

THE IMPACT OF WINE COMPONENTS ON THE CHEMICAL
AND SENSORY PROPERTIES OF WINES

By

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DEDICATION

Soli Deo gloria

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Abstract

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The wine matrix includes a variety of compounds that influence wine quality. The overall objective of this study was to examine the relationship between the matrix components and sensory attributes of wine. To address the issue on wine quality as related to tannin and polymeric pigments, the effect of initial tannin concentration, storage time and temperature on chemical and sensory properties of young bottled Cabernet Sauvignon and Merlot wines was studied. In general, storage treatment resulted in a significant increase in small polymeric pigment (SPP) with a decrease in anthocyanin ($p \leq 0.05$). Tannin concentration was directly related to large polymeric pigment (LPP) and correlated with perceived astringency. Increased perceived bitterness was associated with storage at 32°C for 70 days. In addition to tannin content, further experiments investigated the interaction of tannin with ethanol and fructose concentrations on the headspace concentration of eight odorants in model wine. In general, increased tannin concentration exhibited an enhancement effect while fructose induced a retention effect, both of which were largely dependent upon ethanol concentration. The net magnitude effect was a reduction in the odorants' headspace concentration dominated by ethanol. Odor detection thresholds increased between 2 and 10,000-fold, lowering the odor unit values (OUV). The impact of ethanol, tannin and fructose concentrations on the sensory properties of model red

wines was also evaluated. Principal component analysis (PCA) differentiated the model wines based on PC 1 (floral, fruity and caramel), PC 2 (earthy and herbaceous) and PC 3 (sulfur, spicy, woody, and bitterness). Analysis of variance (ANOVA) results showed a significant impact of ethanol concentration on these principal components ($p \leq 0.05$). Tannin and fructose, and all interaction effects were not significant. These results highlighted the influence of major wine components and their interactions on sensory perception. Viticulturists and winemakers are suggested to consider the information obtained in this research when making decisions to optimize wine quality.

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CHAPTER I

INTRODUCTION

Over the last three decades, the production and consumption of United States (US) wines have demonstrated a consistent growth. The total economic impact of the wine industry reached estimated 4.7 billion dollars with Washington State contributing more than 3 billion dollars (MKF Research LLC 2007). However, despite this satisfactory performance, the demand to maintain production of wines with consistent quality continues to be one increasingly important challenge for the producers. Given the fast changing global wine trends and highly competitive market today, there is a pressing need to invest even more on focused research to meet this challenge.

From the point of view of the wine industry, the perception of wine quality is generally easier to recognize than it is to define. It is often related to the intrinsic aspects of wine, which are consequences of the grape, grape growing and winemaking conditions. The numerous applications of chemical and analytical sensory methods geared towards establishing quality parameters of wine have brought valuable knowledge to understanding perceived quality. In general, the sensory approach includes the evaluation of stylistic purity, visual appeal, subtlety and complexity, aging potential, personality, length, finish, harmony and balance amongst all the components (aroma, taste, flavor and mouthfeel) (Amerine and Roessler 1976, Jackson 2000), all of which are inevitably linked to wine chemical composition.

Wine is a complex alcoholic beverage, which contains numerous compounds, capable of influencing perceived quality of wine. The phenolics are major non-volatile compounds widely known as essential components of red wine quality. Among the phenolic compounds, tannins are of particular importance in red wine (Harbertson et al. 2003). They contribute to perceived

astringency (Landon et al. 2008, Fischer and Noble 1994, Gawel 1998), which is the drying, puckering mouthfeel that results from increased friction between the tongue and the surfaces inside the mouth (Lea and Arnold 1978). However, previous studies have shown that tannin concentration alone is not enough to explain the variation of astringency perception in wines (Ishikawa and Noble 1995, Landon 2007). During aging, anthocyanins react with tannins to form polymeric pigments or pigmented tannins (Somers 1968, Somers 1971, Remy et al. 2000), which are thought to have different protein-binding properties than tannin, thus contributing to the reduction of astringency (Singleton 1992). Storage conditions that enhance aging of wine and production of polymeric pigments may allow investigation of the effects of the polymeric pigments on perceived astringency. A modified protein precipitation assay and bisulfate bleaching method to fractionate these polymeric pigments into large polymeric pigments (LPP) and small polymeric pigments (SPP) developed by Harbertson et al. (2003) may be applied.

The volatile composition of the wine is also one of the important factors determining wine quality. Recent studies indicated that the nature of the wine matrix affects the concentration of various odorants in the headspace, which are responsible for the aroma and flavor of wine. Ethanol, the most abundant volatile compound has been shown to decrease the partition coefficient of various classes of volatile compounds by increasing the solubility of volatile compounds in model wine systems (Whiton and Zoecklein 2000, Hartmann et al. 2002). The suppression effect of ethanol on aroma was demonstrated to correspond to the increase in odor detection threshold in the presence of ethanol (Grosch 2001). This in effect could impact the contribution of aroma volatiles to the overall characteristic of wine aroma and perception. Other sensory investigations on the impact of ethanol on wine aroma perception revealed an increased overall fruity and flowery/floral intensity (Escudero et al. 2007, Guth 1998). An additive effect

of ethanol on fruity and woody aroma intensity was also reported in a simple model solution (Le Berre et al. 2007).

The phenolic compounds have been also reported to affect the release of aroma compounds to some extent. Decreased volatility was observed for 2-methylpyrazine and ethylbenzoate in 1% ethanol/water and aqueous solution, respectively due to gallic acid (Aronson and Ebeler 2004) whereas the same monomeric phenol had no impact on 3-alkyl-2-methoxypyrazines (Hartmann et al. 2002). Jung & Ebeler (2003) evaluated the effect of catechin on flavor volatility and showed a significant reduction in the ion response for hexanal and ethylhexanoate when compared to water but not for heptanone. The addition of tannin was found to have no significant effect on perceived aroma in Cabernet Sauvignon and Chardonnay wines although headspace analyses revealed reduction in odorant volatility (Aronson and Ebeler 2004). In Malbec wines, the perception of fruity, citrus, strawberry, cooked fruit and floral aromas was observed higher in wines with higher polyphenol content (Goldner et al. 2011).

Although studies on the direct influence of some chemical components on sensory properties had been previously reported, little research has focused on the interaction effects of major wine components. Wine is a complex alcoholic beverage, which contains numerous components that may interact with odorants and alter the quantity of volatile compounds available for perception. Robinson et al. (2009) found a significant reduction of peak areas for most volatiles due to increased ethanol concentration, which was slightly increased in the presence of glucose in model solutions. Jones et al. (2008) showed interaction effects of wine proteins, alcohol and glycerol concentration at the lower volatile concentration. Conversely, overall aroma intensity was not impacted by glycerol or ethanol at higher volatile concentration. In another study, perceived bitterness increased with increased ethanol, catechin and to some

extent pH, with the largest effect induced by ethanol. On the other hand, the masking effects of ethanol on sourness were evident only at pH 3.2 while catechin produced no significant effect (Fischer and Noble, 1994). Vidal et al. (2004) showed that the perception of astringency increased with tannin concentration but decreased in the presence of rhamnogallacturonan II.

Thus, the overall research objective was to examine the relationship between the wine matrix components and the sensory and chemical attributes of wine. Specific objectives and hypotheses were:

1. To determine the influence of initial tannin concentration, storage temperature and time on the chemical and sensory properties of Cabernet Sauvignon and Merlot wines. We hypothesize that higher tannin concentration, storage temperature and longer storage time will induce chemical and sensory changes in the wines;
2. To determine the effects of ethanol, tannin and fructose concentrations and their interactions on the headspace concentration of selected odorants and their aroma contribution in a model wine. We hypothesize that the headspace concentration and aroma contribution of odorants will vary with ethanol, tannin and fructose concentrations, and the interactions among these matrix components, in the model wine solutions;
3. To determine the effects of ethanol, tannin and fructose concentrations and their interactions on the aroma, taste, flavor and mouthfeel properties of model wine. We hypothesize that perceived aroma, taste, flavor and mouthfeel ratings of model wines will be different depending on ethanol, tannin, and fructose concentrations and interactions.

This dissertation is divided into 6 chapters. Following this introductory chapter is a review of literature on wine quality and composition, and their effects on wine sensory properties presented in Chapter II. The manuscript entitled “The influence of initial tannin concentration,

storage temp and time on the chemical and sensory properties of Cabernet Sauvignon and Merlot wines” which was published in the American Journal of Enology and Viticulture in 2009 is provided in Chapter III. Chapter IV includes the second manuscript submitted recently to the Journal of Agricultural and Food Chemistry entitled “Effects of ethanol, tannin and fructose on the headspace concentration and potential sensory significance of odorants in a model wine”. The third manuscript entitled “Sensorial impact of ethanol, tannin and fructose concentrations in a model wine” is presented in Chapter V, and will be submitted to American Journal of Enology and Viticulture. Finally, a summary of conclusions and recommendations is presented in Chapter VI.

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CHAPTER II

LITERATURE REVIEW

Wine Quality

From an enological point of view, the term “wine” is defined as “the drink resulting from the fermentation by the yeast-cells, and also in certain cases by the cells of lactic bacteria, of the juice from the crushing or maceration of grape-cells” (Peynaud 1984). Of the grape genus *Vitis*, the species *V. vinifera* is often cultivated for wine production. This alcoholic beverage contains numerous chemical components varying in composition and proportions due to many factors such as the quality of raw material (grape), conditions of winemaking, storage and transport. Wine components include water, alcohols, acids, sugars, phenolics, nitrogenous compounds, vitamins and various volatile compounds, with each constituent capable of contributing unique aromas, tastes and oral sensations to the wine and ultimately, affecting its perceived quality.

The issue of quality has become increasingly important to the wine industry over the past several decades. Worldwide, an oversupply situation has prevailed, consequently pulling down wine prices and decreasing profitable margins for producers. It has been suggested that the wine industry must vigilantly attend to wine quality, in conjunction to marketing efforts in order to lead the competition (Summer et al. 2010). This raises important question as to what is meant by quality regarding wine.

Quality may be understood in different ways. Generally, quality in foods and beverages is defined as the ability of a set of inherent characteristics of a product, system or process to fulfill requirements of customers and other interested parties (ISO 2000). From the perspective of marketing, with reference to wine, there exist two quality dimensions based on the consumer experiences according to Charters and Pettigrew (2007): the external (grapes, production and

marketing) and the internal (pleasure, appearance, gustatory, paradigmatic and potential) quality, the latter being more significant in evaluating the overall wine quality during consumption. However, some marketing studies have defined the concept of quality as a one-dimensional judgment. As cited by Charters and Pettigrew (2007), these studies suggest that perceived quality is a broad overview and exists on a continuum.

Meanwhile, in the wine industry, wine quality is more recognized as related to the intrinsic aspects of wine. The numerous applications of analytical sensory methods geared toward establishing quality parameters of wine have brought valuable knowledge in understanding perceived quality. The sensory evaluation approach includes the evaluation of stylistic purity, visual appeal, subtlety and complexity, aging potential, personality, length, finish, harmony and balance among all components (Amerine and Roessler 1976, Jackson 2000). It was noted that there is hardly an exact definition of each of these terms while a common explanation to describe some terms can be derived from the literature. The “complexity and subtlety” are often referred to as highly valued attributes of the richness of aroma and flavor of wine while the “development or length” refers to changes in the intensity and aromatic character of the wine after pouring. “Balance and harmony” are described in wine having a smooth taste (where one taste does not dominate another) and mouthfeel combined to produce an acceptable overall pleasurable sensation (Jackson 2000, Peynaud 1996).

Wine Components and Wine Quality

Phenolic compounds

The phenolic compounds are major non-volatile components in wines, which are widely recognized as an essential component of wine quality. They provide color (Somers 1971, Somers and Evans 1986), astringency (Landon et al. 2008) and bitterness (Fischer and Noble 1994) to

wine. The phenolic composition of the finished wines depends on the grape and winemaking practices. Polyphenols are unstable compounds, with their reactions starting as soon as the grape is crushed or pressed, continuing throughout winemaking and aging. These reactions may involve interaction or binding with aroma compounds, thus influencing aroma release and perception (Clarke and Bakker 2004).

White wines are essentially composed of phenols found in flesh of the grapes such as gallic acid, hydroxynammic acid esters, catechin, epicatechin, galocatechin gallate, procyanidin and catechin gallate. The content varies and normally found in few mg/L units. On the other hand, red wines are mainly composed of several closely related chemical groups: flavanol-3 group (catechin), flavane(3,4)diol group (leucocyanidin), flavanol-3 (quercetin), anthocyanins and tannins, in addition to those phenolic compounds found in white wines (Margalit 2004; Peynaud 1984).

Among the phenolic compounds, tannins are of particular importance in red wine. Tannins, the most abundant class of phenolics in grapes contribute to perceived astringency. Astringency is an important attribute in wine influencing consumer acceptability. It is often described as the drying, roughing and puckering feeling (ASTM 1989) attributed to the precipitation of a non-covalent complex between salivary-mucoproteins and proline-rich proteins and tannins in the mouth (Noble 1998, Gawel 1998). The cross-linking of mucoproteins results in its precipitation and consequently, reduction in lubrication and increased friction in the mouth (Green 1993) while the proline-rich proteins bind favorably with tannin (Hagerman et al. 1998).

The sensory properties of tannins are thought to be dependent upon their structure, molecular size and concentration. Tannins may be bitter and/or astringent. Bitterness is restricted to small molecules with particular structural features enabling them to enter the receptor and

activate the signal transduction process, whereas astringency depends on the number of protein interaction sites in the molecule (Cheynier et al. 2006). The low molecular weight flavanols are both bitter and astringent (Robichaud and Noble 1990). Tannins become gradually less bitter but more astringent as the molecular weight increases to about 10 units. The perception of astringency was also observed to significantly increase as tannin concentration increased, with a tendency to mask perceived bitterness (Arnold and Noble 1978).

Sugars

Glucose and fructose account for the sweet taste of some wines with recognition thresholds equivalent to 16 g/L and 9.5 g/L, respectively (Margalit 2004). Dry wines generally contain less than 1.5 g/L of these residual sugars dominated by fructose, thus are not perceived as sweet. In contrast, ice wines, which are typically produced from Vidal and Riesling grape varieties contain 140 to 280 g/L range of sugar in the finished wines and are sweet and aromatic (Cliff et al. 2002).

The influence of other components such as ethanol, acids, and tannins on the perception of sweetness has been accounted previously. The sweet sensation has a mitigating effect on perceived sourness and bitterness. In addition, sugar concentration was also reported to increase the volatility of aromatic compounds (Jackson 2000). At the level of sugar in model white wines increased (80 to 250 g/L), approximate equal to that of ice wine, both the perceived density and viscosity increased (Nurgel and Pickering 2006).

Polysaccharides

Polysaccharides in wine occur in wines at concentrations of 500 to 1500 mg/L (Will et al. 1991). These wine macromolecules originate from grape primary cell walls and autolysis of microorganisms such as yeasts used in winemaking or *Botrytis cinerea*, a parasitic mold of the

vine. Those polysaccharides derived from grape cell walls include the arabinogalactan-proteins (AGP) and rhamnogalacturonan II while those from yeast cell walls are mainly mannoproteins. These polysaccharides are classified based on acidity and protein contents, either as neutral or acidic pectic polysaccharides (Pellerin et al. 1993, Pellerin et al. 1995, Pellerin et al. 1996). The interaction between polysaccharides and other wine constituents has been recently investigated. An AGP fraction and a *Saccharomyces* cell wall mannoprotein were reported to contribute to a reduction in protein haze (Waters et al. 1994a, Waters et al. 1994b). The rate of crystallization of potassium hydrogen tartrate was influenced by the presence of wine polysaccharides, specifically the rhamnogalacturonans, which bind to crystal growth sites (Gerbaud et al. 1997).

The role of wine polysaccharides on wine sensory properties has been investigated to a lesser extent. Vidal et al. (2004) evaluated two wine polysaccharides (a mixture of arabinogalactan proteins, mannoproteins and rhamnogalacturonan II) and found that both polysaccharide fractions significantly increased the fullness sensation of the wine. The rhamnogalacturonan II fraction significantly decreased the perceived intensity of astringency whereas the neutral wine polysaccharide fraction had less of an effect on reducing the ratings of these attributes. These results confirmed that the role of polysaccharides on the mouthfeel properties can be either direct, through the impact on mellowness, or indirect, through modulation of tannin astringency. The importance of polysaccharides (yeast mannoproteins) in the enhanced perception of sweetness and roundness was also reported recently in Tempranillo wines (Guadalupe et al. 2007). The ability of yeast macromolecules to bind aroma compounds affecting aroma perception has been evaluated (Chalier et al. 2007, Dufour and Bayonove 1999a). Nevertheless, more studies are needed to better clarify their overall sensorial impact on wine.

Proteins

Protein nitrogen in wine is only about 2% of the total nitrogen content. Almost all amino acids are included in proteins. Most abundant amino acids are alanine, aspartic acid, glutamic acid, glycine, serine and threonine (each between 9-17% of the total amino acids). In general, there is an occasional increase in protein content in wine compared to its must content that is attributable to the yeast during and after fermentation (Margalit 2004). However, recent investigation revealed that the majority of the polypeptides present in wine originated from grape pulp (Ferreira et al. 2000). Proteins have been reported to have average molecular weights in the 10,000-30,000 Dalton range, with low iso-electronic points ($4.1 < pI < 5.8$) (Margalit 2004, Brissonnet and Maujean 1993, Ferreira et al. 2000).

Wine proteins have been known to be the cause of clouding or haze in wines. Bentonite fining appears the most promising clarifying technique to remove proteins in wine (Sanborn et al. 2010). However, some evidence have indicated that wine turbidity cannot be attributed exclusively to the presence of wine proteins (Waters et al. 1991, Waters et al. 1992) but also to a number of other factors, that are of non-protein in origin such as ethanol and pH (Lagace and Bisson 1990), polysaccharides and polyphenols (Vernhet et al. 1996). It has been suggested that the interaction between proteins and other wine components on wine stability should be the focus of future research on wine proteins.

The proteins in wine are also beneficial to winemakers, particularly those involved in the production of sparkling and champagne wines owing to their role in foam formation and foam stability (Brissonnet and Maujean 1993).

Acids

Important non-volatile, fixed acids in wine include tartaric, malic, citric and succinic acids. They are mostly formed during photosynthesis, growth and ripening of grapes. Titratable acidity (TA) in grapes normally ranges from 5 to 16 g/L whereas in wine, lower values are observed at about 5 to 7 g/L range (Zoecklein et al. 1995).

The actual sour taste of a wine depends on the absolute and relative amounts of the various acids, the proportion of acids present in undissociated form or as acid salts, factors that affect the pH of the wine, the sugar and ethanol concentrations. At the same level of acidity, perceived sourness of the common wine acids proceeds in the order as follows: malic > tartaric > citric > lactic (Amerine et al. 1965, Fischer and Noble 1994).

Acids not only elicit sour taste, but they also modify the perception of other taste and mouthfeel sensations. This is especially noticeable in a diminished perception of sweetness demonstrated in a recent study of sourness-sweetness interactions in water, wine and alcohol mixture (Zamora et al. 2006). In particular, Zamora and others (2006) observed a suppressive effect of tartaric acid (solutions adjusted to pH 3.0, 3.4 and 3.8) on the perceived sweetness of fructose. However, in general, sweeteners suppress sourness, and the amount of sourness suppression depends on both components' levels (Schifferstein and Frijters 1990, Bonnans and Noble 1993). In addition to sour taste perception, acids also have astringent properties that have been directly related to pH (Sowalsky and Noble, 1998).

The perception of acidic character of wine is described by as a function of palate balance according to the relationship (Zoecklein et al. 1995):

Sweet ↔ acidity + astringency and bitterness

The role of acids in maintaining a low pH is crucial to the color stability of red wines. As the pH rises, anthocyanins lose their color and may eventually turn blue above pH 4, and then fade to yellow in neutral or alkaline medium (Ribereau-Gayon et al. 2006). Acidity also affects ionization of phenolic compounds. The ionized form of phenols (phenolate) is more readily oxidized than is the nonionized form (protonated phenol). Accordingly, wines of high pH (≥ 3.9) are very susceptible to oxidation and loss of their fresh aroma and young color (Singleton 1987). Acids are also involved in the precipitation of pectins and proteins that otherwise could cloud a finished wine. Conversely, acids can solubilize copper and iron, which can induce haziness (Jackson 2000).

Volatile compounds

Wine aroma is extremely complex. It is the cumulative effect of a diverse group of volatile compounds, which are typically detected at very low concentrations between 10^{-4} and 10^{-12} g/L (Guadagni et al. 1963). These volatile compounds generally include alcohols, esters, aldehydes, ketones, acids, terpenes, phenols and sulfur compounds present in varying concentrations. Wide differences in the proportions and characteristics of aromas can be greatly influenced by both viticultural (including climate, soil, water, cultivar, grape-growing practices.) (Jackson and Lombard 1993) and enological (condition of grapes, fermentation, postfermentation treatments) (Francis and Newton 2005, de Revel et al. 1999, Voilley et al. 1990) factors. Maarse and Vischer (1989) have estimated more than 800 volatile compounds present in wine. However, only a few compounds were reported to be present at concentrations above the perception threshold and thus, responsible for characteristic odors (Cullere et al. 2004, Li et al. 2008, Guth 1997, Zhang et al. 2007). The total content of aroma compounds in wine is approximately 0.8-1.2 g/L (Rapp 1998, Rapp and Mandery 1986).

The volatile compounds that are detected by the olfactory receptors and responsible for the perception of wine aroma and flavor are described below. These compounds have relatively low threshold levels and are commonly reported to be present in substantial amounts. According to different sources, volatile compounds are classified as: 1) primary aroma or aroma arising directly from the grapes and modifications during grape processing, 2) secondary aroma or aroma produced by fermentation, or 3) tertiary aroma or the bouquet resulting from the transformation of the aroma during aging (Rapp and Mandery 1986). Each of these types of aromas is discussed in greater detail below.

The grape-derived components responsible for the primary aroma or varietal character of wines are predominantly localized in the exocarp (skin) tissue. The majority of these compounds are stored as sugar or amino acid conjugates in the exocarp cell vacuoles while some compounds are present as free volatiles. The compounds stored as conjugates are released through the action of glycosidases and peptidases introduced at the time of crushing, pressing and during fermentation thereby increasing the amounts available for perception in the wine (Lund and Bohlmann 2006). Volatile compounds associated with varietal aroma of grapes are comprised of terpenes, norisoprenoids, pyrazines, and carbonyl compounds.

Floral, rose-like aroma in Muscat (Muscat d'Alexandrie, Morio Muscat, and Muscat blanc), grapes (Weisser Riesling, Bukettraube, Guwürztraminer, Fernao Pires and Scheurebe) and wine is due mainly to the presence of monoterpenes such as linalool, geraniol, nerol, α -terpineol and hotrienol occurring in complex combinations (Marais 1983). Differences in the concentrations are dependent on the cultivar, climatic conditions, grape maturity, pH, enzymes, storage time, extraction and winemaking procedures (Marais 1983). While the majority of monoterpenes were detected at high concentrations, their perception thresholds were observed to

be lower in the presence of other terpene compounds than in isolation (Ribereau-Gayon et al. 1975).

Other grape aroma-active compounds structurally related to terpenes are the C₁₃-norisoprenoids, which are responsible for the typical aroma of some grape varieties. They are also considered important in both white and red wines due to their very low odor thresholds (de Pinho et al. 2001, Aznar et al. 2004, Ferreira et al. 2000, Guth 1997). Winterhalter et al. (1999) showed that norisoprenoids in Riesling wines originated from several precursors and were stored as glycoconjugates. Enzymatic oxidation and cleavage of β -carotene (other carotenoids) substances during crushing of grapes and “in-bottle” ageing are thought to produce a diverse group of norisoprenoids (Ribereau-Gayon et al. 2000). These compounds include β -ionone (aroma of viola), damascenone (aroma of exotic fruits), β -damascone (aroma of rose and fruits), β -ionol (aroma of fruit and flowers), 3-oxo- β -ionone (tobacco smell), and vitispirane (aroma of fresher flowery-fruity and/ or exotic flowers and earthy-woody undertone) among others as reviewed by Mendes-Pinto (2009).

The pyrazine compound, 2-methoxy-3-isobutylpyrazine (MIBP), is considered the most important contributor of vegetative aroma character to Sauvignon Blanc and Cabernet Sauvignon grapes and wines. It represents about 80% of the total pyrazines found in wine (Allen and Lacey 1997) and exists at concentrations above its detection threshold (2 ng/L in wine) (Etievant 1991). Descriptive analysis of Cabernet Sauvignon wines has shown that higher intensities of bell pepper and vegetative character aromas were found in wines produced from younger vines compared to older vines (Heymann and Noble 1987). The extent of accumulation was also reported to depend on climatic conditions, with higher levels of pyrazines in wines obtained from grapes grown under warmer conditions (Lacey et al. 1991).

The volatile compounds that contribute to secondary aromas in wine are predominantly by-products of fermentation present at the highest concentration. Fermentation produces ethanol, fusel oil substances (aliphatic alcohols, ethers, acids, aldehydes, etc) and esters, which provide a background to the aroma of any wine (Pisarnitskii 2001). Depending on the fermentation conditions and yeast strains used, the concentrations of these compounds may vary.

Ethanol odor is described as sweet and has a concentration ranging from 7 to 14% in commercial table wines, with a low threshold between 0.01-0.05% v/v (in water) (Williams 1972). It is considered an important matrix component that may have physicochemical and perceptual effects on other volatile compounds. Meanwhile, the higher chain alcohols, fusel fuels have a characteristic pungent odor generally present at concentrations above their threshold. The most important are 3-methyl-1-butanol (whiskey, malt, burnt aroma), 2-methyl-1-butanol (lemon, orange aroma) and 2-methyl-1-propanol (wine, solvent, bitter aroma). These compounds contribute desirable complexity to wine at concentrations below 300 mg/L (Rapp and Mandery 1986). Factors affecting the production of each alcohol include yeast strain, yeast growth, ethanol production, fermentation temperature, must pH, aeration, level of solids, grape variety, maturity and skin contact time (Fleet and Heard 1993).

The characteristic fruity aromas in wine can be largely attributed to numerous acetate esters and ethyl esters of fatty acids formed from the reaction between an organic acid and alcohol during fermentation. Esters formation depends upon the fermentation temperature and alcohol concentration (Killian and Ough 1979). The presence of high amino acids concentration in must was also shown to enhance the production of more volatile esters (Guitart et al. 1999). During wine aging, the concentration of esters increased with time (Marais and Pool 1980). Esters with lower detection threshold include ethyl acetate (pineapple aroma), isoamyl acetate

(banana aroma), ethyl octanoate (fruit, fat aroma), ethyl hexanoate (apple peel, fruit aroma), ethyl butanoate (apple aroma) and 2-phenethyl acetate (rose, honey, tobacco aroma) (Baumes et al. 1986).

Changes in the aroma properties associated with oxidation can be related to the formation of aldehydes. Aldehydes are important to the aroma of wines due to their low threshold levels. In a study of the role of oxidation-related aldehydes in wine aroma, sensory experiments revealed a suppression effect of (E)-2-alkenals on flavor while branched aldehydes enhanced dried fruit aromas and masked the effect of (E)-2-alkenals (Cullere et al. 2007). Ketones are also produced during fermentation, although in small amounts. Yeasts produce diacetyl, estimated to be between 0.2-0.3 mg/L in wine and contribute to buttery aroma. The action of spoilage lactic acid bacteria during winemaking may increase the concentration of diacetyl to 1-4 mg/L to produce an off-odor (Sponholz 1993).

Wine contains between 500-1000 mg/L volatile acids, of which more than 90% consist of acetic acid (Henschke and Jiranek 1993). Acetic acid is formed during yeast fermentation as a result of the oxidation of acetaldehyde. In addition, high levels of acetic acid may be due to the presence of acetic and lactic acid bacteria acid. This vinegary, pungent compound is regarded as objectionable at concentrations above 0.7-1.1 g/L, depending on the style of wine, with an optimal value of 0.4 g/L (Henick-Kling 1993).

The off-odors often described as animal, stable, horse sweat and medical can be attributed to volatile phenols in wine present above their threshold levels. Volatile phenols may result from the decarboxylation of coumaric and ferulic acid esters from the grapes and subsequent reduction reactions during malolactic fermentation or in the presence of *Brettanomyces* sp. (Chatonnet et al. 1992). Vinylphenols (4-vinylguaiacol, 4-vinylphenol) and

ethylphenols (4-ethylguaiacol, 4-ethylphenol) are considered significant in this group of compounds, with concentration ranges in wine found to be between 0-2.8 mg/L (Castro-Meijas et al. 2003).

Most important sulfur compounds in wines can be found at $\mu\text{g/L}$ levels while thresholds may be at ng/L levels. For instance, hydrogen sulfide may be present at 370 $\mu\text{g/L}$ and impart rotten egg odor. Its perception threshold in wine was observed at concentrations between 1 ng/L and 150 $\mu\text{g/L}$ (Mestres et al. 2000). Other sulfur compounds that have an impact on wine aroma include some thioesters, thiols, thiolanes, esters of sulfur containing acids, thiazols, a mercaptal and an acetamide with a range of descriptors from asparagus, cabbage, corn, molasses, onion to rubber aroma (Goniak and Noble 1987, Rapp and Mandery 1986). Their formation is closely linked to yeast metabolism (Rauhut 1993).

During wine ageing, the loss of the grape and fermentation-derived characters of the young wine is replaced by the development of an aged bouquet (“tertiary aroma”). Depending on storage conditions, several chemical reactions may influence the aroma profile of the mature wine. Different processes involved in aroma evolution during aging of red wines in oak barrels and stainless steel, were previously described by Jarauta and co-workers (2005). These authors demonstrated that in addition to wood extraction, important changes in volatile concentration during storage may arise from reactions such as oxidation of wine alcohols and amino acids, formation of ethyl phenols by microorganisms, sorption processes, and condensation of acetaldehyde with polyphenols.

Among the compounds released from wood, the *cis*- β -methyl- γ -octalactones are observed to be most important. In a study relating evolution of volatile compounds during red wine (60% Tempranillo, 20% Cabernet Sauvignon and 20% Garnacha) maturation in twice-used French oak

barrels, it was found that the *cis*-oak lactone reached highest level at 119 µg/L after 18 months of storage (Garde-Cerdan et al. 2002). This concentration exceeds the threshold of natural *cis* isomer of 24 µg/L and 57 µg/L in white and red wine, respectively (Brown et al. 2006). Previous sensory study in barrel-aged wines showed a positive correlation between the *cis*-isomer lactone concentration and aroma descriptors such as ‘coconut’ in Chardonnay, and ‘coconut’, ‘vanilla’, ‘dark chocolate’ and ‘berry’ in Cabernet Sauvignon (Spillman et al. 2004).

Medicinal and horsey aroma characteristics of barrel-aged wines due to accumulation of 4-ethylphenol are often associated with yeast contamination. Some studies showed that the concentration of 4-ethylphenol increased with aging at levels above the threshold limit of 620 µg/L commonly found in used barrels (Chatonnet et al. 1992). Wines (blend of Tempranillo, 41% and Cabernet Sauvignon, 59%) aged for one year in used American and French oak barrels (for 5 years) reached an average 4-ethylphenol concentration of 1400 µg/L and 1657 µg/L, respectively (Garde-Cerdan et al. 2002). In another study, 4-ethylphenol was also found in wines (blend of Tempranillo (60%), Cabernet Sauvignon (20%), Garnacha (20%)) aged in twice-used French oak barrels. At the end of 18 months storage in the barrels, the highest concentration of 4-ethylphenol was observed at 1064 µg/L.

Acetaldehyde is responsible for the pungent, ethereal character of Sherry wines produced by long biological or oxidative ageing in oak casks (5-12 years). The chromatographic analysis of aroma fractions of Sherry wines made under different ageing conditions showed the highest concentrations of acetaldehyde in the Fino (545 mg/L), followed by Amontillado (183 mg/L), and Oloroso (126 mg/L) Sherry-type wines (Zea et al. 2001, Moreno et al. 2005). All of these concentrations exceeded the reported threshold of 100 mg/L in wine, suggesting its important contribution to pungent aroma particularly in Fino wines (Etievant 1991).

The isoprenoid degradation product, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) contributes to the bottle-aging character of Riesling wines. Simpson & Miller (1983) evaluated varietal grape juices exposed to high temperature to simulate quick wine ageing and found TDN concentrations particularly in Riesling grape juice to be higher (28-65 $\mu\text{g/L}$) than the published threshold level of 20 $\mu\text{g/L}$ (Simpson 1978). As suggested by Winterhalter et al. (1999), the level of TDN can be used as to estimate the aging potential of Riesling wine. TDN has a characteristic kerosene-like odor when it accumulates above the threshold value (Simpson 1978).

Increased levels of dimethyl sulfide (DMS) in wines during bottle aging were associated with reduction of DMSO (dimethyl sulfoxide) acting as precursor (de Mora et al. 1993). In aged wines, DMS is often present above the published perception threshold (25 $\mu\text{g/L}$ in white wine) (Goniak and Noble 1987) and can have a negative effect on the quality of wines (Spedding and Raut 1982, Simpson 1979). Higher concentrations of DMS in white wines were associated with asparagus, corn, and molasses aromas (Goniak and Noble 1987), with possible quince and metallic notes in aged port wines (Silva-Ferreira et al. 2003).

Understanding the complexity of wine aroma has been a challenge to wine researchers for the past 70 years. Since the first wine aroma studies conducted in the 1940's as cited by Rapp and Mandery (1986), a considerable amount of research have been performed to expand the knowledge of wine aroma. Advances in the aroma extraction, concentration, separation, and detection methods applied in wine volatile analysis in general, have been reviewed by several authors (Munoz-Gonzalez et al. 2011, Ebeler 2001, Ebeler and Thorngate 2009). These methods have brought numerous compounds to be identified, characterized and quantified in wines, with the majority of compounds having little to no sensory impact. The following section will discuss

some analytical approaches used to determine the relationships among composition and content of volatile compounds and aroma properties of wines.

For many years, gas chromatography-olfactometry (GC/O) has been a valuable analytical tool used by wine researchers to provide a hierarchical list of aroma compounds according to their potential aroma significance in wines. GC-O combines instrumental and descriptive sensory techniques to determine the odor activity (present at concentration below or above sensory detection threshold), description (smell), and time of odor activity and intensity of the odor of volatile compounds. The quality of information obtained generally depends on the volatile compound isolation or extraction procedure and the quantitative methods used, as previously described in the literature (Zellner et al. 2008, Plutowska and Wardencki 2008, Delahunty et al. 2006).

GC-O methods that have been developed and applied in wine volatile analysis can be divided into the three categories of dilution to threshold, direct intensity, and detection frequency methods. These techniques have been employed to characterize odor active compounds in Cabernet Sauvignon (Falcao et al. 2008), Grenache (Ferreira et al. 1998), Spanish aged red (Cullere et al. 2004), Pinot Noir (Fang and Qian 2005) and Riesling (Komes et al. 2006) wines. In addition, GC-O analyses have been applied extensively to identify differences among wines (Botelho et al. 2007, Ferreira et al. 2001, Gorbuz et al. 2006, Lopez et al. 1999, Bernet et al. 2002, Kotseridis et al. 2000, Kotseridis and Baumes 2000).

The combined use of GC-O and odor activity values (OAV) has also been explored in estimating the relative importance of component odorants. Following GC-O screening for impact odorants, the OAV of each compound may be calculated by dividing the concentration of a compound by its odor threshold to estimate the odor activity and relative strength. In line with

this approach, Ong and Acree (1998, 1999) employed GC-O techniques to determine the odor-active compounds in Gewürztraminer wines and lychee fruits (fresh and canned). GC-O results of both wine and fruit showed 12 common odorants with higher activity, which authors indicated to be responsible for the lychee aroma of Gewürztraminer wine. Subsequently, OAV's were calculated to determine the relative potency of the odorants. Among these compounds, they found that *cis*-rose oxide, β -damascenone, linalool, furaneol, ethyl isohexanoate, and geraniol to be more potent compounds based on calculated OAV's (>1) (Ong and Acree 1999). Further investigation using headspace solid phase microextraction (SPME) technique revealed the significance of *cis*-rose oxide, linalool, and geraniol, which were present at high concentrations in Gewürztraminer wines (Ong and Acree 1999). However, similar with other GC-O studies mentioned above, no sensory studies were carried out to validate the results.

Until recently, few wine research studies have included sensory validation of GC-O data and/or OAV to determine the real impact of key odorants taking into account synergistic, enhancement or suppression effects of different odorants as well as other wine matrix components. Such studies include preparation of aroma recombination models by addition of the target odorants selected according to their high OAV's or dilution factors (FD) in base wine or in various combinations of water/ethanol mixtures. The aroma models are then compared to the original wine for similarity or difference by triangle or duo-trio tests (Pineau et al. 2009, Aznar et al. 2001). Other studies have included omission or addition experiments evaluating aroma models in terms of changes in odor by omission (Ferreira et al. 2002, Guth 1997, Guth 1998) or addition (Escudero et al. 2004) of individual odor compounds. Interestingly, while most studies found satisfactory results in evaluating the importance of individual odorants in mixtures using OAV's and FD's, Escudero and co-workers (2004) observed no change in the aroma of dry,

young wine after the addition of compounds high in OAV. They explained that the results were in part due to the buffering capacity of wine in the presence of relatively high concentrations of ethanol, ethyl esters, fusel alcohols, volatile phenols, β -damascenone and fatty acids with relatively similar aroma properties.

Studies on Wine Matrix Effects on Wine Volatiles

Effect of ethanol

Ethanol, one of the alcohols produced during fermentation, represents a major component of the wine matrix after water with concentrations ranging between 8.5 and 15% in table wines (Robinson 1999). It is the most abundant volatile component capable of moderating the taste and mouthfeel properties of wines (Fischer and Noble 1994, Nurgel and Pickering 2006).

Many studies have been undertaken to understand the effect of ethanol on wine aroma by altering the solubility of aroma compounds in simple model wine systems. Preliminary analytical studies focused on the measurement of the activity and partition coefficient of volatile compounds using the static headspace technique, coupled with gas chromatography for analysis. In one study, the volatile compounds, isoamyl acetate, ethyl hexanoate, n-hexanol and β -ionone were found to have lower activity coefficient in the artificial wine composed of organic acids (0.4% tartaric acid, 0.3% malic acid and 0.01% acetic acid), salts (0.0025% magnesium sulfate and 0.01% potassium sulfate) and 10% ethanol than in water (Voilley et al. 1991). In another study, decreases of 30-35% in the partition coefficient of volatile compounds were found when ethanol concentration was increased from 5 to 80 mL/L in a model wine (10 g/L glycerol, 1 g/L potassium at pH 3.2) (Fischer et al. 1997). Later on, Conner and coworkers (1998) showed a log-linear decrease in the activity coefficients of ethyl esters as the ethanol concentration increased from 17 to 80% in MilliQ water. At ethanol concentration below 17%, the activity coefficient of

these same compounds did not change. These authors related the results to structural changes in ethanol/water mixture from an ethanol-monomodispersed-in-water system to water-monomodispersed-in-ethanol system. At ethanol concentrations above 17%, the aggregation of ethanol molecules reduced the hydrophobic hydration of the alkyl chain, thus increasing the solubility of esters. Hartmann and associates (2002) studied pyrazine compounds extracted with a divinylbenzene (DVB)/carboxen/polydimethylsiloxane (PDMS) SPME fiber in model solutions (with 0, 5, 10, 15 and 20% ethanol/water mixture and 2 g/L potassium bitartrate) and found a significant reduction in the recovery of volatile compounds. A ten-fold decrease in recovery was observed for isopropyl-, *sec*-butyl- and isobutyl-pyrazines when ethanol concentration was increased from 0 to 20%. Similarly, a consistent decrease in the extraction yield of terpenoids was observed with increasing ethanol contents from 0 to 18% in water with tartaric acid (5 g/L), particularly for the most polar compounds such as nerolidol, β -ionone, geraniol and α -ionone (Camara et al. 2006). In another study using SPME-GC for the analysis of aroma compounds representing alcohols, acids, esters, norisoprenoids, and phenolics spiked in ethanol/water mixture with 1 mM tartaric acid, an increase of ethanol concentration from 11 to 14% was sufficient to decrease the relative response of the analytes by at least 20%, except for 3-methyl-1-butanol (Whiton and Zoecklein 2000).

Aznar et al. (2004) measured the effect of ethanol on the headspace partitioning of 11 compounds using their optimized atmospheric pressure chemical ionization-mass spectrometry (APCI-MS) methods. They reported that the hydrophobicity of the compound, normally expressed in $\log P$ values was also a determining factor influencing the partition of volatiles in the headspace of ethanolic solutions. In 12% ethanol/water solutions, these authors observed a linear correlation between the decrease in volatile headspace concentration and the $\log P$ values

but only to some extent. For non-polar molecules such as ethyl octanoate and limonene ($\log P > 3$), decrease in the headspace concentration was not apparent as compared with polar compounds ($\log P \leq 3$) such as diacetyl, furfuryl alcohol, c-3-hexenol, 3-methyl butanol, ethyl butyrate, ethyl isovalerate, linalool, 1-octen-3-one, and octanal.

Meanwhile, there were few studies reported in the literature that focused on the dynamic volatile headspace analysis to evaluate the effect of ethanol on volatile delivery. Tsachaki et al. (2005) observed a constant ethanol headspace concentration above 0.01, 4, and 12% ethanol/water solutions during headspace dilution analysis. Further investigation on the dynamic headspace dilution profile of target carbonyl compounds, alcohols, esters and terpenes showed a relatively higher absolute headspace concentration of these volatiles in the presence of 12% ethanol than in water, in contrast with results in static equilibrium studies. According to the authors, the impact of ethanol on volatiles under non-equilibrium conditions can be explained by a phenomenon referred to as the Marangoni Effect. Following the Marangoni Effect, the destabilization of the ethanol/water system caused by ethanol evaporation, results in a continuous transfer of ethanol and aroma compounds from the bulk phase to the interface maintaining the volatile headspace concentration during the dilution process. In a subsequent physical modeling study, Tsachaki et al. (2008) found that the enhanced delivery and preservation of high headspace concentration of volatiles was due primarily to the increase in mass transfer in the liquid phase by ethanol.

Many sensory investigations on the impact of ethanol on wine aroma perception also have been published. In reconstructed model wines with characteristic aroma of G uwerztraminer wines, reduction in the amounts of ethanol (100 to 0 g/L) resulted in an increased overall fruity and flowery/floral intensity (1.5 to 3) as scored by 6 trained panelists in a scale ranging from 0 to

3, with 0=none and 3=strong (Guth 1998). Consistent with their results, Escudero et al. (2007) observed that increasing concentrations of ethanol from 0 to 14.5% dramatically suppressed the perception of fruitiness in the synthetic wines (mixtures of water/ethanol at different levels with 5 g/L tartaric acid adjusted at pH 3.5) with 9 fruity compounds added. At 0% ethanol in the mixture, the aroma was described as strong and apple-like and this decreased in intensity as ethanol concentration approached 12%. At 14.5% ethanol, the fruity aroma was no longer detected. In a study on more complex Malbec wines, the aroma intensity decreased with increasing ethanol level except for the herbaceous attribute. The sensory perception of the aroma at low ethanol concentration (10.0 to 12.0%) was described as fruity while at higher ethanol (14.5-17.2%), the odor was defined as herbaceous (Goldner et al. 2009). However, in much simpler mixtures evaluated by Le Berre et al. (2007), an additive effect of ethanol on fruity and woody aroma intensity was reported in model solutions containing only isoamylacetate and whiskey lactone. The panelists (n=15) in the study perceived greater intensity of these odors in the ethanol/water solution (12%, v/v) than in MilliQ water.

The suppression effect of ethanol on aroma was demonstrated to correspond to the changes in odor detection threshold in the presence or absence of ethanol. Odor detection threshold is the lowest concentration at which the panelist can perceive or detect an aroma sensation. For a given aroma compound existing at higher quantities in a food product, a low detection threshold would indicate a potential stronger impact in the product aroma. As reviewed by Grosch (2001), GC-O results showed a dramatic increase in threshold values (suppression effect) in the presence of ethanol (55.6 mg/L) compared to aqueous solution (with no ethanol). The increase in odor threshold ranges between factors of 10 for ethylhexanoate and

312 for methylpropanol. This in effect could impact the contribution of aroma volatiles to overall characteristic of wine aroma and perception.

Finally, increased alcohol content in the wines (3-14%) has also been shown to increase the maximum perceived viscosity intensity (I_{\max}) of white wine (Pickering et al. 1998) which may indirectly affect aroma perception. The I_{\max} value for viscosity at 3% ethanol was found to be statistically different from the values obtained for 7, 10, 12, and 14% ethanol. However, a recent study on the main effect of ethanol (11.6, 12.6, and 13.6% v/v) on perceived viscosity, aroma and flavor intensity of Riesling wines was found not significant (Gawel et al. 2007). The lack of agreement on the results can be due in part to the wider difference in alcohol range used in the two studies.

Effect of phenolic compounds

The phenolic composition of the finished wines depends on the grape and winemaking practices. Polyphenols are unstable compounds, with their reactions starting as soon as the grape is crushed or pressed, continuing throughout winemaking and aging. These reactions may involve interaction or binding with aroma compounds influencing aroma release and perception. Experiments using both exponential dilution techniques and nuclear magnetic resonance (^1H NMR) spectroscopy showed a hydrophobicity-driven, weak bimolecular aroma-phenolic compound interaction (Dufour and Bayonove 1999b). Increased concentrations of catechin and epicatechin results in higher affinity for benzaldehyde, than for 3, 5 dimethoxyphenol (Dufour and Bayonove 1999b). Additional NMR analyses confirmed that aroma-phenolic interaction was due to the presence of π - π stacking between galloyl ring and the aromatic ring of aroma compounds, of which stability was provided by hydrogen bonding (Jung et al. 2000). In addition, Jung et al. (2000) observed that the chemical structure of the aroma and phenolic compounds

influenced the strength of interaction. Based on the evaluation of thermodynamic parameters and spatial orientation of aroma/phenol complexes, gallic acid displayed a higher binding affinity for the aromatic compounds, vanillin, 2-methylpyrazine, and ethylbenzoate than for naringin. However, in terms of binding capacity of aroma compounds with the phenolic compounds, 2-methylpyrazine and vanillin had stronger interactions with gallic acid and naringin, than did ethylbenzoate.

Few studies using HS-SPME/GC-MS technique demonstrated different effects of monomeric phenols on the partitioning of aroma compounds. Gallic acid (10 mM) significantly decreased the headspace concentration of 2-methylpyrazine and ethyl benzoate in 1% ethanol/water and aqueous solution, respectively (Aronson and Ebeler 2004) while in another study, gallic acid (1 g/L) had no impact on 3-alkyl-2-methoxypyrazines (Hartmann et al. 2002). Jung and Ebeler (2003) evaluated the effect of catechin (10 g/L of water) on flavor volatility by using a similar technique (combined HS-SPME and GC-MS). Their results showed a significant reduction in the ion response for hexanal and ethylhexanoate (~10-20%) when compared to water but not for heptanone, wherein a 15% increase in volatility was observed.

Information on the effect of interactions between aroma compounds and condensed tannins is limited. One study by Dufour and Bayonove (1999b) investigated the influence of wine condensed tannins on volatility of some volatiles using dynamic headspace technique. Addition of tannin (0-5 g/L) to a 10% ethanol/aqueous tartrate solution (v/v, with 2 g/L) plus volatile compounds resulted in increased volatility (“salting out”) of limonene. In contrast, benzaldehyde volatility slightly decreased, whereas isoamylacetate and ethylhexanoate were not affected.

Recently, sensory approaches were employed to explore the changes in aroma perception due to the action of polyphenols. Aronson and Ebeler (2004) prepared model solutions of ethyl benzoate (2-16 mg/L of 1% ethanol/water mixture) and 2-methylpyrazine (60-300 mg/L of water) to study their interactions with gallic acid (10 mM) and naringin (2 mM). Based on the sensory data from 10 trained assessors, they found that both gallic acid and naringin decreased the perceived aroma intensity of 2-methylpyrazine while naringin had a greater negative effect on ethyl benzoate, in agreement with the results of HS-SPME/GC-MS analysis. The interaction effects between aroma and polyphenols were also studied by adding extracted tannins (1500 mg/L) to Cabernet Sauvignon and Chardonnay wines. In contrast, the same assessors were not able to differentiate the wines with or without tannin based on aroma via the duo-trio test even though a significant decrease in flavor volatility was determined using headspace analysis. Authors pointed out a limitation of insufficient panel training. Furthermore, the different results obtained in model and real wines may be attributed to a large difference in the ethanol content in the wines (13.2 % - 14.5%) when compared to model solutions (1% ethanol content).

Lund et al. (2009) conducted sensory difference tests (*R*- index methodology) to evaluate the effect of polyphenols on the perception of key aroma compounds found in New Zealand Sauvignon Blanc wines. For each aroma compound, difference threshold (the lowest concentration at which a panel could perceive a significant difference for any increase in the concentration of the aroma compounds) values were determined with or without addition of polyphenol. Results from the 15 trained panelists showed a suppression effect (increase in the threshold) on the perception of isobutyl methoxypyrazine, 3-mercaptohexanol and ethyldecanoate after addition of catechin (12 mg/L), caffeic acid (102 mg/L) and quercetin (10 mg/L) in dilute base wine (6.25% ethanol, 4 g/L residual sugar, 3.25 g/L titratable acidity, at pH

3.2). Caffeic acid was found to enhance (decrease the threshold) the perception of 3-mercaptophexanol while catechin had a slight enhancing effect on the mercaptophexanol acetate perception.

Most recent documentation on the assessment of the effect of polyphenols on aroma perception was a study conducted on unadulterated Malbec wines (Goldner et al. 2011). In comparison with previous works, their study evaluated wines which had naturally low (1.4-3.2 g/L) and high (5.4-7.2 g/L) polyphenol concentrations. Significant results were obtained with lower panelist perception of fruity, citrus, strawberry, cooked fruit and floral aromas in wines containing high polyphenols compared to the low polyphenol content, indicating a matrix effect. However, the accompanying headspace SPME GC-MS data provided no significant difference between wines. Authors stated that the contradictory results may be due to an incomplete extraction of aroma compounds by PDMS SPME fiber.

Effect of polysaccharides

Polysaccharides present in wine originate from the primary cell walls of grapes (arabinogalactan-proteins and RG-I and II) and microorganisms such as yeasts (mainly mannoproteins (MP) used in winemaking (Vidal et al. 2003)). Owing to its variety of sources, wine polysaccharides differ in composition and structure. Based on acidity and protein content, wine polysaccharides can be classified as: neutral pectic substances (type II arabinogalactans (AGs), arabinogalactan-proteins (AGPs), branched (1,5- α -L-arabinans and type I arabinogalactans) or acidic pectic polysaccharides (homogalacturonans and rhamnogalacturonans), as reported by Dufour and Bayonove (1999a). The presence of high concentration of polysaccharides in wine (500 to 1500 mg/L) (Will et al. 1991) has many consequences including interaction with wine volatile compounds.

The influence of different polysaccharides on the aroma of wine has been investigated to a lesser extent. Lubbers et al. (1994) evaluated the effect of interaction between polysaccharide-rich yeast cell walls and volatile compounds using an artificial model wine. Headspace analysis data revealed a decrease in concentration of all the test compounds- isoamyl alcohol, octanal, ethyl hexanoate and ethyl octanoate. When the equilibrium dialysis method was employed to determine the extent of binding of volatiles on yeast walls, results showed greater binding capacity of a more hydrophobic volatile, β -ionone, as compared to ethyl hexanoate. The authors suggested that the degree of binding is of a hydrophobic nature.

In another study, Dufour and Bayonove (1999a) found effects of different polysaccharides on two esters using exponential dilution technique. These authors investigated the effect of AGPs, monomeric and dimeric RG-II, and MPs isolated from a red wine on the activity coefficient of isoamyl acetate and ethyl hexanoate. No significant difference was found on the volatility induced by the wine polysaccharides (5-20 g/L) in model wines (11.8% ethanol/aqueous tartrate solution with 2 g/L potassium hydrogen tartrate). However, at higher concentrations of polysaccharides (30 g/L), they found slight retention (decreased activity) of these volatiles in the presence of protein-rich polysaccharides, AGPs and MPs and a weak salting-out effect (increased activity) due to uronic acid rich fractions AGP4, monomeric RG-II and dimeric RG-II.

The effect of interactions between aroma compounds and mannoproteins was further assessed using combined dynamic and static headspace, and sensory techniques (Chalier et al. 2007). In this study, the authors attempted to use a lower concentration of mannoproteins (0.15 g/L) to avoid formation of mannoprotein aggregates, which were hypothesized to decrease access to binding sites available for the volatiles (Landy et al. 1995). In general, their results

showed that the presence of whole and fraction of mannoproteins in 12% ethanol/aqueous tartrate model solution significantly decreased the volatility of all aroma compounds up to 80% as measured by static headspace analysis (hexanol, ethyl hexanoate and β -ionone) except for isoamyl acetate. They observed that the two commercial yeast strains (*Saccharomyces cerevisiae* ICV D21 and ICV D80) produced mannoproteins with different strengths of interaction with the aroma compounds. They further suggested that the presence of both glycosidic and peptidic parts in the structure of mannoproteins may have influenced the interactions. A preliminary sensory evaluation test results from 4 assessors confirmed the ability of mannoproteins to decrease the intensity of aroma compounds, supporting the headspace GC analytical results.

Higher Order Interaction Effects Between Wine Components and Aroma Compounds

The complexity of wine can be thought as a consequence of the complex interactions between different chemical components that make up the wine. Some studies presented below provide information on the existence of higher order interactions between wine components influencing the aroma compounds.

In model wines, Voilley and Lubbers (1998) investigated the relationship between the surface hydrophobicity of proteins (bovine serum albumin (BSA), ovalbumin and trypsin inhibitor) and the binding of aroma compounds (ethyl hexanoate, β -ionone and γ -decalactone) in the presence of 10% ethanol. The binding of aroma compounds with BSA decreased by a factor of 2, 1.09 and 1.37 for ethyl hexanoate, γ -decalactone and β -ionone, respectively. On the other hand, the binding of β -ionone with ovalbumin was reduced by a factor of 2. Further study of the surface hydrophobicity of these proteins revealed a decrease in the number of binding sites for BSA from 22 to 10 and from 40 to 19 for albumin, indicating the role of ethanol in modifying

the protein conformation consequently changing the binding capacity of proteins with aroma compounds (Voilley and Lubbers 1998).

In a recent study, the effects of ethanol (14%), glucose (240 g/L), glycerol (10 g/L), proline (2 g/L) and catechin (50 mg/L) on the volatility of 20 aroma compounds were evaluated using a full factorial design (Robinson et al. 2009). Significant two-way interactions between ethanol and glucose, ethanol and glycerol, and glycerol and catechin on the volatility of aroma compounds were reported. Results obtained from the interaction between ethanol and glucose showed that the relative peak area for all volatile compounds except limonene was reduced by increasing ethanol concentration while peak area increased in the presence of glucose. A significant interaction effect between ethanol and glycerol was also observed for 2-isobutyl-3-methoxypyrazine, linalool, nerol, 2-phenethyl acetate, β -damascenone, α -ionone, β -ionone and eugenol. The enhancement effect of glycerol the relative peak areas of the volatile compounds was only observed in the absence of ethanol. The interaction effect between catechin and glycerol although statistically significant, the magnitude of the effect was low (4-7% difference in relative peak areas) (Robinson et al. 2009).

Higher order wine matrix interactions occurring in model wine system was also explored through sensory methods of analysis. Jones et al. (2008) applied a multi-factorial design to assess the influence of interactions among selected levels of wine proteins (0 and 112 mg/L), polysaccharides (0 and 170 mg/L), volatile compound mixture (30 and 70%, v/v), glycerol (0 and 10 g/L) and ethanol (11 and 13%, v/v) on perceived aroma intensity. Their results showed that overall aroma and individual aroma attributes, estery and floral were dependent on the concentration of the volatiles, glycerol and ethanol. At lower volatile concentrations, ethanol had a suppressing effect in the absence of glycerol. Conversely, at higher volatile concentration,

overall aroma intensity was not impacted by glycerol nor ethanol. While authors mentioned Weber's psychophysical law to explain the effects due to volatile concentration, they were not able to elucidate the rationale behind interacting roles by ethanol and glycerol. These two factors were also implicated in either polysaccharides, volatiles or protein (three-way interaction), with the lowest overall aroma observed in the presence of polysaccharides and glycerol at the lowest alcohol level.

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CHAPTER III

**INFLUENCE OF TANNIN CONCENTRATION, STORAGE TEMPERAURE, AND
TIME ON CHEMICAL AND SENSORY PROPERTIES OF
CABERNET SAUVIGNON AND MERLOT WINES**

Abstract

Storage conditions that may influence the chemical and sensory properties of young bottled Cabernet Sauvignon and Merlot wines were studied. Low and high tannin wines (≤ 400 mg/L and ≥ 800 mg/L catechin equivalents, respectively) stored at 23°C for 0 day (baseline) and at either 27°C or 32°C for 40, 55, and 70 days were used for chemical and sensory analysis. In both low and high tannin wines, storage at 32°C resulted in significant increase in small polymeric pigment (SPP) ($p \leq 0.05$) with a corresponding decrease in anthocyanin concentrations over time, which was more pronounced in Cabernet Sauvignon. In both varieties, high tannin wines contained more large polymeric pigment (LPP) than the low tannin wines ($p \leq 0.05$). Generally, titratable acidity and pH were not affected by storage treatments. A trained sensory panel ($n = 21$) gave higher astringency ratings to high tannin wines than low tannin wines for both varieties, which remained constant throughout the study. An increased perception of bitterness was associated with storage at 32°C storage for 70 days, while alcohol burn intensity was comparable in Cabernet Sauvignon. No significant differences in bitterness and alcohol burn intensity were found in Merlot. Results indicate that storage temperature and storage time contributed to changes in the chemical composition of typically aging red wines but did not impact perceived astringency. Tannin concentration was positively correlated with perceived astringency ($r = 0.882$) in Cabernet Sauvignon, while SPP and LPP had lower correlation with perceived astringency for both varieties.

Introduction

Phenolics are widely recognized as an essential component of red wine quality. They contribute to astringency, one of the key sensory attributes influencing consumer acceptability of red wine (Lattey et al. 2007). Astringency is a complex of sensations that involves shrinking, drawing, or puckering of the epithelium after exposure to substances such as alums or tannins (ASTM 2004). Among phenolic compounds, the polymeric flavan-3-ols, or condensed tannins, are the most important class because of their significant contribution in perception of wine astringency (Fischer and Noble 1994, Gawel 1998, Lea and Arnold 1978).

Previous studies have shown that tannin concentration is not sufficient to explain the variation of astringency perception in wines (Ishikawa and Noble 1995, Landon 2007). Polyphenols associated with astringency have molecular weights between 500 and 3000 Da (Bakker 1998, Lesschaeve and Noble 2005). However, low molecular weight compounds have also been found to elicit astringency, including 5-*O*-caffeoylquinic acid and flavan-3-ol monomers (Naish et al. 1993, Peleg et al. 1999).

In young red wines, color is enhanced through copigmentation, the complex formation between self-associations of anthocyanins and colorless cofactors (Levengood and Boulton 2004, Boulton 2001). During aging, anthocyanins react with tannins to form polymeric pigments or pigmented tannins (Somers 1968, Somers 1971, Remy et al. 2000), which are thought to have different protein-binding properties than tannin, and thus may contribute to reduction of astringency (Singleton 1992).

Storage conditions that decrease anthocyanin content increase polymeric pigment (Dallas and Laureano 1994, Somers 1971). In Shiraz and Grenache wines, a higher storage temperature (25°C versus 3°C) for 100 days greatly influenced the rapid formation of polymeric pigments

(Somers and Evans 1986). In another study, anaerobic heat treatment (42–45°C) of Shiraz wines promoted changes in wine color and phenolic composition and reduced astringency after 10 days (Somers and Pocock 1990).

The objective of this study was to clarify the factors that influence perceived astringency during aging of Cabernet Sauvignon and Merlot wines. Specifically, this work determined the influence of initial tannin concentration, storage temperature, and storage time on the chemical composition and sensory properties of young bottled wines. We used a protein precipitation assay and bisulfate bleaching method to fractionate polymeric pigments into large polymeric pigments (LPP) and small polymeric pigments (SPP) (Harbertson et al. 2003). In a preliminary study, storage at 35°C for 21 days altered polymeric pigment content of Cabernet Sauvignon wines while tannin levels remained the same (Landon 2007). In addition, wine precipitate was observed. Trained panelists found significant difference in astringency among low, medium, and high tannin wines, both heat treated and not treated. However, trained panelists found no significant difference in astringency between the heat-treated and untreated wines of the same tannin content (Landon 2007). To better understand the impact of polymeric pigment on wine sensory characteristics, our current study examined the storage of wines for 40, 55, and 70 days at either 27°C ± 1 or 32°C ± 1 and allowing for differentiation of wines with the same tannin content but varying polymeric pigment content. Lower heating temperatures were selected to minimize precipitation. A trained panel was used to examine the relationship among polymeric pigment, anthocyanin, and tannin and perceived astringency, bitterness, and alcohol burn.

Materials and Methods

Wine samples and procedures

Merlot (2007) and Cabernet Sauvignon (2007) wines made from grapes grown in

Columbia Valley were obtained from Snoqualmie Winery (Prosser, WA). Wines were prepared according to typical commercial practices, and wine chemical characteristics for each variety at the time of bottling are shown (**Table 1**). Within each variety, 14 bottles (750 mL) of low tannin (≤ 400 mg/L catechin equivalents) and 14 bottles (750 mL) of high tannin (≥ 800 mg/L catechin equivalents) wine were used. Two bottles of each concentration were held at room temperature (23°C) and served as baseline. The remaining bottles were stored under two temperature conditions that would allow formation of polymeric pigments, which are stable to degradation and precipitation: 12 bottles were stored at $27^{\circ}\text{C} \pm 1$ and 12 bottles were stored at $32^{\circ}\text{C} \pm 1$. All wines were stored horizontally with cork, in the dark. Chemical and sensory analyses were conducted at room temperature ($23^{\circ}\text{C} \pm 1$) at 0 day (baseline) and after storage for 40, 55, and 70 days.

Chemical analyses

Tannin (mg/L catechin equivalents) and SPP and LPP (absorbance units at 520 nm) measurements were conducted as previously described (Hagerman and Butler 1978) with modifications (Harbertson et al. 2003). Anthocyanins (mg/L malvidin-3-glucoside equivalents) were measured using a previous method (Picciotto 2002).

Bovine serum albumin (BSA, Fraction V powder), sodium dodecyl sulfate (SDS, lauryl sulfate, sodium salt), triethanolamine (TEA), ferric chloride hexahydrate, potassium metabisulfite, and (+)-catechin were purchased from Sigma (St. Louis, MO). The materials used for preparing buffers were purchased from J.T. Baker (Phillipsburg, NJ) and included hydrochloric acid, malic acid, sodium chloride, sodium hydroxide, 100% ethanol, quinine sulfate, and glacial acetic acid. A Genesys 10 UV scanning spectrophotometer (Thermo Electron Corporation, Madison, WI) was used, together with a Vortex-Genie 2 (Scientific Industries,

Table 1. Composition of Cabernet Sauvignon and Merlot wines at bottling.

Parameters	Cabernet Sauvignon		Merlot	
	Low Tannin	High Tannin	Low Tannin	High Tannin
pH	3.71	3.79	3.75	3.61
Titrateable acidity (g/L)	0.59	0.54	0.58	0.60
Free SO ₂ (mg/L)	56	47	42	35
Total SO ₂ (mg/L)	65	61	54	46
Reducing sugars (%)	0.12	0.12	0.13	0.11
Alcohol (% vol)	13.9	13.8	13.9	13.8
Tannin (mg/L CE) ^a	203	800	185	844
Anthocyanin (mg/L M-3-GE) ^b	452.4	491.6	333.8	452.0
Small Polymeric Pigment (AU) ^c	1.56	1.62	1.18	1.08
Large Polymeric Pigment(AU) ^c	0.45	1.08	0.53	1.63

^aCE = catechin equivalents

^bM-3-GE = malvidin-3-glucoside equivalents

^cAU = absorbance units

Bohemia, NY), and Galaxy 140 centrifuge (VWR, Bridgeport, NJ). Titratable acidity was measured by titrating 0.1 N NaOH into 5 mL sample (pH 8.4 endpoint), while pH was obtained using a digital pH meter (Fisher Scientific, Waltham, MA). All measurements were performed in triplicate.

Sensory training and evaluation

Twenty-one (9 males and 12 females) volunteer wine drinkers between 22 and 61 years formed the trained sensory panel. Panelists were recruited from Washington State University and the Pullman community and all consumed red wine at least once a week. Panelists were screened for taste disorders that could influence their responses by measuring their responses to astringent and bitter standards to determine sensitivity (see below). The panelists received minimum background information about the study to reduce potential bias and were simply informed they would be evaluating red wines over a two-month period. All panelists signed an informed consent form and received nonmonetary incentive after each training and evaluation session. The project was approved by the Washington State University Institutional Review Board for human subject participation.

Panelists completed a total of eight one-hour training sessions over a two-month period, including review sessions before each formal sensory evaluation session. Astringency and bitterness were defined to establish a common knowledge about the parameters and avoid confusion. Astringency was defined as the drying, puckery feeling that results from increased friction between the tongue and the surfaces inside the mouth (Lea and Arnold 1978), while bitterness was described as the sensation perceived at the back of the tongue.

Training was conducted through presentation of standard solutions prepared in base wine (Livingston Red Rosé, Gallo, Modesto, CA). Astringency standards used were 0.6 g tannic acid

and 0.25 g/L alum (low), 2.1 g tannic acid and 0.875 g/L alum (medium), and 3.6 g tannic acid and 1.5 g/L alum (high). Bitterness standards used were 5 mg/750 mL quinine sulfate (low), 20 mg/750 mL quinine sulfate (medium), and 35 mg/750 mL quinine sulfate (high). Merlot and Cabernet Sauvignon wines of varying tannin levels were also used for training. Based on panelist suggestion, alcohol burn (the extent of hot, chemical burning mouth feel sensation) was added for evaluation. Standards used for low and high alcohol burn intensity were base wine (9.5% alcohol) and Cabernet Sauvignon wine (13.5% alcohol), respectively.

For each variety, four sensory evaluation sessions were conducted on separate days within a period of two months: after bottling and after 40, 55, and 70 days of storage. In each session, panelists were presented with reference and eight samples consisting of two wines for each level of tannin and storage temperature. The base wine used during training served as the reference for medium astringency and bitterness intensity and low alcohol burn, provided for tasting before evaluation. The wine samples were labeled with three-digit codes and presented to panelists one at a time in a randomized serving order. Panelists rated the perception of intensity of astringency, bitterness, and alcohol burn sensations on a 15-cm structured line scale: 0 to 5 cm for low, 5.1 to 10 cm for medium, and 10.1 to 15 cm for high intensity. The 25-mL wine samples were served at 23°C in an ISO/INAO (International Standards Organization) tasting glass and covered with a petri dish for one hour before testing. Panelists were given a forced 20-sec break time between samples while rinsing their mouth with distilled water and eating unsalted crackers and were given another 2-min forced break after the fourth sample to refresh their palate. Evaluations were done in individual booths under red lights to mask color differences among samples. All instructions, scale presentations, and data collection were carried out using a computerized sensory software program (Compusense 5, Guelph, Canada).

Data analysis

A three-way analysis of variance (ANOVA) and Tukey's multiple comparisons of means were used to analyze both chemical and sensory data with SAS statistical software (SAS Institute, Cary, NC). The ANOVA performed on chemical data used a fixed effects model with tannin, temperature, and time as fixed effects. The ANOVA performed on sensory data used a mixed effects model, assuming panelists as random effect, and tannin, temperature, and time as fixed effects. Pearson's correlation test was used to describe the relationship between sensory and chemical data (XLSTAT, Addinsoft, Paris). Significance level was defined as $p \leq 0.05$.

Results

F ratios were obtained from the three-way ANOVA model (**Table 2**). The data show that tannin concentration, storage temperature, and time effects were significant ($p \leq 0.05$) for all phenolic compounds in Cabernet Sauvignon and Merlot, except temperature for LPP. The interaction effects tannin-by-temperature, tannin-by-time, and temperature-by-time were also significant for majority of the compounds and varied depending on the variety. From this point, subsequent interpretation of results was based on significant interaction effects.

Anthocyanins

For both wines, high tannin wines had higher anthocyanin concentrations than low tannin wines (**Figure 1**). For both low and high tannin wines, storage at 27°C resulted in significantly higher anthocyanin concentrations than storage at 32°C, except at 40 days of storage for Merlot. In Merlot, at 70 days of storage, the high tannin wines had significantly lower anthocyanin compared with the low tannin wine stored at the same temperature (32°C), whereas storage at 27°C resulted in no significant difference between the low and high tannin wines. Anthocyanin concentration generally decreased in both Cabernet Sauvignon and

Table 2. F-values from the analysis of variance (ANOVA) of protein precipitation assay data in Cabernet Sauvignon and Merlot wines (n = 24).

Sources of variation	Anthocyanins		Small Polymeric Pigment (SPP)		Large Polymeric Pigment (LPP)		Tannin	
	Cabernet Sauvignon	Merlot	Cabernet Sauvignon	Merlot	Cabernet Sauvignon	Merlot	Cabernet Sauvignon	Merlot
Tannin	2041.7**	155.7**	54.6**	31.8**	234.2**	1430.1**	7966.0**	33562.9**
Temperature	1441.7**	730.2**	245.8**	129.8**	1.5	0.03	233.1**	61.5**
Time	122.7**	209.5**	1.9	15.9**	50.8**	42.0**	8.3**	61.5**
Tannin x Temperature	19.4**	50.1**	1.1	15.3**	3.1	4.4	15.6**	0.9
Tannin x Time	2.6	53.4**	5.1*	3.4	44.9**	4.4*	2.7	52.2**
Temperature x Time	60.2**	153.1**	2.4	9.4	5.2*	0.5	7.5**	16.0**
Tannin x Temperature x Time	2.3	1.0	0.4	0.6	5.9*	0.2	1.8	0.9
MSE	352.6	161.4	29.2	21.4	41.1	139.0	750.5	3080.6

*, ** indicate significance at $p \leq 0.05$ and $p \leq 0.01$, respectively.

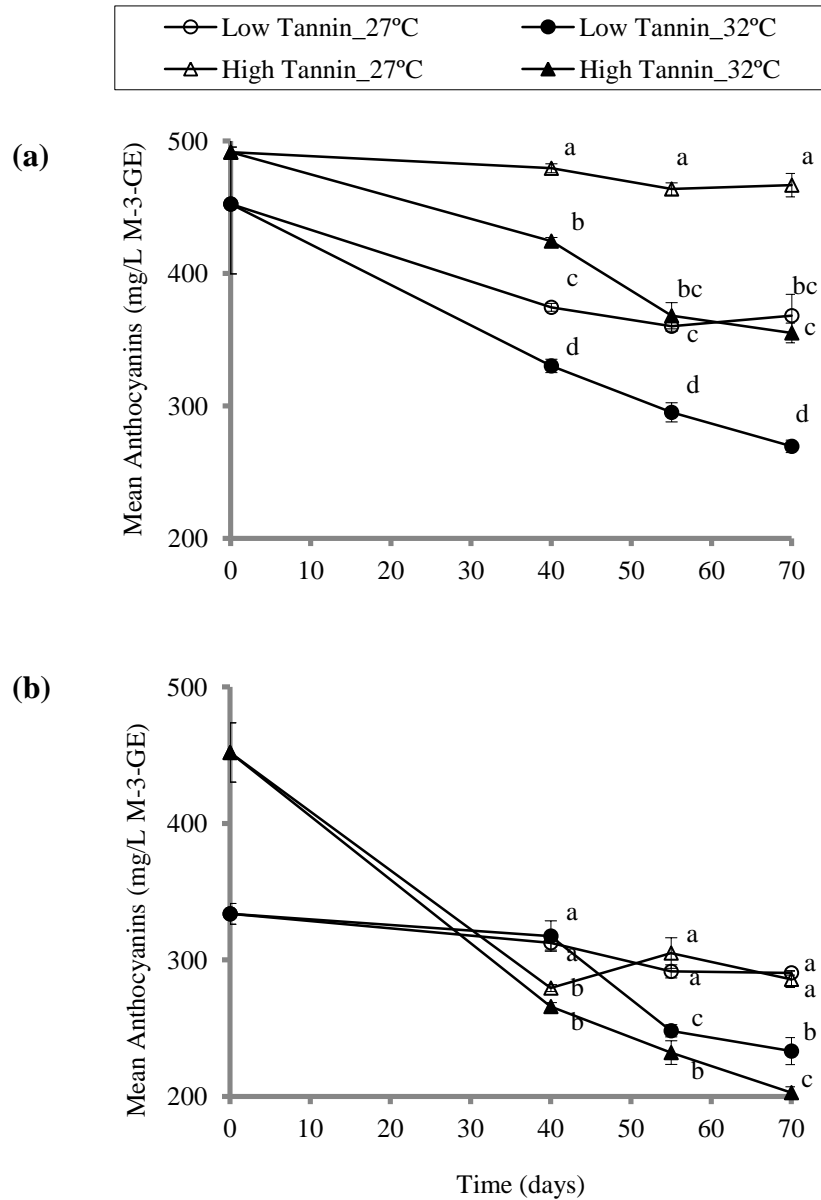


Figure 1. Mean concentration of anthocyanins in heat-treated, low and high tannin content wines stored for 40, 55 and 70 days: **(a)** Cabernet Sauvignon, $n = 24$ and **(b)** Merlot, $n = 24$. Different letters represent significant difference at each time point, as determined by Tukey's HSD ($p \leq 0.05$).

Merlot wine as storage time increased.

Small polymeric pigments

Storage at 32°C resulted in significantly higher SPP formed than at 27°C (**Figure 2**). In Cabernet Sauvignon wines with low tannin content, SPP increased from 0 to 40 days by 12.2% and 19.9% for wines stored at 27°C and 32°C, respectively. High tannin wines stored at 27°C had a slight increase in SPP (0.6%), while storage at 32°C resulted in a 10.5% increase. After 40 days, there was no significant change in SPP until 70 days of storage ($p > 0.05$). In Merlot wines stored at 27°C, SPP levels increased from baseline values to 40 days of storage (27%), after which time they did not significantly change ($p > 0.05$). However, wines stored at 32°C had 41.8% more SPP at 40 days than the baseline, followed by a decrease at 55 days of storage ($p \leq 0.05$).

Large polymeric pigments

Initial LPP content in Cabernet Sauvignon and Merlot wines was higher in high tannin wines than in low tannin wines (**Figure 3**). With storage at both 27°C and 32°C, LPP content of the high tannin wines increased. In Cabernet Sauvignon, at 70 days, high tannin wines stored at 27°C were significantly higher in LPP than wines stored at 32°C. However, no significant differences in LPP were observed between the low tannin wines stored at either 27°C or 32°C for 40, 55, or 70 days. In Merlot, LPP content of wines stored at 27°C was not significantly different from the wines stored at 32°C ($p > 0.05$). However, storage time significantly influenced LPP content of low and high tannin wines stored at both temperatures, with LPP showing a significant increase with storage time ($p \leq 0.05$).

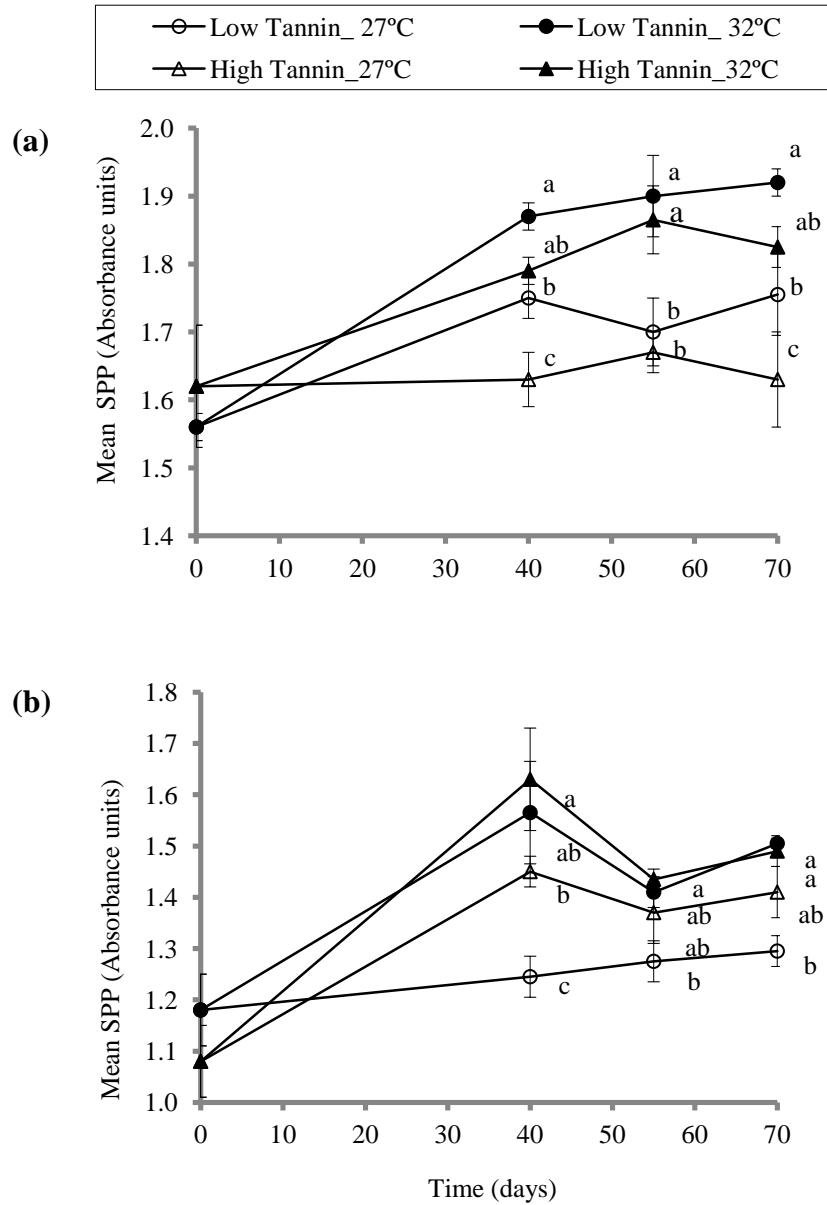


Figure 2. Mean concentration of small polymeric pigment (SPP) in heat-treated, low and high tannin content wines stored for 40, 55 and 70 days: **(a)** Cabernet Sauvignon, $n = 24$ and **(b)** Merlot, $n = 24$. Different letters represent significant difference at each time point, as determined by Tukey's HSD ($p \leq 0.05$).

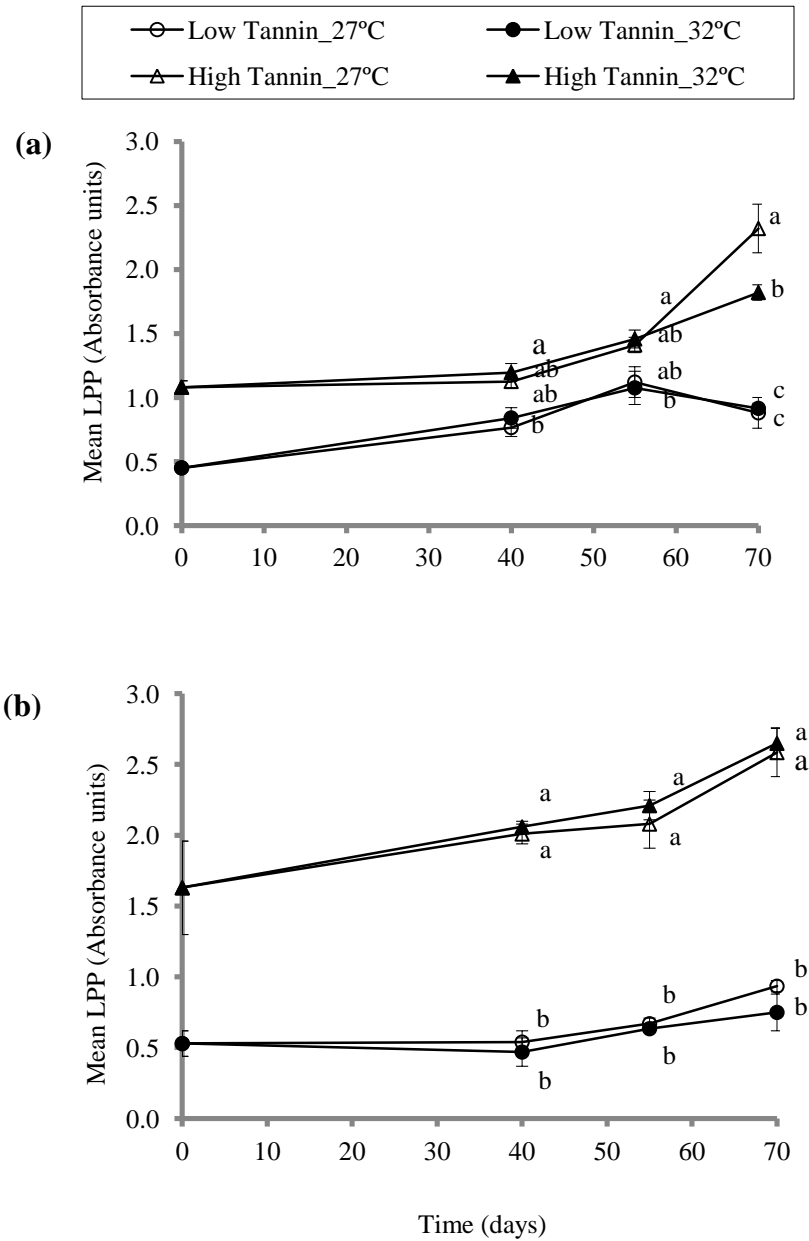


Figure 3. Mean concentration of large polymeric pigment (LPP) in heat-treated, low and high tannin content wines stored for 40, 55 and 70 days: **(a)** Cabernet Sauvignon, $n = 24$ and **(b)** Merlot, $n = 24$. Different letters represent significant difference at each time point, as determined by Tukey's HSD ($p \leq 0.05$).

Tannin

At all storage times, the high tannin wines had significantly higher tannin concentration than the low tannin wines ($p \leq 0.05$) (**Figure 4**). Generally, within each wine (low or high), the tannin levels of wines stored at 27°C were significantly higher than those stored at 32°C. Tannin levels of low and high tannin wines at either 27°C or 32°C decreased from the baseline but did not significantly change from 40 to 70 days ($p > 0.05$).

Titrateable acidity and pH

In Cabernet Sauvignon, titrateable acidity was not significantly different at either the low or high tannin levels or at any of the storage temperatures or storage times ($p > 0.05$). However, higher pH values were found in high tannin wines (pH 3.82) than in low tannin wines (pH 3.70). In Merlot, lower titrateable acidity values were found in low tannin wines (0.54) than in high tannin wines (0.58), while a higher pH was observed in low tannin (3.68) compared to high tannin wines (3.61) ($p \leq 0.05$) (data not shown).

Sensory analyses

From the trained panel data, F values were determined from the analysis of variance data for astringency, bitterness, and alcohol burn sensory perceptions in wines (**Table 3**). In Cabernet Sauvignon wines, the panelist, tannin concentration, storage temperature, storage time, and interaction between tannin concentration and storage time significantly impacted the perception of astringency ($p \leq 0.05$). Mean astringency ratings of low tannin wines were lower than ratings of high tannin wines (**Figure 5a**), consistent with findings reported elsewhere (Landon et al. 2008, Peleg et al. 1999). At each storage time, panelists gave consistently higher astringency scores to wines stored at 27°C than wines stored at 32°C, regardless of tannin level. However, the difference was not significant. At 40 days, there was no clear distinction between the

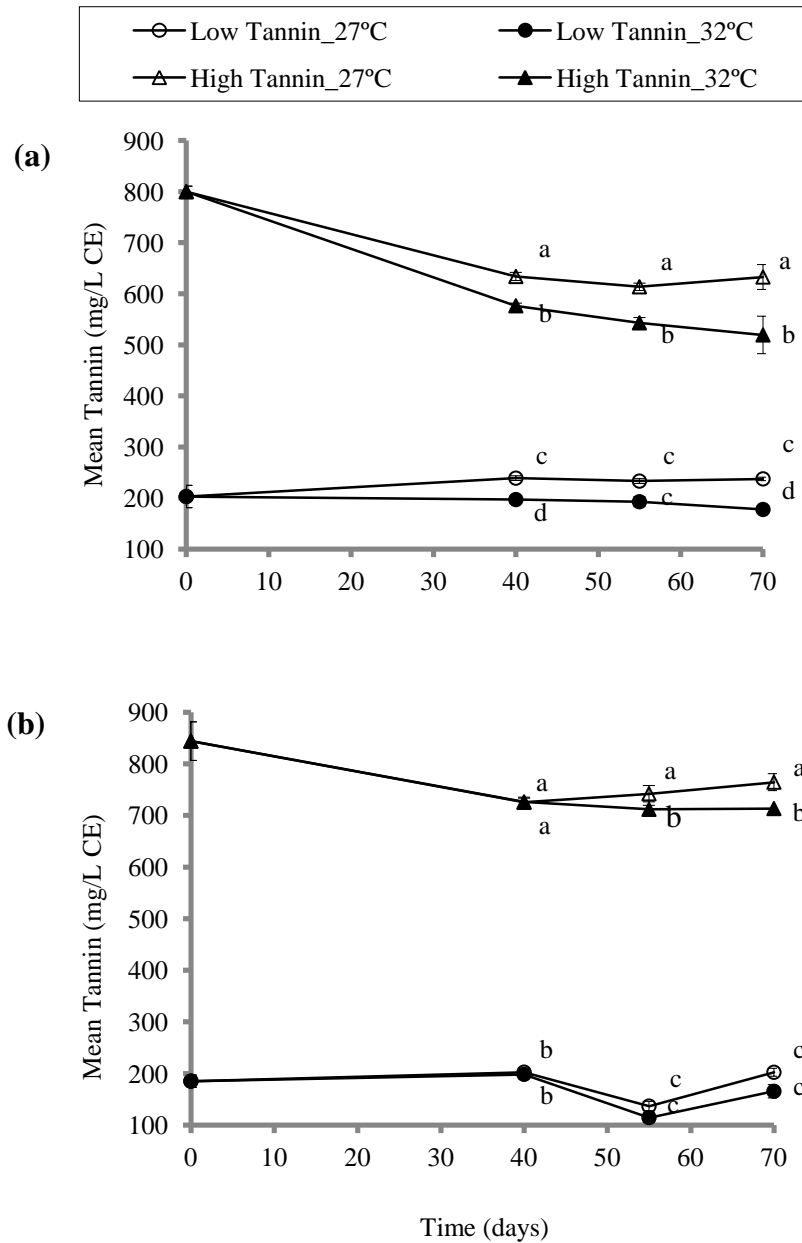


Figure 4. Changes in tannin concentration following heat treatment at either 27°C or 32°C and storage for 40, 55 and 70 days: **(a)** Cabernet Sauvignon, $n = 24$ and **(b)** Merlot, $n = 24$. Different letters represent significant difference at each time point, as determined by Tukey's HSD ($p \leq 0.05$).

Table 3. F-values from the analysis of variance (ANOVA) of the trained panel data (n = 21) for astringency, bitterness and alcohol burn sensory perceptions in Cabernet Sauvignon and Merlot wines.

Sources of variation	Astringency		Bitterness		Alcohol Burn	
	Cabernet Sauvignon	Merlot	Cabernet Sauvignon	Merlot	Cabernet Sauvignon	Merlot
Panelist	4.84**	2.44**	6.32*	3.54**	12.8**	10.9**
Tannin	86.8**	75.3**	0.05	1.66	0.55	1.06
Temperature	9.14**	10.8**	5.75*	0.96	0.75	0.09
Time	3.27*	1.11	11.0**	0.23	3.96*	0.22
Tannin x Temperature	0.88	1.15	1.21	0.64	0.48	0.71
Tannin x Time	3.75*	19.1**	0.28	0.13	0.07	0.27
Temperature x Time	0.76	20.2**	1.23	2.74	0.21	0.07
Tannin x Temperature x Time	2.56	1.36	0.39	0.93	0.03	0.19
MSE	6.88	7.13	5.05	2.63	8.61	7.13

*, **indicate significance at $p \leq 0.05$ and $p \leq 0.01$, respectively.

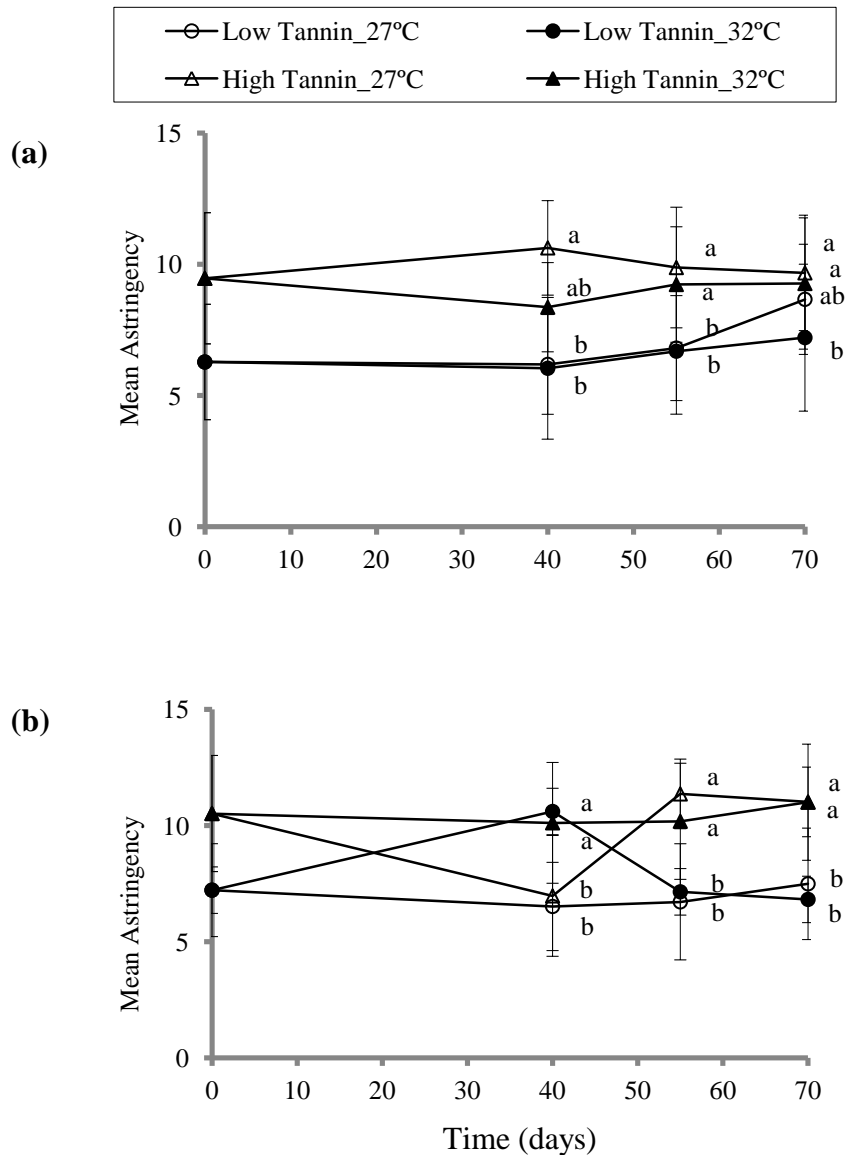


Figure 5. Perceived astringency ratings (on a 15-cm line scale) of heat-treated, low and high tannin wines after storage for 40, 55 and 70 days evaluated by the trained panel, $n = 21$: **(a)** Cabernet Sauvignon and **(b)** Merlot. Different letters represent significant difference at each time point, as determined by Tukey's HSD ($p \leq 0.05$).

astringency ratings of low and high tannin wines. Significant difference was found at 55 days between low and high tannin wines stored at 27°C or 32°C. As storage time increased, perceived astringency increased significantly in the low tannin wines stored at 27°C, while at 32°C storage the perceived astringency of wines of the same tannin concentration was comparable. In high tannin wines, no significant change in astringency perception was observed over time. Thus, storage at either 27°C or 32°C for 40 to 70 days did not significantly impact perceived astringency.

In Merlot wines, the panelist, tannin concentration, storage temperature, and storage time and interactions between tannin concentration and storage time and between storage temperature and storage time significantly influenced the perception of astringency ($p \leq 0.05$) (**Figure 5b**). At 40 days of storage, the panelists were able to distinguish between astringency of wines stored at 27°C and 32°C. Low and high tannin wines stored at 27°C were rated significantly lower in perceived astringency compared to low and high tannin wines stored at 32°C. However, the low tannin wines stored at 27°C or 32°C for 55 and 70 days were perceived as significantly less astringent than the high tannin wines.

Panelist, storage temperature, and storage time significantly affected bitterness sensation in Cabernet Sauvignon wines ($p \leq 0.05$), with no significant tannin concentration and interaction effects observed ($p > 0.05$). Significantly higher mean bitterness scores (10.68) were observed in higher temperature wines (32°C) than the wines stored at 27°C (10.01), which tended to increase with storage from 40 to 70 days. For alcohol burn, the panelist and storage time effects were both significant ($p \leq 0.05$). The mean panelist ratings of alcohol burn significantly increased when evaluating wines stored for 40, 55, and 70 days (7.39, 7.61, and 8.26, respectively). However, these ratings were relatively similar corresponding to medium alcohol burn intensity based on

the 15-cm line scale. Tannin concentration, storage temperature, and interaction effects were not significant.

In Merlot, only the panelist effect on bitterness and alcohol burn was significant ($p \leq 0.05$). Overall, results showed that at each storage time and storage temperature, the panelists were not able to detect significant difference between the wines of different tannin concentrations or storage temperature conditions based on perceived bitterness or alcohol burn sensations.

Relationships between chemical and sensory variables

To determine whether phenolic components could explain the sensory characteristics of the wine and vice versa, Pearson's correlation analysis was performed (**Table 4**). For both varieties, perceived astringency was positively correlated with tannin and LPP concentration, consistent with a previous report (Landon et al. 2008). Bitterness and alcohol burn perception had relatively low degrees of association with SPP, LPP, anthocyanin, and tannin content.

The examination of the relationship between the wine chemical and sensory properties also revealed marked differences between varieties. In Cabernet Sauvignon, a strong positive correlation was observed between anthocyanin and astringency, while in Merlot these parameters were negatively correlated. However, significant correlation was only found in Cabernet Sauvignon ($p \leq 0.01$), perhaps partly because of the magnitude difference in the anthocyanin content between varieties across all treatments and the unclear trend in the changes in anthocyanins in Merlot. The limited number of wines used in this study may have contributed to the few significant correlations observed.

Table 4. Correlation coefficients between wine chemical and sensory characteristics.

	Astringency		Bitterness		Alcohol Burn	
	Cabernet Sauvignon	Merlot	Cabernet Sauvignon	Merlot	Cabernet Sauvignon	Merlot
Small Polymeric Pigment	-0.543	0.470	0.498	-0.404	-0.276	0.326
Large Polymeric Pigment	0.619*	0.677*	0.243	-0.377	0.583*	0.509
Anthocyanin	0.738**	-0.091	-0.530	0.095	0.140	-0.361
Tannin	0.882**	0.685*	-0.156	-0.408	0.230	0.548

*, ** indicate significance at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Discussion

Previous research has indicated the strong influence of storage factors such as light and oxygen on wine during aging (Somers and Evans 1986, Recamales et al. 2006). The present study examined storage of wines in corked bottles in the absence of light; thus, the effects observed could be attributed to initial tannin concentration, storage temperature, and time. The influence of tannin concentration, storage temperature, and storage time on the chemical composition and sensory properties of wines varied with variety. The differences between Cabernet Sauvignon and Merlot wines were not surprising given the inherent difference in the genetic composition of the grapes from which the wines were made (Ribéreau-Gayon et al. 2000). While differences were observed, similar trends were apparent that could be further examined and validated in other wine varieties.

Results of this study showed that, as expected, anthocyanin concentrations gradually decreased in wines while SPP and LPP were being formed. The anthocyanins were progressively transformed into more stable oligomeric and polymeric pigments, typical of young wines undergoing maturation and aging (Somers and Pocock 1990). Data showed that the difference in anthocyanin level was a function of storage temperature, with more anthocyanin participating in condensation reactions at an elevated temperature, thus resulting in less anthocyanin remaining in the wines. Different mechanisms have been proposed by many authors involving anthocyanin reactions to form the oligomeric and polymeric pigments (Somers 1971, Bakker and Timberlake 1997). A recent study showed that LPP were more favorably formed than SPP during bottle aging for three years (Adams et al. 2004). However, the present results indicate there was not a great difference in LPP content before and after the storage treatment compared with SPP

content, possibly because significant levels of anthocyanin may have been converted to LPP before treatment application.

The storage temperatures that were used differentiated wines of the same tannin concentration. Storage at 32°C resulted in significantly lower tannin concentration than storage at 27°C. Heat treatment may have caused some modification in the tannin or pigments formed, which have a more bitter taste than the parent compound. The loss of high molecular weight tannins is believed to be a result of the acid-catalyzed C-C bond-breaking process in the wines, forming low molecular weight species (Vidal et al. 2002). In Cabernet Sauvignon, the shorter chain-length products of this reaction may account for increased bitterness perception observed in this study.

While the conversion of tannin and formation of polymeric pigments occurred in the wines, the expected reduction in perceived astringency was not achieved. Previous work in our laboratory showed that trained panelists were able to distinguish between the low and medium and low and high tannin wines. The present study shows that polymeric pigment and tannin concentrations were significantly different between wines; however, the magnitude of difference may have been below the detection limit of the panelists and thus was not great enough to result in differences in perceived astringency. Clearly, the presence of higher tannin and LPP found in both varieties contributed to higher perceived astringency.

Conclusion

This research showed that aging reactions in Merlot and Cabernet Sauvignon wines, which increased the level of SPP while decreasing anthocyanins, were primarily due to higher temperature of storage (32°C versus 27°C). Increase in LPP was influenced more by tannin concentration than by storage time or temperature. In Cabernet Sauvignon, sensory perception of

bitterness increased with storage time at 32°C. While perceived astringency decreased over the aging period in both wine varieties, the change was not great enough to impact perceived astringency. Our results support earlier studies that show the usefulness of tannin concentration to predict perceived astringency. Future work may focus on determining the optimum storage time and temperature to investigate further changes in SPP, LPP, and tannin content and their influence on perceived astringency.

Acknowledgments

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CHAPTER IV
EFFECTS OF ETHANOL, TANNIN AND FRUCTOSE ON THE HEADSPACE
CONCENTRATION AND POTENTIAL SENSORY SIGNIFICANCE OF
ODORANTS IN MODEL WINES

Abstract

The effects of ethanol, tannin and fructose concentrations on the headspace concentration of eight selected odorants were investigated using headspace solid phase microextraction (HS-SPME) and gas chromatography- mass spectrometry (GC-MS). The results showed significant three-way interaction effects for the majority of odorants ($p \leq 0.05$). In general, increased tannin concentration exhibited an enhancement effect while fructose induced a retention effect, both of which were largely dependent upon ethanol. The net magnitude effect was a substantial reduction in the headspace concentration of odorants with the dominant contribution from ethanol. Reduction in odorant recovery was determined to be a function of molecular weight. Subsequent gas chromatography-olfactometry (GC-O) analysis revealed differences in the estimated odor thresholds of odorants in model wines. Threshold values increased between 2 and 10,000-fold consequently, lowering the odor unit values (OUV) of odorants. These results highlighted the significant impact that wine matrix interactions can have on wine aroma quality.

Introduction

Wine aroma is one of the major determining factors influencing consumer acceptance.¹⁻² As with other food products, the perception of wine aroma is related predominantly to the nature and concentration of aroma compounds in the gaseous phase above the wine. More than 800 volatile compounds have been estimated to be present in wine³ while only a few compounds exist at concentrations above the sensory perception threshold.⁴⁻⁷

Several studies conducted to understand wine aroma perception have focused on the main effects of the wine matrix composition using static headspace solid phase microextraction (HS-SPME) and dynamic headspace analytical techniques. Ethanol, a major component of the wine matrix has been shown to decrease the partition coefficient of various classes of volatile compounds by increasing the solubility of volatile compounds in model wine systems.⁸⁻¹² Aznar et al.¹³ measured the effect of ethanol on the headspace partitioning of 11 compounds and reported that the hydrophobicity of the compound was also a determining factor influencing the partition of volatile compounds in the headspace of ethanolic solutions. The suppression effect of ethanol on aroma was determined to correspond to the changes in odor detection threshold in the presence or absence of ethanol. As reviewed by Grosch,¹⁴ GC-O results showed a dramatic increase in threshold values (suppression effect) in the presence of ethanol (55.6 mg/L). The increase in odor threshold ranged between factors of 10 for ethylhexanoate to a factor of 312 for methylpropanol.¹⁴

Investigations using both exponential dilution techniques and nuclear magnetic resonance (¹H NMR) spectroscopy showed a hydrophobicity-driven, weak bimolecular aroma-phenolic compound interaction.¹⁵ Additional NMR analyses confirmed that the aroma-phenolic interaction was due to the presence of π - π stacking between a galloyl ring and the aromatic ring of aroma compounds, of which stability was provided by hydrogen bonding.¹⁶ Few studies using HS-SPME/GC-MS technique demonstrated different effects of monomeric phenols on the partitioning of odorants.¹⁷⁻¹⁸ Dufour & Bayonove¹⁵ investigated the influence of wine condensed tannins on the volatility of some volatile compounds using a dynamic headspace technique. The authors found that addition of tannin (0-5 g/L) resulted in increased volatility of limonene and a slight increase in benzaldehyde volatility but had no effect on isoamylacetate and

ethylhexanoate. Lund et al.¹⁹ conducted sensory difference tests (*R*-index methodology) to evaluate the effect of polyphenols on the perception of key odorants found in New Zealand Sauvignon Blanc wines. The results showed an increase in the threshold of isobutyl methoxypyrazine, 3-mercaptohexanol and ethyldecanoate after the addition of catechin (12 mg/L), caffeic acid (102 mg/L) and quercetin (10 mg/L) in a dilute base wine. In another study, panelist found lower perception of fruity, citrus, strawberry, cooked fruit and floral aromas in Malbec wines containing high polyphenols (5.4-7.2 g/L) compared to the low polyphenol content (1.4-3.2 g/L), indicating a matrix effect.²⁰

However, few interaction effects among wine components have been described in the literature. Wine is a complex alcoholic beverage, which contains numerous components that may interact with odorants and alter the quantity of volatile compounds available for perception. Robinson et al.²¹ found a significant reduction of peak areas for most volatiles due to ethanol, and this effect was slightly increased in the presence of glucose in model solutions. In another study using model white wine, Jones et al.²² demonstrated the influence of interactions among major wine components on perceived aroma intensity. Their results showed more evident interaction effects of wine proteins, alcohol and glycerol concentration at the lower volatile concentration. Ethanol and glycerol were also shown to be involved in either polysaccharides or protein interactions, with the lowest overall aroma observed when polysaccharides and glycerol were present at lower ethanol concentration (11%, v/v).

Thus, the aim of the present study was to investigate the possibility of higher order interaction effects among the wine components, ethanol, tannin and fructose, which may impact the headspace concentration of selected odorants and their relative potential significance to the aroma of model wine.

Materials and Methods

Chemicals and reagents

All odorants including the internal standards (IS) were purchased from Sigma-Aldrich (St. Louis, MO): 1-octen-3-one (50 wt. % in 1-octen-3-ol), β -damascenone (1.1-1.3 wt. % in 190 proof ethanol), 2-phenylethanol ($\geq 99\%$), 3-methyl-1butanol, dimethyl disulfide, 1-hexanol, 2-methoxyphenol, eugenol, 1-pentanol (IS) and 1-dodecanol (IS) ($\geq 98\%$). Pure ethanol (100%) was obtained from Decon Labs, Inc. (King of Prussia, PA) while grape tannin, Biotan, was provided by Laffort Company (Sonoma, CA). D-(-)-fructose, L-(+)-tartaric acid, NaCl and NaOH were procured from Sigma-Aldrich (St. Louis, Mo). Milli-Q water used was purified (Millipore, Bedford, MA).

Model wine preparation

Thirty (30) different model wine solutions replicated five times were prepared based on a full-factorial design used to assess the effect of ethanol (8, 10, 12, 14, and 16 %, v/v), grape tannin, Biotan (500, 1000 and 1500 mg/L) and fructose (200 mg/L and 2000 mg/L). All model wines contained tartaric acid (5000 mg/L) and were spiked with eight selected odorants of different physico-chemical and aroma properties commonly found in red wines: 50 mg/L 3-methyl-1butanol, (caramel/cooked), 4 mg/L dimethyl disulfide (chemical/sulfury), 2 mg/L 1-hexanol (herbaceous/green), 1 mg/L 1-octen-3-one (earthy/mushroom), 4 mg/L methoxyphenol (woody/medicinal), 14 mg/L 2-phenylethanol (floral/rose), 0.5 mg/L eugenol (spicy/clove), and 2 mg/L β -damascenone (fruity) (**Table 5**). The above concentrations of odorants were selected as they are within the range present in wines that could be analyzed by gas chromatography. For each odorant, a stock solution was prepared every two weeks in 50 % ethanol/Milli Q (v/v) water and stored at 5 °C in a 5 mL amber vial sealed with a PTFE/Silicone septum until the solution

Table 5. Physicochemical and sensory properties of eight odorants used in the study.

Compound	CAS Registry No.	MW	BP (°C)	Log P value ^a	Aroma Descriptors ^b	Aroma Category ^c
3-methyl-1-butanol	123-51-3	88.15	131.1	1.16	whiskey, malt, burnt	caramel
dimethyl disulfide	624-92-0	94.19	109.8	1.77	onion, cabbage	chemical
1-hexanol	111-27-3	102.2	157.6	2.03	green, flower	herbaceous
1-octen-3-one	4312-99-6	126.2	162.0	2.37	mushroom, metal	earthy
2-methoxyphenol	90-05-1	124.1	205.0	1.32	smoke, medicine	woody
2-phenylethanol	60-12-8	122.2	218.2	1.36	honey, spice, rose	floral
eugenol	97-53-0	164.2	253.2	2.27	clove, honey	spicy
β -damascenone	23726-93-4	190.3	265.1	4.21	apple, rose, honey	fruity

^aHydrophobic constant³²^bFlavornet³³^cNoble et al.³⁴

was used. Prior GC-MS analysis results showed no change in odorant concentration within two weeks of storage. The pH of the model wines was adjusted to 3.4 with 1 M NaOH.

HS-SPME/ GC-MS analysis

The volatiles were isolated and concentrated using the headspace solid-phase microextraction (HS-SPME) technique. A sample consisting of two (2) milliliter of each model wine was spiked with the odorants and then introduced into a 10-mL amber vial sealed with a magnetic stainless steel screw cap (PTFE/silicone septum). All samples were saturated with 32.5% (w/v) (0.65g) NaCl and volatiles were extracted using a CTC Combi-PaL autosampler (Zwingen, Switzerland). The optimized parameters wherein optimum odorant concentration was obtained for a reasonable extraction time were: pre-incubation time for 5 min, incubation temperature at 30°C, agitator speed at 250 rpm, agitator-on time for 5 s, agitator-off time for 2 s, extraction time at 30 min and desorption time for 5 min. Prior to use, a polydimethylsiloxane/divinylbenzene (PDMS/DVB) coated- fiber (65 μ m) was conditioned at 250°C for 30 min.

Samples were then analyzed using a GC 6890N chromatograph coupled with a mass spectrometer (MS 5975) (Agilent technologies, Avondale, PA) functioning in the EI mode. The following GC column was used: HP-5MS (30.0 m x 250 μ m x 0.25 μ m). The injection was done in splitless mode at 200°C for 5 min. Helium carrier flow was set at the rate of 1 mL/min. The detector temperature was 250°C. The GC oven was programmed as follows: 35°C for 3 min; 0.50°C/min ramp to 60°C; 2°C/min ramp to 120°C; 10°C/min ramp to 210°C; 20°C/min ramp to 230°C; and held at 230°C for 10 min. Data were collected in scan mode (33 to 300 m/z). Compounds were identified by comparison of each mass spectra with those of pure reference

compounds and confirmed using the NIST mass spectra library provided by the Chemstation software (version E.02.00.493).

The quantitation was performed by internal standard calibration procedure using 10 mg/L 1-pentanol and 0.25 mg/L 1-dodecanol as internal standards (IS). A five-point standard curve covering the concentration range at which the odorants were detected was constructed for each odorant in each model system by linear regression analysis. The unknown concentration of the odorant was determined from the standard curves by examining the ratio of the target odorant concentration to the IS concentration that corresponded to the ratio of their integrated total ion chromatogram (TIC) peak areas multiplied by the IS concentration. Analyses were conducted in triplicate.

Odor threshold and odor unit value (OUV) determination

The detection threshold of the eight aroma compounds considered in the study was measured in the selected eight different model wines by using a combination of the HS-SPME/gas chromatography- olfactometry (HS-SPME/GC-O) technique. Odorants were diluted for 5 to 6 times by a factor of 10 in the prepared model wine solutions. The model wine solutions varied in concentrations of ethanol (10 and 14%, v/v), tannin (500 and 1,500 mg/L) and fructose (200 and 2000 mg/L). Tartaric acid was added at 5,000 mg/L and the pH was adjusted to 3.4 with 1 M NaOH.

GC-O analysis

The volatile compounds were extracted and concentrated using a 65 μ m PDMS/DVB fiber, following the optimized condition parameters described previously for the SPME/GC-MS analysis. The samples were analyzed using the same GC column and GC-MS instrument equipped with an olfactometry port connected by a flow splitter to the column exit.

Chromatographic conditions were identical to those used for the GC-MS analysis with slight modification of the GC temperature program as follows: initial temperature at 35° C; 6°C/min ramp to 200°C; 30°C ramp to final temperature at 230°C. The final temperature was held for 2 min with a total GC run of 35 min.

Four assessors (1 male and 3 females) between 21 and 51 years old, and who had previous experience in descriptive analysis of the same model wines, participated in the study. All judges were trained during the preliminary sessions (3, one-hr sessions) to identify and detect the target eight compounds by sniffing the GC effluents coming out of the olfactory port for 25 min. During the formal evaluation, model solutions were analyzed with odorants prepared at decreasing concentrations. The assessors were asked to record the odor description of each odorant perceived. Individual best estimate threshold values were determined for each panelist by obtaining the geometric mean of the highest concentration missed and the next higher concentration.²³ The geometric mean of the individual best estimate threshold values was calculated and reported as the group threshold. The OUV for each odorant compound was computed as the ratio of its concentration to its odor threshold as cited by Buttery et al.²⁴ in respective model wine matrices.

Statistical data analysis

Three-way analysis of variance (ANOVA) (ethanol, tannin and fructose) and Tukey's HSD multiple comparisons of means were performed on the GC-MS data using XLSTAT statistical software (XLSTAT version 2011; 2.01). Significance was defined as $p < 0.05$. Mean odorant recovery in each type of wine was determined and principal component analysis (PCA) was applied to explore the relationship among odorants and model wines with different concentrations of ethanol, tannin and fructose (SAS Institute, Cary NC).

Results and Discussion

The present study assessed the effects of ethanol, tannin and fructose, and their corresponding interactions on the headspace partitioning of eight wine-impact odorants in a model system using HS- SPME/GC-MS analysis. Ethanol levels used in this investigation reflected the concentration range found in commercial red wines. The low (500 mg/L), medium (1000 mg/L), and high (1500 mg/L) grape tannin, Biotan concentrations were selected based in part on the groupings established previously for the low, medium and high tannin commercially available red wines²⁵. Fructose concentrations used were within the range present in wines considered as “dry”.²⁶

The recovery of odorants in the headspace of model solutions was quantified using an internal standard calibration method as described in the materials and methods section. For each odorant, 30 calibration curves were obtained from 30 different model solution matrices constructed by linear regression analysis. The effect of sample matrix on the headspace SPME during compound quantification was previously noted¹² and thus, for this study, calibration standards were prepared in the model solution similar to the sample matrix. The calibration curves were linear with mean determination coefficients across all model wine matrices as follows: for 3-methyl-1-butanol, $r^2 = 0.9303$; for dimethyl disulfide, $r^2 = 0.9593$; for 1-hexanol, $r^2 = 0.9579$, for 1-octen-3-one, $r^2 = 0.9135$; for 2-methoxyphenol, $r^2 = 0.9670$; for 2-phenylethanol, $r^2 = 0.9502$; for eugenol, $r^2 = 0.9672$; and for β -damascenone, $r^2 = 0.9729$.

Matrix effects.

Table 6 presents a summary of the ANOVA results showing statistical significance of the observed effects from ethanol, tannin and fructose variables. As expected, all of the selected

Table 6. Statistical significance of the influence of interactions of ethanol, tannin, fructose.

Sources of variation	df	Probability (<i>p</i>) values							
		3-methyl-1-butanol disulfide	1-hexanol	1-octen-3-one	2-methoxy-phenol	2-phenyl-ethanol	eugenol	β -damascenone	
Ethanol (Et)	4	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Tannin (T)	2	<0.0001	0.65	0.013	0.0001	<0.0001	0.08	<0.0001	0.534
Fructose (F)	1	0.044	<0.0001	<0.0001	0.78	<0.0001	<0.0001	<0.0001	<0.0001
Et x T	8	<0.0001	0.0001	<0.0001	0.308	0.011	<0.0001	0.002	0.707
Et x F	4	<0.0001	0.092	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
T x F	2	<0.0001	0.0001	<0.0001	<0.0001	<0.0001	0.002	<0.0001	<0.0001
Et x T x F	8	0.154	0.011	<0.0001	0.164	<0.0001	0.001	<0.0001	<0.0001

p values ≤ 0.05 are significant

eight odorants in the model wines studied were affected by ethanol, tannin and fructose to varying degrees.

The odorant, 3-methyl-1-butanol was significantly influenced by the concentration of ethanol ($p < 0.001$), tannin ($p < 0.001$) and fructose ($p < 0.05$). In addition, all two-way interactions were significant ($p < 0.001$). Upon closer inspection of these interactions, tannin concentration showed no significant effect on odorant recovery at higher ethanol concentrations (14 and 16%). However, at lower ethanol concentration (8, 10 and 12%), significantly higher recovery of odorant was obtained in the presence of low tannin than in medium or high tannin concentration (**Figure 6a and 6b**). Increasing tannin level resulted in decreased odorant concentration but only at the low level of fructose. At the high fructose level, the recovery of 3-methyl-1-butanol decreased as tannin was reduced from low to medium and then increased again as tannin increased from medium to high tannin. On the other hand, odorant recovery significantly decreased as ethanol increased at high fructose concentration. At the low fructose level, reduction of odorant recovery was less pronounced with increased ethanol concentration.

Both ethanol and fructose had significant main effects on decreasing the headspace concentration of dimethyl disulfide ($p < 0.001$) although both factors were also implicated in two-way interactions with tannin. In addition, a higher order interaction was also found involving ethanol, tannin and fructose ($p < 0.05$). The low tannin model wines had lower dimethyl disulfide recoveries than the high tannin wines at either 10% or 14% ethanol at high fructose concentration. This effect was reversed when ethanol concentration reached 16% (**Figure 7a and 7b**). In general, the suppression of ethanol on volatile compound recovery was more pronounced when fructose concentration was high. The lowest concentration of dimethyl disulfide was seen in the presence of high tannin and fructose at the highest alcohol level (16%).

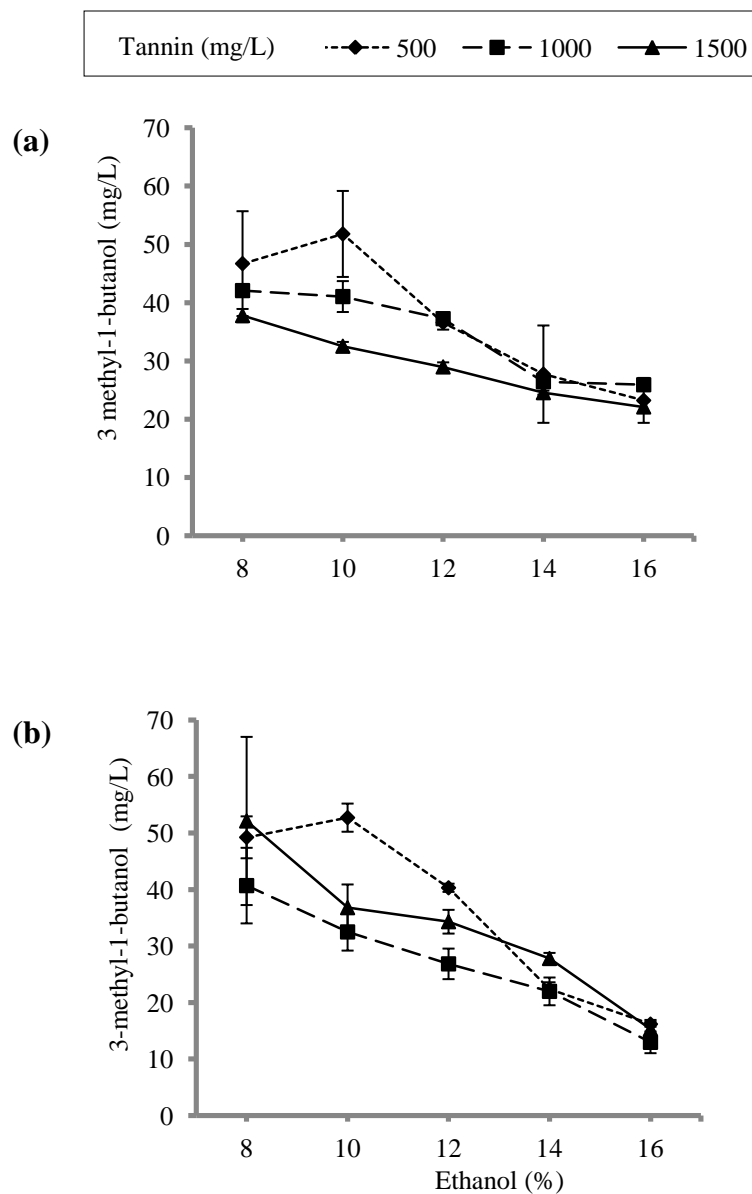


Figure 6. Concentrations of 3-methyl-1-butanol from model wines with different ethanol, tannin and fructose concentrations **(a)** low fructose, 200 mg/L **(b)** high fructose, 2000 mg/L, extracted using headspace SPME with a PDMS/DVB coated fiber. Error bars denote standard deviations ($n = 3$ to 5).

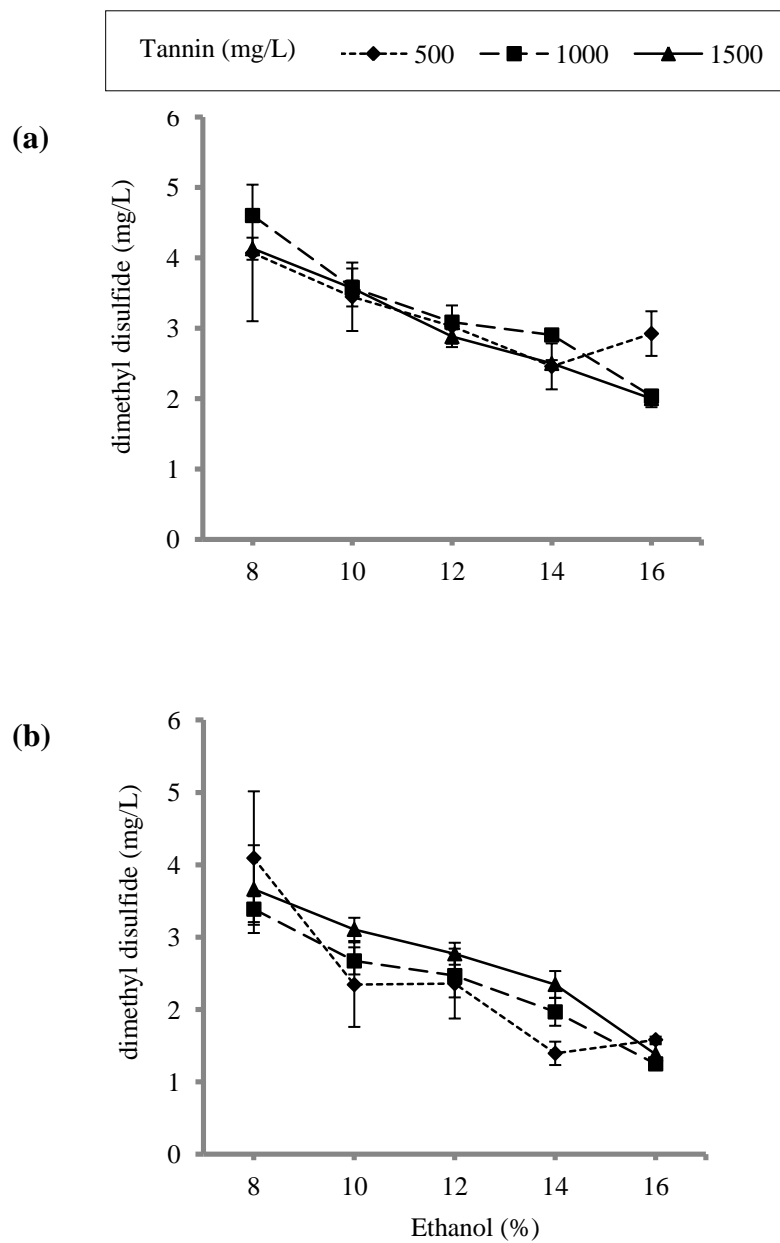


Figure 7. Concentrations of dimethyl disulfide from model wines with different ethanol, tannin and fructose concentrations **(a)** low fructose, 200 mg/L **(b)** high fructose, 2000 mg/L, extracted using headspace SPME with a PDMS/DVB coated fiber. Error bars denote standard deviations (n = 3 to 5).

The effects of ethanol, tannin and fructose and their interactions on the recovery of 1-hexanol were all statistically significant (predominantly $p < 0.001$). The concentration of 1-hexanol in model wines decreased at lower ethanol concentrations (8 and 10%) when tannin levels increased. However, at a higher concentration of ethanol (12 and 14%), increasing tannin resulted in a slight increase in recovery of the odorant (**Figure 8a and 8b**). Generally, at the same level of ethanol, higher concentrations of the 1-hexanol were recovered in the presence of low fructose than in high fructose.

Ethanol and tannin were the factors with an observed main effect on the headspace concentration of 1-octen-3-one ($p < 0.001$ and $p = 0.001$, respectively). However, these factors were also implicated in two-way interactions with fructose. Further examination of the ethanol x fructose data showed that at the lower ethanol concentrations (8 and 10%), a significantly higher concentration of 1-octen-3-one was recovered when the fructose level was low. In contrast, at 12% ethanol, a higher recovery of 1-octen-3-one was obtained from model wines containing high compared to low fructose levels. At the higher level of ethanol (14 and 16%), no effect of fructose was observed. No clear effect was observed from the interaction between tannin and fructose (**Figure 9a and 9b**).

As with 1-octen-3-one, the main and all interaction effects on 2-methoxyphenol involving ethanol, tannin and fructose were significant ($p < 0.001$). In general, a sharp reduction of odorant recovery was observed with increasing ethanol and fructose. This depressive effect was more apparent at high fructose concentrations (**Figure 10b**). At low fructose and lower ethanol content (8% and 10%), odorant concentrations in low and high tannin content model wines were comparable. The suppression effect of tannin was clearly demonstrated when the ethanol content reached 12% ethanol (**Figure 10a**).

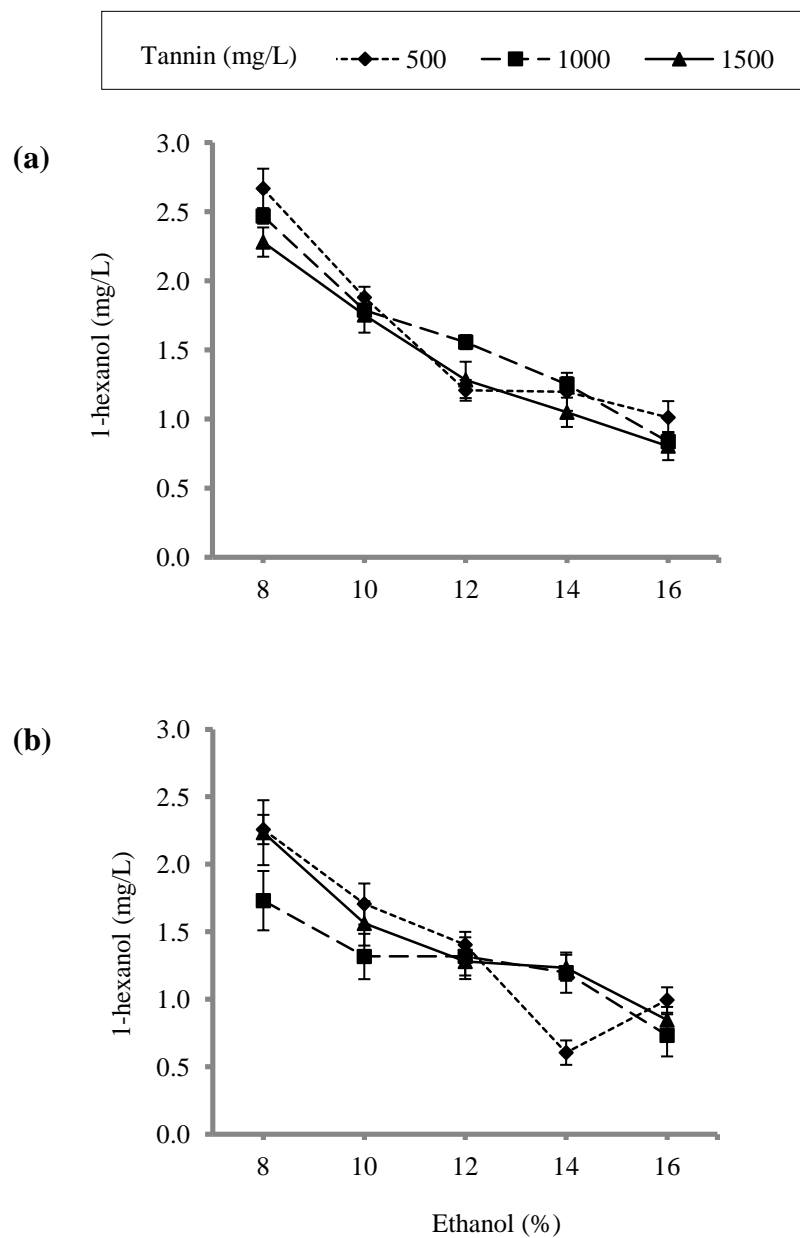


Figure 8. Concentrations of 1-hexanol from model wines with different ethanol, tannin and fructose concentrations **(a)** low fructose, 200 mg/L **(b)** high fructose, 2000 mg/L, extracted using headspace SPME with a PDMS/DVB coated fiber. Error bars denote standard deviations (n = 3 to 5).

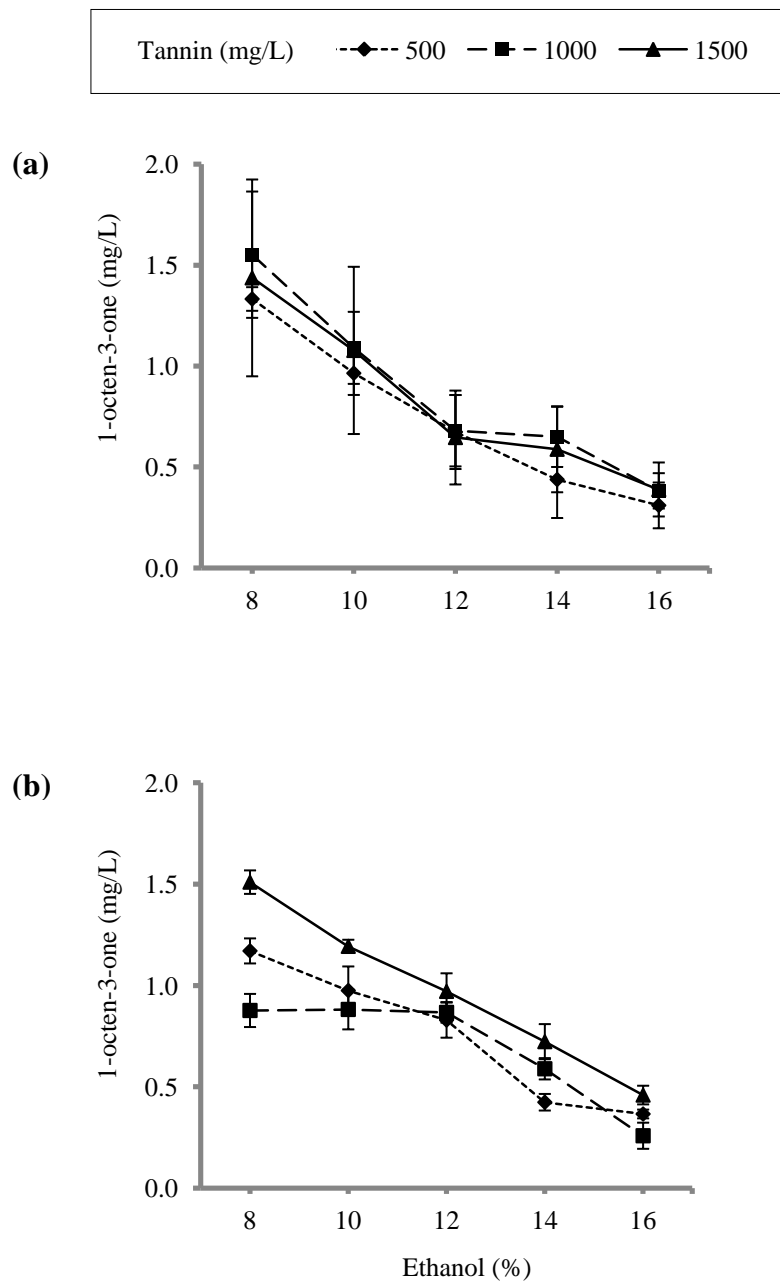


Figure 9. Concentrations of 1-octen-3-one from model wines with different ethanol, tannin and fructose concentrations **(a)** low fructose, 200 mg/L **(b)** high fructose, 2000 mg/L, extracted using headspace SPME with a PDMS/DVB coated fiber. Error bars denote standard deviations (n = 3 to 5).

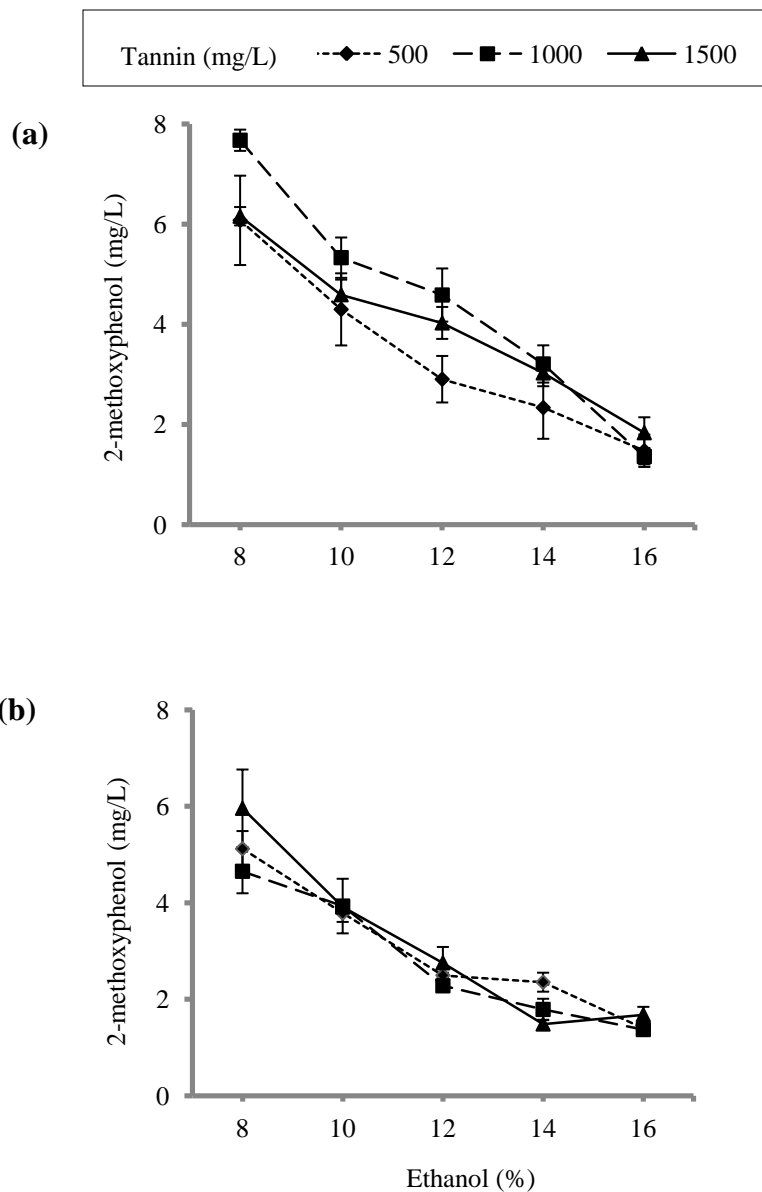


Figure 10. Concentrations of 2-methoxyphenol from model wines with different ethanol, tannin and fructose concentrations **(a)** low fructose, 200 mg/L **(b)** high fructose, 2000 mg/L, extracted using headspace SPME with a PDMS/DVB coated fiber. Error bars denote standard deviations (n = 3 to 5).

Ethanol and fructose were the only factors exerting a main effect on the recovery of 2-phenylethanol ($p < 0.001$). However, a higher order interaction between ethanol, fructose, and tannin was also observed (**Figure 11a and 11b**). The general trend was that increasing ethanol led to a reduction in 2-phenylethanol recovery, with lower recovery of the odorant at a high fructose level. With few exceptions, the difference in recoveries due to tannin concentration was not significant ($p > 0.05$). A significant tannin effect was found at 12 and 14% ethanol and low fructose concentration, wherein higher tannin resulted in higher odorant recovery ($p < 0.05$).

The concentration of eugenol was highly influenced by all factors and their interactions (predominantly, $p < 0.001$). Increasing ethanol concentration from 8 to 16% negatively impacted the recovery of the odorant but only at the low fructose level (**Figure 12a**). At high fructose level, this trend was only evident when the tannin concentration was high (**Figure 12b**). Also, at the high fructose level, high tannin concentration enhanced the recovery of the odorant when ethanol concentrations were at 8, 10 and 12% in model wines.

β -damascenone was highly affected by the main effects of ethanol and fructose ($p < 0.001$). Two-way and three-way interactions were also significant except for the ethanol and tannin interaction ($p > 0.05$). Higher ethanol level significantly decreased the concentration of β -damascenone extracted from the model wines. The negative impact of ethanol on the recovery of the odorant was more influenced by the high amount of fructose than the tannin counterpart (**Figure 13a and 13b**). Tannin concentration did not show significant effects on the recovery of the odorant except when fructose was low and ethanol was at its lowest concentration (8%). Under this condition, odorant recovery increased with an increase in tannin concentration from low to medium. A further increase in tannin concentration resulted in a significantly lower

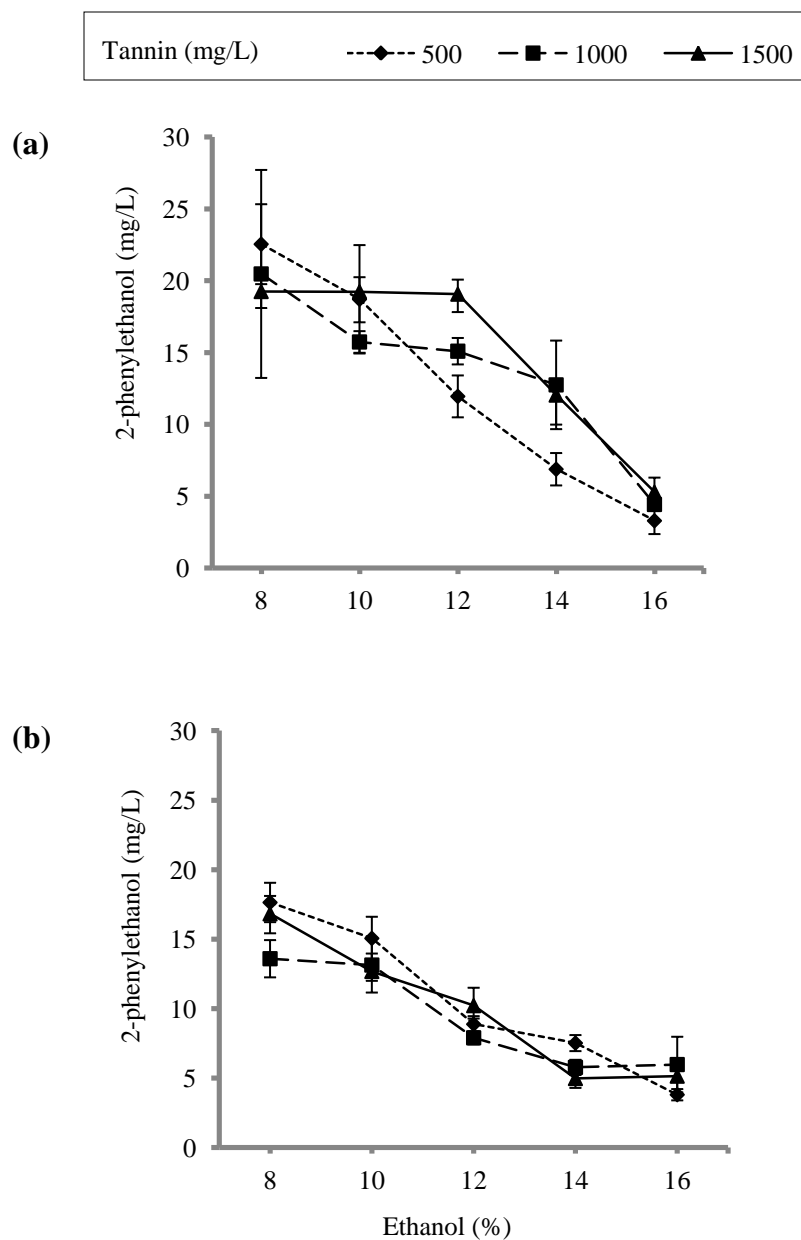


Figure 11. Concentrations of 2-phenylethanol from model wines with different ethanol, tannin and fructose concentrations **(a)** low fructose, 200 mg/L **(b)** high fructose, 2000 mg/L, extracted using headspace SPME with a PDMS/DVB coated fiber. Error bars denote standard deviations (n = 3 to 5).

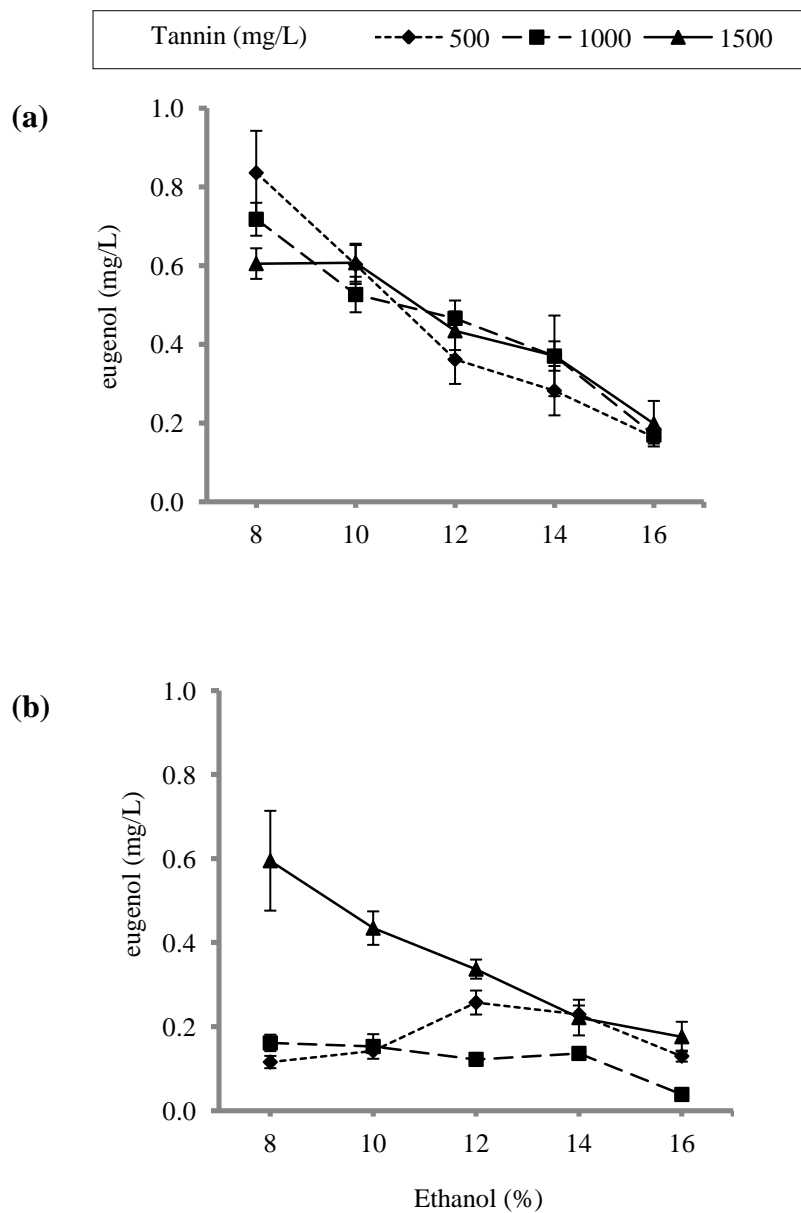


Figure 12. Concentrations of eugenol from model wines with different ethanol, tannin and fructose concentrations **(a)** low fructose, 200 mg/L **(b)** high fructose, 2000 mg/L, extracted using headspace SPME with a PDMS/DVB coated fiber. Error bars denote standard deviations (n = 3 to 5).

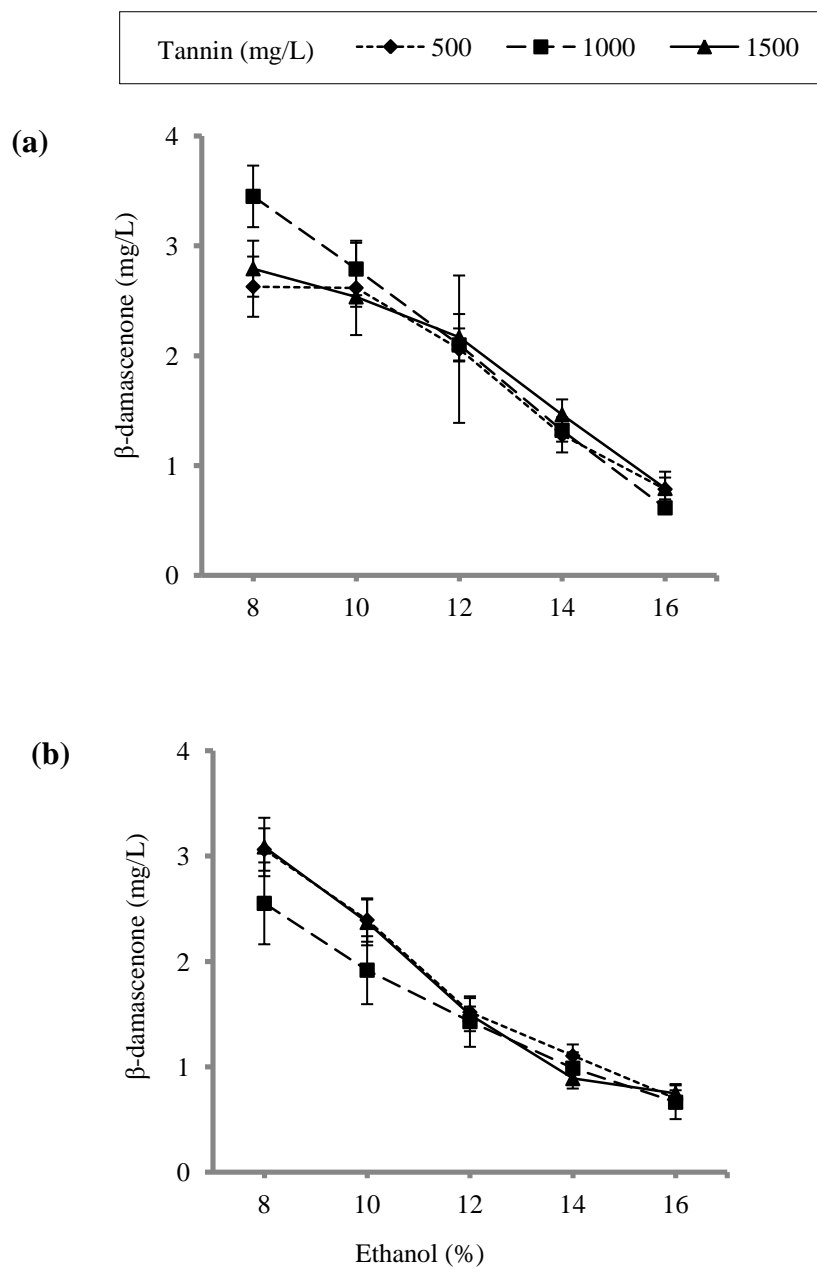


Figure 13. Concentrations of β -damascenone from model wines with different ethanol, tannin and fructose concentrations **(a)** low fructose, 200 mg/L **(b)** high fructose, 2000 mg/L, extracted using headspace SPME with a PDMS/DVB coated fiber. Error bars denote standard deviations (n = 3 to 5).

recovery, which was comparable with the recovery of β -damascenone in the presence of low tannin.

The above ANOVA results showed that the impact of interactions among ethanol, tannin and fructose on volatile compound partitioning varied with the odorant, suggesting the influence of some physicochemical nature of the individual compound. A linear relationship between percent relative reduction in odorant concentration due to ethanol, tannin and fructose concentrations and compound molecular weight was found. The percent reduction in odorant recovery at the highest ethanol, tannin and fructose concentration relative to the recovery at the lowest level of these factors was observed to be higher for the larger, less volatile compounds such as in 1-octen-3-one (72.4%, MW=126.20), 2-methoxyphenol (74.3%, MW=124.12), 2-phenylethanol (74.9%, MW=122.16), eugenol (70.7%, MW=164.20), and β -damascenone (75.5%, MW=190.28). A decrease in volatile recovery was less pronounced on the low molecular weight, more volatile compounds such as 3-methyl-1-butanol (57.2%, MW=88.15), dimethyl disulfide (58.0%, MW=94.20) and 1-hexanol (61.7%, MW=102.17). The greater retention observed with higher molecular weight compounds may be due to their lower rate of diffusion through the matrix as compared to the lower molecular weight compounds. Consequently, this might lead to an unbalanced perception of aroma with mainly smaller compounds dominating the headspace, a possibility that requires further sensory investigation.

Overall, these findings clearly indicate the complex interactions between odorants and wine components. Results of ANOVA showed that ethanol concentration was consistently implicated in the main effects for all odorants and compared with the other factors, displayed the largest impact on the headspace recovery of volatile compounds. Fructose and tannin factors were also implicated in the main effects but to a lesser extent than ethanol. However, all the

factors studied were generally involved in a significant three-way interaction, indicating that the impact on odorant recovery should be explained in terms of the combined effects of ethanol, tannin and fructose.

A general view of the combined effects of ethanol, tannin and fructose on the headspace recovery of odorants can be described. Ethanol demonstrated a suppression effect which can be attributed by its ability to act as co-solvent with water, enhancing the solubility of the odorants.²⁷ Higher fructose concentration also reduced odorant recovery. The greater retention of odorants in the solution driven by the increase in fructose may have been due to the hydration of more fructose molecules, which depleted the available water molecules. This possibly increased the effective concentration of ethanol and resulted in more of the odorants kept in solution.

In contrast, tannin displayed an enhancement effect, which allowed a greater release of the odorants into the headspace, but this was limited by the ethanol concentration. At 8, 10 and 12% ethanol concentrations, the salting-out effect was more enhanced. It is possible that at relatively lower ethanol concentrations, more incidence of tannin self-aggregation occurred,²⁸⁻²⁹ which led to a decrease in the potential binding sites for odorants. As the ethanol concentration increased, self-association of tannins decreased²⁸ thereby increasing hydrophobic interactions between tannin and odorants. This resulted in a reduction in the headspace recovery of odorants as observed.

Considering all the possible mechanisms involved, the net magnitude effect from the increase in ethanol, tannin and fructose was a strong reduction in headspace concentration of all odorants with the dominant contribution arising from ethanol as observed. Since the perception of wine aroma and flavor is dependent predominantly on the concentration of odorants in the

headspace above the wines, the treatment effects imply a considerable reduction of impact among these compounds on the perceived wine aroma and flavor during consumption.

PCA analysis

The relationship between odorants to each other and among model wine samples were further analyzed using PCA (**Figure 14**). The effect of ethanol concentration was found to be larger than the effect of tannin and fructose concentrations and in agreement with the trends found using ANOVA. The first principal component (PC 1) separated the model wines as a function of ethanol (85% variation) whereas the second principal component (PC 2) distinguished the wines by fructose and to a lesser extent by tannin concentration (6% variation). Model wines with lower ethanol concentrations (8 and 10%) were characterized by higher headspace recovery of 2-phenylethanol, 2-methoxyphenol, dimethyl disulfide, 1-hexanol, 1-octen-3-one, and β -damascenone. At higher fructose concentration, the recovery of eugenol decreased while 3-methyl-1-butanol increased. The increase in tannin concentration showed less effect on odorant recovery in model wines with higher ethanol (14 and 16%) than those with lower ethanol content (8, 10 and 12%).

Effects on threshold and odor unit values

The odor threshold values obtained for all odorants determined in different model wine solutions were generally higher than the values previously reported (**Table 7**). The suppression effect of the combination of ethanol, tannin and fructose observed on the headspace recovery of odorants indeed contributed to the increase in odor threshold values. At either 10% or 14% ethanol, high tannin and high fructose concentrations resulted in higher odor thresholds for the majority of odorants. The highest threshold value was found in the model wines containing 14% ethanol, high tannin (1500 mg/L) and high fructose (2000 mg/L). The threshold value increased

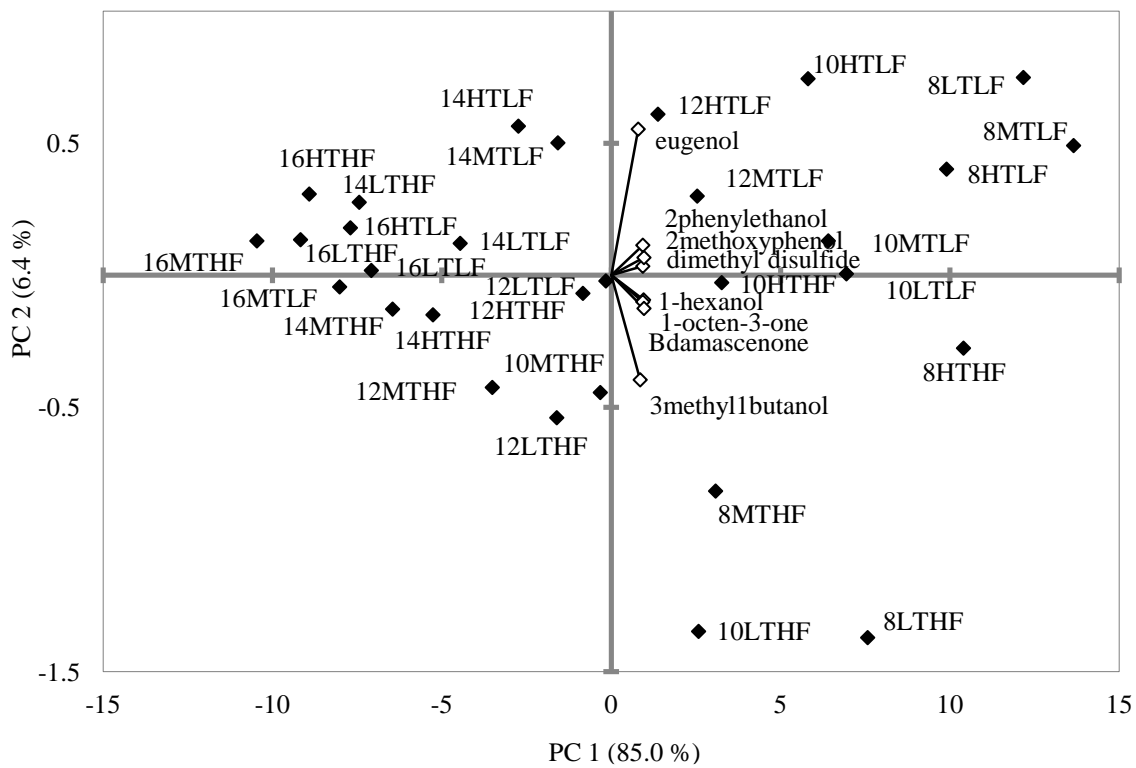


Figure 14. Principal component analysis showing the relationships among odorants and model wines. 8, 10, 12, 14, and 16 = percent ethanol (v/v), LT=Low tannin, MT=Medium tannin, HT=High tannin, LF=Low fructose, HF=High fructose.

Table 7. Odor thresholds^a of aroma compounds in different model wine solutions.

Odorants	10% Ethanol				14% Ethanol				Lit ^b	
	Low Tannin		High Tannin		Low Tannin		High Tannin			
	Low Fructose	High Fructose	Low Fructose	High Fructose	Low Fructose	High Fructose	Low Fructose	High Fructose		
3-methyl-1-butanol	28.1	158.1	158.1	281.2	158.1	158.1	158.1	500	500	30 ^c
dimethyl disulfide	2.2	12.6	12.6	22.5	12.6	12.6	22.5	12.6	22.5	0.029 ^d
1-hexanol	112	2000	2000	2000	2000	2000	2000	2000	2000	8 ^c
1-octen-3-one	5.6E-06	3.2E-04	3.2E-04	5.6E-04	5.6E-05	3.2E-04	3.2E-04	3.2E-04	1.8E-03	1.5E-06 ^e
2-methoxyphenol	0.01	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.0001 ^c
2-phenylethanol	0.4	4.4	0.4	21	1.4	4.4	1.4	1.4	44	10 ^c
eugenol	8.9E-07	1.6E-03	1.6E-04	8.9E-03	2.8E-05	2.8E-03	1.6E-04	8.9E-03	5.0E-00 ^c	
β-damascenone	3.6E-04	2.0E-03	2.0E-03	3.6E-03	3.6E-04	3.6E-03	2.0E-03	3.6E-03	3.6E-03	5.0E-05 ^c

^agroup best estimate threshold (mg/L) obtained from GC-O analysis, n = 4.

^bthreshold values (mg/L) from the literature: ^cin 10% ethanol/water solution⁶; ^din white wine, 12.6% ethanol³⁵;

^e10% ethanol/water solution³⁷

by 2-fold for 2-methoxyphenol, 10-fold for dimethyl disulfide and β -damascenone, 18-fold for 1-hexanol and 3-methyl-1-butanol. Other compounds had larger increases in the threshold value such as in the case of 2-phenylethanol, 1-octen-3-one and eugenol, which increased by 110-fold, 321-fold and 10,000-fold, respectively.

The effect of the wine matrix on the potential contribution of individual odorants in aroma perception was also evaluated. Previous studies showed changes in the odor qualities of mixtures resulting from modifications in matrix composition.³⁰⁻³¹ **Table 8** provides the log odor unit values U_o of each compound present in the specified wine matrix. A more positive value ($\log U_o > 0$) indicates a higher degree of likelihood of contributing to the aroma profile while a negative value ($\log U_o < 0$) would imply an unlikely contribution. Results showed that model wines containing 10% ethanol, low tannin and low fructose had the highest OUV's for all odorants. These model wines can be characterized with strong "spicy/clove" and "earthy/mushroom" contributed by eugenol and 1-octen-3-one ($\log U_o > 5.0$), respectively. However, the addition of high tannin and high fructose at either 10% or 14% ethanol resulted in a lowering of the calculated odor unit values, indicating a reduction in the contribution of odorants to the overall aroma of a model wine. Based on the OUV's, the model wines containing higher concentrations of wine components had a different aroma profile contributed mainly by 1-octen-3-one (earthy), 3-methyl-1-butanol (caramel/cooked) and β -damascenone (fruity) ($\log U_o > 2.0$).

In conclusion, the study highlighted the significant interaction effects of ethanol, tannin and fructose concentrations on the headspace recovery of odorants and in turn, on their sensory significance. The interactions are complex and thus, future work should focus on investigations at the molecular level to improve understanding of the chemical nature of interactions. In

Table 8. Log odor unit values (OUV)^a of aroma compounds in different model wine solutions.

Odorants	10% Ethanol				14% Ethanol			
	Low Tannin		High Tannin		Low Tannin		High Tannin	
	Low Fructose	High Fructose	Low Fructose	High Fructose	Low Fructose	High Fructose	Low Fructose	High Fructose
3-methyl-1-butanol	3.9	3.1	3.1	2.8	3.6	2.5	2.9	2.4
dimethyl disulfide	2.2	-0.7	-0.5	-0.9	-0.7	-1.2	-0.7	-1.0
1-hexanol	-1.8	-3.1	-3.0	-3.1	-3.2	-3.2	-3.3	-3.2
1-octen-3-one	5.2	3.5	3.5	3.3	3.9	3.1	3.3	2.6
2-methoxyphenol	2.5	2.2	2.3	2.2	2.2	2.0	2.1	1.8
2-phenylethanol	1.6	0.5	1.6	-0.2	0.7	0.2	0.9	-0.9
eugenol	5.8	1.9	3.6	1.7	4.0	1.9	3.4	1.4
β -damascenone	3.9	3.1	3.1	2.8	3.6	2.5	2.9	2.4

^aLog U_o = log of odorant concentration divided by odor threshold in the given model wine solution

general, tannin had an enhancement effect while fructose induced a retention effect, both of which were largely dependent on ethanol concentration. High ethanol, tannin and fructose concentrations caused greater losses of odorants particularly the larger compounds, which may lead to an imbalance in perceived aroma quality during consumption. A marked increase in the aroma threshold values and decrease in the potential aroma contribution of odorants supported the negative impact of these wine parameters. Considering these consequences on wine aroma quality arising from wine matrix interactions may assist viticulturists and winemakers to make necessary adjustments in their practices as needed.

Acknowledgments

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CHAPTER V

SENSORY IMPACT OF INTERACTIONS AMONG ETHANOL, TANNIN AND FRUCTOSE IN A MODEL RED WINE

Abstract

The impact of ethanol (0, 8, 10, 12, 14 and 16% v/v), tannin (500, 1000 and 1500 mg/L) and fructose (200 and 2000 mg/L) concentrations on the sensory properties of model red wines was investigated. Using a trained panel ($n = 12$), the intensity of aroma, flavor, taste and mouthfeel characteristics of the different wines ($n = 36$) were determined. Significant ($p \leq 0.05$) positive correlations were found between an aroma and its flavor counterpart descriptor, fruity ($r = 0.76$), woody ($r = 0.69$), caramel ($r = 0.75$), sulfur ($r = 0.64$), herbaceous ($r = 0.83$), earthy ($r = 0.81$), floral ($r = 0.79$), and spicy ($r = 0.76$). When principal component analysis (PCA) of the ratings was applied, model wines were differentiated based on PC 1 (floral, fruity and caramel), PC 2 (earthy and herbaceous) and PC 3 (sulfur, spicy, woody, and bitterness). The analysis of variance (ANOVA) results showed a significant impact of ethanol concentration on these principal components ($p \leq 0.05$) while tannin and fructose concentration effects, and all interaction effects were not significant. Model wines with lower ethanol concentration (0, 8 and 10%) had higher floral, fruity, caramel aroma and flavor scores than those with 16%. Higher scores for earthy and herbaceous aroma and flavor were observed with 0, 8, 10, 12% ethanol concentration compared to those containing 14 and 16%. In contrast, sulfur, spicy, woody aroma and flavor, and perceived bitterness were lowest with 0 and 8% ethanol, with higher sensations detected as the ethanol concentration increased from 8 to 16%. These results showed the importance of ethanol in modulating the sensory perception and resulting quality.

Introduction

Wine is a complex alcoholic beverage containing both volatile and non-volatile components, which may interact with each other and influence the perception of its intrinsic quality. The combination of factors such as grape variety, climate, sites, viticultural and enological practices has been shown to alter the concentration and distribution of these components in the wine matrix and ultimately, the overall quality of wine produced (Jackson and Lombard 1993).

The assessment of wine components that influence the sensory properties of wines has been the focus of recent wine research studies. Ethanol, the most abundant volatile wine constituent was shown to modify the taste (sweetness, bitterness), mouthfeel (hotness, fullness, viscosity) (Fischer and Noble 1994, Nurgel and Pickering 2006, Nurgel and Pickering 2005), aroma and flavor sensations (Guth et al. 2007). On the other hand, the colour quality, perceived bitterness and astringency in red wines have been attributed to the non-volatile component, phenolic compounds. In addition, the influence of polyphenols on odorants contribution to aroma perception was also demonstrated (Lund et al. 2009, Goldner et al. 2011). Other wine macromolecules, the polysaccharides, particularly the mannoproteins, were thought to contribute to the fullness and decreased astringency of red wines (Vidal et al. 2004) as well as in the suppression of perceived intensity of aroma compounds (Chalier et al. 2007).

Few research studies directed towards studying the interaction effects among wine components have shown the extent to which individual compounds can influence the sensory aspects of wine in the presence or absence of another compound. Jones et al. (2008) reported that the overall aroma, ester and floral aroma intensity were dependent on the concentration of volatiles, glycerol and ethanol. At lower volatile concentration, ethanol had a suppressing effect

but only when glycerol was absent. Conversely, at higher volatile concentration, overall aroma intensity was impacted by neither glycerol nor ethanol. In another study, perceived bitterness increased with increased ethanol, catechin and to some extent pH, with the largest effect induced by ethanol. However, the masking effects of ethanol on sourness were evident only at pH 3.2 while catechin produced no significant effect (Fischer and Noble 1994). Vidal et al. (2004) showed that the perception of astringency increased with tannin concentration but decreased in the presence of rhamnogallacturonan II.

The current study aimed to investigate the effects of ethanol, tannin and fructose and their interactions that may affect the aroma, taste, flavor and mouthfeel properties of wine. The concentrations of the ethanol, tannin and fructose were selected to cover the range found in red wines. A descriptive sensory analysis of model wines with varied concentrations of these components was carried out using a full factorial design. To assist in the interpretation of sensory data, a correlation-based principal component analysis (PCA) was initially applied followed by the analysis of variance (ANOVA) to discriminate among the model wines.

Materials and Methods

Model wine samples

Thirty-six (36) synthetic-based model wines were prepared with 0, 8, 10, 12, 14, and 16% v/v ethanol (Decon Labs, Inc., PA), 500, 1000 and 1500 mg/L tannin (Biotan, Laffort Company, CA), and 200 and 2000 mg/L fructose (Sigma-Aldrich, CA). Tartaric acid (500 mg/L, Sigma-Aldrich, CA) was added to all wines prior to adjustment to pH 3.4 with 1 M NaOH (AAA Chemicals, Inc., TX). Eight selected odorants (Sigma-Aldrich, CA), were spiked at fixed concentrations: 250 mg/L 3-methyl-1-butanol (caramel), 0.002 mg/L dimethyl disulfide (sulfur), 1 mg/L 1-hexanol (herbaceous), 0.0001 mg/L 1-octen-3-one (earthy), 0.02 mg/L methoxyphenol

(woody), 30 mg/L 2-phenylethanol (floral), 0.5 mg/L eugenol (spicy), and 0.03 mg/L β -damascenone (fruity) (**Table 9**). For each odorant, stock solution was initially prepared every week in 50% ethanol/Milli Q (v/v) water and stored at 5°C in 5 mL amber vial sealed with PTFE/Silicone septum until ready to use. Previous gas chromatography-mass spectrometry analysis results showed no change in odorant concentration within two weeks of storage. Each replicated preparation of all the 36 model wines was prepared and stored in a 1 L screw cap glass bottle (Pyrex[®], Corning, Inc.) in a dark room at 23°C for at least 12 hours prior to sensory analysis.

Sensory evaluation

Twelve students and staff from the Washington State University (4 females and 8 males, aged 21 to 55) participated in the sensory evaluation. Panelists were recruited based on interest, availability, frequency of wine consumption (at least a week) and sensitivity to aroma and flavor of wines.

Prior to formal evaluations, trainings (10, one-hr sessions) were conducted to ensure that panelists evaluate the wines in a similar, reproducible manner. During the training sessions, panelists were exposed to solutions with similar characteristics as the experimental model wine samples. Panelists developed skills to recognize 8 aroma and flavor, and 2 taste and mouthfeel attributes. They were also trained how to evaluate intensities of each attribute by utilizing a 15-cm unstructured line scale on paper ballots, and on the computer during practice sessions in individual booths under red lighting. The sensory descriptive terms, references standards and the corresponding intensity ratings used were shown in **Table 10**. The intensity ratings for each prepared standard were agreed upon by consensus. Taste and mouthfeel descriptors were defined

Table 9. Physico-chemical and sensory properties of odorants used in the study.

Compound	CAS Registry No.	MW	BP (°C)	Log P value ^a	Aroma Descriptors ^b	Aroma Category ^c
3-methyl-1-butanol	123-51-3	88.15	131.1	1.16	whiskey, malt, burnt	caramel
dimethyl disulfide	624-92-0	94.19	109.8	1.77	onion, cabbage	chemical
1-hexanol	111-27-3	102.2	157.6	2.03	green, flower	herbaceous
1-octen-3-one	4312-99-6	126.2	162.0	2.37	mushroom, metal	earthy
2-methoxyphenol	90-05-1	124.1	205.0	1.32	smoke, sweet, medicine	woody
2-phenyl ethanol	60-12-8	122.2	218.2	1.36	honey, spice, rose, lilac	floral
eugenol	97-53-0	164.2	253.2	2.27	clove, honey	spicy
β-damascenone	23726-93-4	190.3	265.1	4.21	apple, rose, honey	fruity

^aHydrophobic constant obtained from Estimation Program Interface (EPI) Suite SoftwareTM (U.S. Environmental Protection Agency)

^bFlavornet (www.flavornet.org)

^cNoble et al. (1987)

Table 10. Aroma, flavor, taste and mouthfeel reference standards and their corresponding intensity ratings used during panel training.

Attributes	Reference standard	Rating ^a
Aroma and flavor		
caramel	90 mg/L 3-methyl-1-butanol	10.0
sulfur	0.1 mg/L dimethyl disulfide	10.0
herbaceous	24 mg/L 1-hexanol	10.0
earthy	0.01 mg/L 1-octen-3-one	13.0
fruity	0.1 mg/L β -damascenone	12.5
floral	40 mg/L 2-phenylethanol	10.0
woody	0.1 mg/L 2-methoxyphenol	12.5
spicy	0.1 mg/L eugenol	10.0
Taste		
sourness	2 g tartaric acid/L milliQ water	15.0
bitterness	0.1 g quinine sulfate/L milliQ water	15.0
Mouthfeel		
drying	2 g tannic acid + 0.875 g alum/0.75L milliQ water	15.0
heat	20% ethanol/milliQ water (v/v)	15.0

^aMean observations for each attribute of aroma, flavor, taste and mouthfeel on a 15-cm intensity line scale (n=12)

to avoid confusion. Perceived sourness and bitterness were described as the sensation predominantly perceived along the sides of the tongue and at the back of the tongue, respectively. The term drying was defined as the feeling of lack of lubrication in the mouth whereas heat was referred to as either warm (parching) or hot (numbing) sensation. Panel performance was monitored by calculating the standard deviations of each individual panelist and the panel as whole. Feedback regarding the results of practice evaluation in the booths (two, 30-minute sessions) in the form of computerized graphs was provided to each panelist on the next training session.

The trained panelists evaluated the 36 wines in duplicate during the formal evaluation sessions. Each sample was assigned a 3-digit number and served in an ISO/INAO (International Standards Organization) tulip-shaped wine tasting glasses, covered with petri dish at ambient temperature (approximately 23°C). Samples were individually presented to all panelists under red lights in a balanced, random order. Panelists evaluated 6 model wines per session, with a forced 1-min break after each wine and a 10-min break after every three wines to reduce sensory fatigue. They were given unsalted crackers and milliQ water for cleansing and rinsing their palates between each sample. Each panelist attended 12 sessions to complete the evaluation of 72 samples. The panelists rated intensities of the 20 attributes on computer using Compusense software, version 5.0 (Guelph, Canada).

Statistical analysis

Pearson's correlation analysis was carried out on individual intensity ratings to determine the relationships among sensory descriptors. Subsequently, factor analysis (principal components method) was applied to explore the underlying factors responsible for the covariation among the observed variables in model wines using three factors with varimax rotation. A fixed-effect

analysis of variance (ANOVA) was performed to determine the impact of panelist, ethanol, tannin and fructose concentrations and their interactions on the three principal components. Multiple comparisons of treatment means for significant factors were done using Fischer's LSD. All data analyses were carried out using SAS (Statistical Analysis Systems, Cary, NC) with significance defined at $p \leq 0.05$.

Results and Discussion

Correlation and principal component analysis (PCA)

The Pearson's correlation analysis among sensory descriptors revealed strong, high associations between aroma attributes and the appropriate flavor counterpart descriptor ($p \leq 0.05$) (**Table 11**). These results were in agreement with Pierce and Halpern (1996), who reported that the orthonasal and retronasal responses to an odorant could be similar since the orthonasal and retronasal olfaction share a common olfactory pathway.

A correlation-based PCA was applied to the intensity ratings of a trained panel on 20 sensory attributes measured on 36 model wines. The analysis generated 6 principal components (PC) with Eigen value > 1 , however only three (3) PCs were retained, which accounted for 49% of the total variance. Further extraction of more than three components did not provide substantial benefits to the explained variability. The retained components were subjected to VARIMAX rotation to bring them into closer alignment with the original variables (Lawless and Heymann 1999). **Figure 15** shows projections of correlations between the PC and the original attribute measurements. Sensory attributes with loadings > 0.5 on a particular PC was considered to have a strong influence on that PC and thus, were used for data interpretation. PC 1 was positively loaded with floral, fruity and caramel aroma and flavor and contributed 28.0% of variation. PC 2 with positive loadings for earthy and vegetal aroma and flavor contributed 12.0%

Table 11. Significant correlations ($p \leq 0.05$) found between sensory descriptors (overall N= 864).

Attributes		<i>r</i>	<i>p</i>
Aroma	Flavor		
Fruity	Fruity	0.76	<0.0001
Woody	Woody	0.69	<0.0001
Caramel	Caramel	0.75	<0.0001
Sulfur	Sulfur	0.64	<0.0001
Herbaceous	Herbaceous	0.83	<0.0001
Earthy	Earthy	0.81	<0.0001
Floral	Floral	0.79	<0.0001
Floral	Fruity	0.57	<0.0001
Spicy	Spicy	0.76	<0.0001

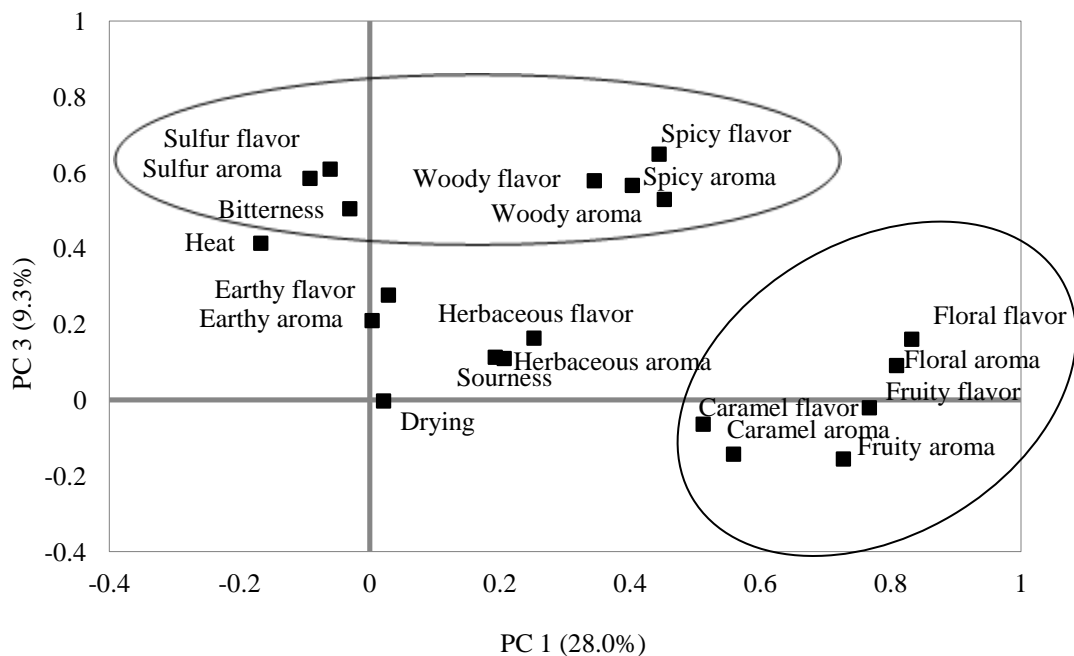
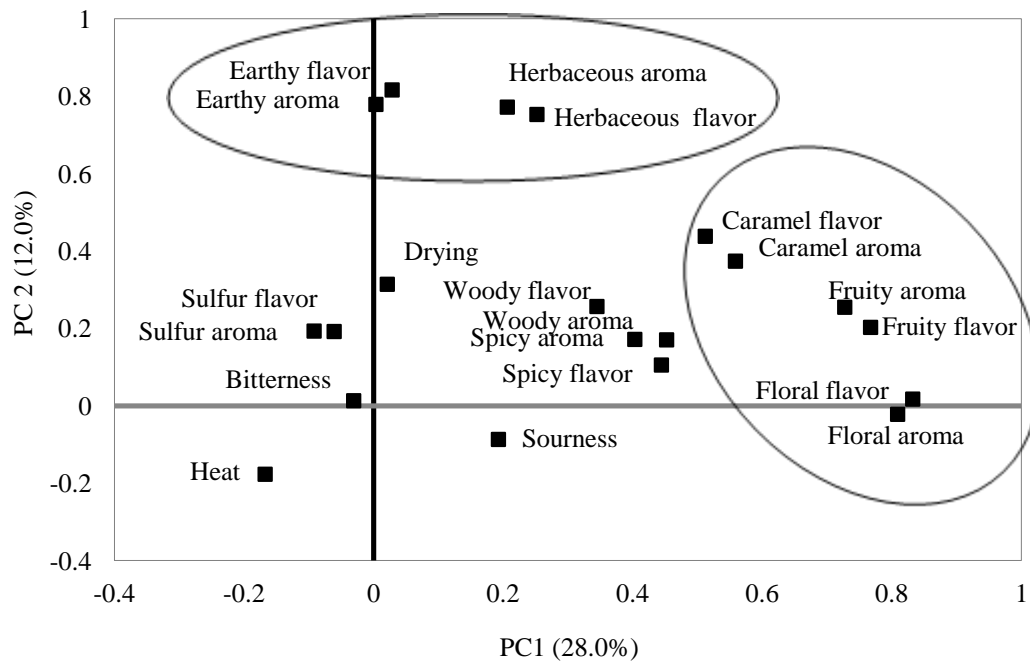


Figure 15. Principal component analysis plot of model red wine sensory attributes projected on PC 1 and 2 (above), and PC 1 and 3 (below) with VARIMAX rotation.

of variation. PC 3 was positively related to sulfur, woody, spicy aroma and flavor and perceived bitterness contributing 9.3% of variation among model wines.

Impact of ethanol, tannin and fructose

The analysis of variance (ANOVA) results was carried out on the factor scores for the retained principal components to determine the significance of the effects of ethanol, tannin and fructose and their interactions on the PCs (**Table 12**). A significant panelist effect was noted for the PCs indicating that panelists may have used different parts of the intensity scale for rating the attributes, which is commonly found even for supposedly highly trained panel (Lawless and Heymann 1999). The effect of ethanol was significant for PC 1 ($p = 0.001$), PC 2 ($p = 0.001$) and PC 3 ($p < 0.000$). However, neither tannin nor fructose main effects were found significant ($p > 0.05$). The observed effects of these wine components may not be sufficiently large to be perceived by the panel.

A graphical representation of the effect of ethanol concentration on the scores loaded for the three PCs is presented in **Figure 16**. Generally, PC 1 and PC 2 were negatively impacted by the increase in ethanol concentration. Model wines with 0, 8 and 10% ethanol had significantly higher floral, fruity, caramel aroma and flavor (PC 1) than those with 12, 14 and 16% ethanol concentration. Considering the earthy and vegetal aroma and flavor (PC 2), significantly higher scores were obtained from wines with 0, 8, 10, 12% ethanol concentration than with 14 and 16%. The suppression effect of ethanol on the perception of fruitiness observed in the present study supported previous studies (Guth 1998, Escudero et al. 2007, Grosch 2001). Higher ethanol content (14.5 to 17.2%) was also demonstrated to decrease the fruity aromas; however, this was accompanied by increasing herbaceous notes in red wines (Goldner et al. 2009).

Table 12. F-ratios, probability values and mean square error (estimated variance) from the analysis of variance (ANOVA) results for the factor scores data of the three principal components (PC) (overall N= 864).

Sources of variation	df	PC 1		PC 2		PC 3	
		F ratio	<i>p</i>	F ratio	<i>p</i>	F ratio	<i>p</i>
Panelist	11	129.93	<0.0001	141.16	<0.0001	26.65	<0.0001
Ethanol (Et)	5	4.34	0.001	4.43	0.001	55.02	<0.0001
Tannin (T)	2	2.71	0.067	1.06	0.346	0.45	0.635
Fructose (F)	1	1.53	0.216	1.23	0.267	1.04	0.405
Et x T	10	0.92	0.517	0.74	0.688	0.53	0.468
T x F	2	1.73	0.178	0.67	0.513	0.29	0.918
Et x F	5	1.26	0.280	1.29	0.268	0.50	0.604
Et x T x F	10	1.21	0.277	0.80	0.631	0.98	0.456
MSE		0.37		0.36		0.612	

p values ≤ 0.05 are significant

PC 1 represents floral, fruity, caramel aroma and flavor

PC 2 represents earthy, herbaceous aroma and flavor

PC 3 represents sulfur, spicy, woody aroma and flavor, bitterness

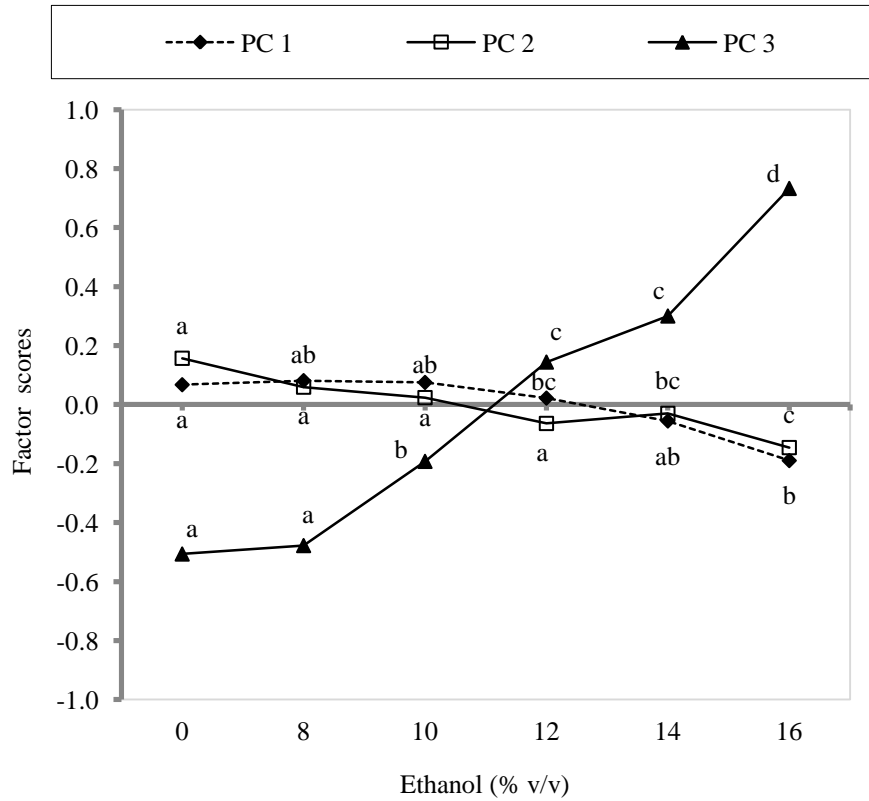


Figure 16. Impact of ethanol concentration on the sensory attributes of model red wines grouped as PC 1 (floral, fruity, caramel aroma and flavor), PC 2 (earthy, herbaceous aroma and flavor) and PC 3 (sulfur, spicy, woody aroma and flavor, bitterness).

On the other hand, ethanol concentration was observed to positively impact sulfur, spicy, woody aroma and flavor as well as perceived bitterness (PC 3). Lowest scores were obtained from model wines with 0 and 8% ethanol, which tended to increase with increasing ethanol concentration. At 16% ethanol, highest scores for these descriptors were found.

Above results demonstrated the changes in the perceived intensity of aroma and flavor of model wines due to increased ethanol concentration. At lower ethanol concentrations (8 and 10 %), the model wines were described as more fruity, floral, caramel, vegetal and earthy. However, at higher concentrations (14 and 16%), these were characterized with more sulfur, spicy and woody characteristics. These observations may be explained in part by the perceptual interactions between odorant compounds responsible for the aroma and flavor (Laing and Wilcox 1983), which were mediated by the ethanol effect. In addition, results showed that the perceived bitterness was enhanced with increased ethanol concentration. Several authors have indicated the same effect of ethanol on bitterness in wines (Fischer et al. 1994) and wine-like solutions (Fontoin et al. 2008, Vidal et al. 2004).

Conclusions

Changes in the sensory perception of model wines particularly the aroma and flavor, and bitterness were largely impacted by ethanol concentration, indicating the importance of controlling ethanol during the winemaking process to improve quality. The suppression and enhancement effect of increasing ethanol concentration on the perceived intensity of sensations varied depending on the attribute. Tannin and fructose concentrations had no significant sensory impact. Findings suggest further sensory investigations on real wines to validate these results.

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CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

The impact of selected wine components on the sensory and chemical properties of wine was studied. This information may be used to assist viticulturists and winemakers to assess their current practices and make adjustments as needed to improve wine quality. The significance of the study in the field of wine research and in the wine industry was described in Chapter I whereas an overview of the different wine components known to affect the chemical and sensory properties of wines and specific studies on wine matrix interactions reported in the literature were presented in Chapter II. Results of the three separate studies as well as the detailed discussions were provided in Chapters III, IV, and V.

In the first study, the strong correlation between tannin concentration and perceived astringency supported previous studies indicating that tannin content in the wine remains a good predictor of perceived astringency. The observed changes in SPP and LPP due to storage treatments were not large enough to influence the perception of astringency. Future work may focus on determining the optimum storage conditions needed to elicit changes in SPP and LPP and clarify their influence on perceived astringency.

Moving beyond studying only polymeric pigments, the subsequent studies examined the overall impact of wine matrix on wine chemical and sensory properties. Findings indicated the presence of higher order interactions between odorants and wine components using static headspace analysis. The combined effects of high ethanol, tannin and fructose were significant in reducing the headspace concentration of odorants potentially available for perception. Among the wine components studied, ethanol concentration had the largest impact on the odorants, mediating the effects of tannin and fructose. The evaluation of the impact of ethanol, tannin and

fructose on the odor unit values was successfully achieved through the combination of GC-MS and GC-O techniques, allowing a more reliable estimation of the potential strength of the odorant relative to the wine matrix. For all odorants, the odor detection thresholds increased as the ethanol, tannin and fructose concentrations increased, suggesting the lowering of the potential contribution of each individual odorant in aroma perception. Study on the exact nature of interactions to understand the mechanisms of interactions between wine constituents may be further explored.

Finally, the sensory work in the third study described the significant impact of ethanol on the sensory perception of model wines, particularly how ethanol disturbed the balance in the aroma and flavor, and bitterness attributes. In contrast to the analytical study, tannin and fructose concentrations and their interactions with ethanol had no significant sensory impact. It would be interesting to extend this research and confirm these results in more complex wines, by comparing the aromas of those, which have naturally low and high ethanol and tannin concentrations.

In addition, investigations of the effect of interactions of volatile matrix components in the wine other than ethanol should also provide more information on the perceptual changes occurring during wine consumption. Previous studies have indicated that odorant-odorant interactions can be additive or suppressive or with masking effects. Future studies may focus on addition or omission tests to determine changes in aroma by adding or removing target group of related wine aroma compounds (similar structure and odor) into/from the selected wine matrix, thus looking beyond the contribution of individual compounds. This is based on the current knowledge that wine aroma (e.g. fruity, floral note) is a cumulative effect of nuances elicited by several compounds (esters, monoterpenes).

Considering the significant impact of ethanol particularly on the wine aroma and flavor perception, future studies should include evaluating the effect of its interaction with other wine components. The sensory impact of the polysaccharides, another major macromolecule in wine, has been given less attention. These polysaccharides from either yeasts secretions during alcoholic fermentation or yeast autolysis during lees ageing may have many consequences in the wine, including interaction with wine aroma compounds. It would be interesting to determine the extent of the combined effects of ethanol and polysaccharides in modifying aroma and flavor perception.