturation. In this study, the amount of yeast glucan and mannan in wines was generally lower than grape-derived SCWCs. It is important to point out that in both Chardonnay and Cabernet Sauvignon wines, yeast SCWCs were not the key determinants of positive mouthfeel attributes. Even though they contributed to the overall sensation in the mouth, they correlated with burn mouthfeel in Cabernet Sauvignon wines and had minimal impact on mouthfeel in Chardonnay wines. This may not hold true in commercial 'sur lie' aged white wines because permitted, favorable additives such as yeast mannoprotein derivatives can contribute to the changes in the SCWC content and composition of the wines.

6.1.2. Changes in SCWCs in Grapes

Tracing these wine SCWC polysaccharide families back to their grape berries of origin (Chapter 5), no major changes in grape-derived SCWC content were observed during development in Chardonnay and Cabernet Sauvignon grape berries. However, significant variations in SCWC composition were observed. In both cultivars, HG was present in higher proportions than RG I/II and AG I/II, and HM was the most abundant hemicellulose. Similar proportions pectic polysaccharides and AGs were observed in wines, but the abundance of hemicelluloses varied.

The XG, RG I/II, and AG I/II appeared to follow similar trends in both grape berries and wines at the same ripening stages throughout. This suggests that these polysaccharide families resist fermentation and are stable in the wine matrix.

6.2. Potential Applications of the Findings

The knowledge obtained here will be extremely valuable for the wine industry by enabling measurement and monitoring of key polysaccharide precursors of grapes that are positively or negatively correlated with desirable wine mouthfeel sensations in the wine. While the development of new grape cultivars could result in selection of superior mouthfeel traits, the traditional breeding process is costly and could take decades. Thus, it is more advantageous to select existing commercial grape cultivars naturally rich in mouthfeel marker compounds or employ winemaking techniques capable of increasing the concentration of SCWCs in wine for wine making and blending. Cabernet Sauvignon wines had higher levels of SCWCs compared with Chardonnay wines. This indicates that grape skins contain appreciable amounts of SCWCs. Popular Bordeaux style long-duration winemaking process, which intentionally extends soaking of grape skins and seeds in cold juice ('cold soak') or fermenting juice ('extended maceration'), can enhance the extraction of SCWCs available in grape skins. It should be noted that this option needs to be manipulated carefully to avoid over-extraction of undesirable chemical compounds like bitter-taste seed tannins. Exogenous materials, such as commercial enzymes and fining agents, are often used to improve the quality and the

efficiency of the winemaking process. The introduction of these exogenous materials into either juice or wine can affect the polysaccharide structures. For example, industrial pectinase preparations can break down the grape pectic polysaccharides present in juice and wine when used at optimum conditions.

Based on the results reported in Chapters 3 and 4, simple assays could be developed in both grape extracts and wine to predict mouthfeel characteristics and to manipulate the SCWCs accordingly. For example, the neutral polysaccharides XG, AGI/II are positive contributors to mouthfeel, so analysis of monosaccharide compositions by anion exchange chromatography could be used to quantitate desirable monosaccharides. This analytical method is more straightforward than linkage analysis.

Conversely, winemakers could select against undesirable mouthfeel sensations, such as bitter and sour tastes, through simple marker assays of HG or galacturonic acid residues using the m-hydroxydiphenyl-sulfuric acid method, with galacturonic acid as the standard. This colorimetric assay could be based on that of Blumenkrantz and Asboe-Hansen (1973), which is cost efficient and rapid and hence it could be practically applied for the wine industry. However, it would be important to ensure that the test samples were free of interfering compounds such as anthocyanins.

The research methods reported here could also be applied to future studies of other commercially important grape varieties such as Syrah and Pinot Grigio to compare the sensory mouthfeel impact of polysaccharides of the different grape sources. The SCWC constituents used to predict mouthfeel would need to be validated for the specific grape variety and winemaking process.

6.3. Methods Development

Details of the methods developed during the course of this work are described in Chapter 2 and challenges around the methods have been described throughout the thesis. For example, chemical analyses of the SCWCs from grapes and wine presented some unusual problems that are not usually experienced in linkage analyses of well-characterized polysaccharides found in plant and

fungal cell walls. In particular, the calculations used to deduce the polysaccharide composition of the samples from glycosyl linkage values were based on the calculations described in Pettolino et al. (2012). However, there were an unusually high number of unassigned glycosyl residues, especially terminal residues. This observation suggested that the parent polysaccharides were subjected to either extensive depolymerization during the winemaking process or that they had unusually high substitution levels. Thus, the calculations were revised as described in Chapters 3, 4 and 5.

One major advantage of the linkage analysis performed here is that it allows the estimation of the classes and origins of the polysaccharides and their degraded oligosaccharide products in grapes and wine, based on well-characterized cell wall polysaccharides commonly found in plants and wine yeast (*Saccharomyces cerevisiae*). For example, the origin of Man residues can be distinguished because 4-linked Man residues are found exclusively in plants, whereas (1,2/1,3; 1,6)-linked Man residues are diagnostic of yeast mannan and mannoproteins.

However, linkage analysis has its limitations. It is known that the anomeric configuration of glycosyl residues is destroyed during the process and an unknown anomeric configuration can complicate polysaccharide assignment when dealing with complex, heterogeneous cell wall materials. In this study, 4-linked Glc residues, which can be constituents of more than one polysaccharide type, were assigned to (1,4)- α -glucan of yeast cell walls. It is important to acknowledge that those 4-linked Glc residues might have originated from (1,4)- β -glucans, which would include oligosaccharides of degraded XG or cellulose. Furthermore, linkage analysis does not provide any information about the sequence of glycosyl residues and the structures of glycoconjugates where the glycosyl residues are linked to lipid or protein molecules are difficult to determine.

In addition, many wines have low but measurable levels of bacterial and fungal contamination and some of the unassigned residues encountered during the analyses might originate from these microorganisms on the grape surface or on contaminated winemaking equipment. For example, some of the unassigned linkages such as 2-Galp reported in Chapters 3, 4 and 5 might be attributable to the presence of bacterial exopolysaccharides.

6.4. Future Structural Analyses of SCWCs

To overcome these limitations and to confirm the SCWC structures reported in this study complementary analytical techniques are clearly required. Nuclear magnetic resonance spectroscopy (NMR) can be used to identify the anomeric configuration of glycosyl residues and the degree of esterification of non-derivatized complex carbohydrates. However, NMR has its shortcomings because the degree of sample purity and spectra resolution can affect data interpretation. Matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry has shown to be a rapid tool for profiling mixtures of complex carbohydrates. Size exclusion chromatography with integrated refractive index detector, viscometer and light scattering detectors can provide useful information about the molecular weight, size and structure of complex carbohydrates. Enzymes that are specific for certain cleavage sites of the oligo- or polysaccharides can also be exploited in structure elucidation. However, none of these instrumental analytical techniques can independently solve all complex carbohydrate structural problems, especially when these polymers are assayed in complex mixtures. In many cases, large quantities or purified substituents would be required. Therefore, a combination of different techniques would be necessary to obtain more complete structural information on these grape and wine SCWCs.

6.5. Future Approach to the Assessment of Sensory Characteristics

The correlations between wine chemistry and sensory evaluation established in this study arose from the application of multivariate data analysis that simultaneously examined the relationship patterns of multiple variables. However, correlation does not imply causation. Further work to understand the cause and effect of each polysaccharide and oligosaccharide type in relation to the sensory outcome is needed to validate the findings. This can be achieved by isolating a specific SCWC by means of solvent extraction or by fractionating the alcohol insoluble residues using a series of chromatographic techniques such as anion exchange, size exclusion and affinity chromatography, and subjecting the purified or partially purified SCWC to sensory evaluation.

It is possible, for example, that winemakers might wish to add a purified or enriched preparation of XG to enhance mouthfeel in their wines. However, before adding such an XG preparation, one would need to take into account both the safety and the allowance of the use of non-food grade solvents or non-standard winemaking processes to obtain the studied materials for the sensory evaluation. In the U.S, the Alcohol and Tobacco Trade and Tax Bureau (TTB) is the primary governing body of the wine industry and has promulgated strict regulations on authorized juice and wine additives, treating materials and processes. Commercial food grade polysaccharides that have the chemistry properties closest to the wine polysaccharides could be the alternative source. In either case, the threshold levels of the SCWC additive would need to be defined. The polysaccharide of choice could be spiked into a model wine at different dose levels for sensory evaluation in order to identify its sensory attribute and detection threshold. Elaborate sensory evaluation methods comprising a large group of experienced tasters are required to support the objectives of such a dosing study. The staircase method with ascending and descending concentrations, and the forced-choice method of limits, both of which involve either stimulus of the whole mouth or local applications in the mouth, may be applicable here. The drawback of conducting this type of sensory evaluation is the significant time and financial commitment required.

Sensory descriptive analysis is a powerful tool for product development and wine style attainment evaluations. Trained sensory descriptive panels are capable of characterizing and quantifying the sensory attributes of wine. However, this cannot be used to predict consumer liking because it is recognized that deviations exist between wine manufacturer's idea of wine quality and that of consumers. In the wine market, consumer sensory testing is conducted because it provides valuable information for determining wine quality, including the identification of key sensory attributes such as mouthfeel that drive consumer wine purchase decision. Hedonic sensory testing is generally the most commonly used sensory method to gauge consumer liking of a wine blend.

Wine composition is highly complex. Therefore, all relevant scientific aspects must be considered to fully understand the factors affecting sensory perception. This study suggests that

SCWCs, aside from their mouthfeel impact, may indirectly modulate the volatile aroma molecules in wines through covalent and non-covalent chemical interactions. In addition, multimodal interactions may influence the correlation between chemical compounds that have no clear impact on taste, mouthfeel or aroma, as observed in the correlation between HG and bitter taste in Chardonnay wines. The chemical measurements do not always entirely explain the sensory differences observed in the wines.

The physical measurements of wine tribology could be another method that can be used to predict wine sensory properties. Oral tribology is an emerging research area and has gained increasing attention in the food and beverage industry. Tribology is the study of lubrication, friction and wear of surfaces in relative motion. Tribology measurements can provide meaningful insights to several “texture” related sensory perceptions of mouthfeel (Pradal and Stokes, 2016). It has been reported that tribology measurements of food systems can provide insights of mouthfeel sensory perceptions during consumption (Stokes et al., 2011). This approach is worthy of exploration since the sensory mouthfeel experience occurring during wine consumption involves the physical and chemical interactions of the wine constituents, possibly including SCWCs, at oral interfaces.

Preliminary experiments investigating wine tribology were conducted at the University of Queensland during the present study to explore the feasibility of using a tribometer to measure the physical properties of wines. The first attempt was to investigate if wine displays a Stribeck curve spanning the entire range of lubrication under the experimental conditions. This experiment utilized the existing tribometer configuration and operating parameters at Professor Jason Stokes’s laboratory at the University of Queensland. The preliminary results suggested that each red and white wine displayed a Stribeck curve with a small degree of differences between them (Figure 6.1). However, the reproducibility of the experiment and the interpretation of the results were challenging, because there were no previous data in this area for reference. Nonetheless, this technique is non-destructive, straightforward and faster than the standard chromatography procedures required for the

measurements of SCWCs present in wines. More work in this area would be necessary to understand if the physical properties of wines can predict the sensory experience during wine consumption.

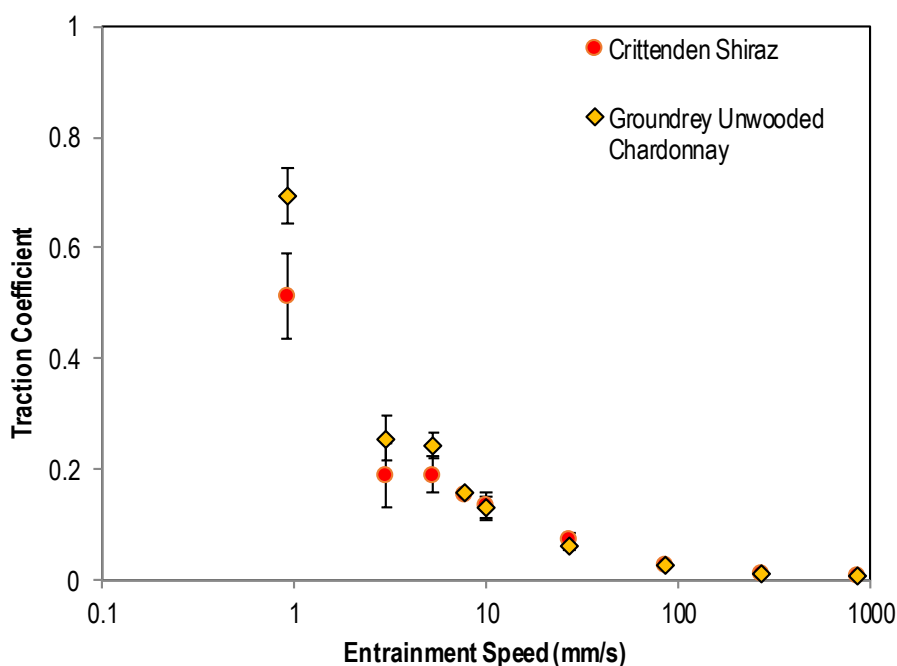


Figure 6.1. Measured tribological properties (Stribeck curve) of wines as a function of entrainment speed with hydrophobic-hydrophobic polydimethylsiloxane tribopairs. At the lowest speed, Chardonnay wine had a higher traction coefficient (could imply less “lubricating effect”) than Shiraz wine. However, at the higher speeds, they were the same.

6.6. Viticultural Management and Mouthfeel

The results described in Chapters 3, 4 and 5 of this thesis will enable the wine industry to optimize grape harvest timing to ensure that the key polysaccharide precursors of the SCWCs are present in high abundance in the berries to make wines offering targeted mouthfeel attributes. This approach allows for the delivery of a more wine-like mouthfeel than other non-grape commercial mouthfeel additives such as gums or wood tannins. The wine industry could strategically apply viticultural practices and winemaking techniques to successfully grow grapes that contain more desirable polysaccharides to produce wines that meet consumer expectations of sensory experience. In addition, new products offering a unique mouthfeel could be created by blending when a known

SCWC composition is used to best predict the sensory target. This understanding will open doors to novel wine mouthfeel management that is not limited to tannins and astringency.

6.7. Literature Cited

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