EFFECT OF PRE-FERMENTATION COLD SOAK ON THE CHEMICAL AND SENSORY QUALITIES OF AROMATIC WHITE WINES

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EFFECT OF PRE-FERMENTATION COLD SOAK ON THE CHEMICAL AND SENSORY QUALITIES OF AROMATIC WHITE WINES

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The aroma profiles of Riesling, Gewürztraminer, and Traminette grapes are largely derived from monoterpenes found in grape pulp and skin. To optimize the extraction of monoterpenes and other volatile compounds, a winemaking technique called pre-fermentation cold soak (CS) is sometimes used. Unfortunately, CS may also allow for the extraction of undesirable bitter compounds, including monomeric phenolics. To address this concern, monomeric phenolic concentrations were measured in wines produced with varying CS durations. Among the compounds analyzed, (+)-catechin and (-)-epicatechin were most affected by CS duration. Because total monomeric phenolic concentrations tended to increase with increasing CS duration, wine bitterness intensity was quantified by a trained panel. Sensory evaluation results, however, suggest that CS duration and bitterness intensity are not directly related.

Following the CS study, detection thresholds of (+)-catechin and (-)-epicatechin in model and commercial wines were determined in order to learn more about the contributions of these compounds to wine sensory characteristics. (+)-Catechin detection thresholds in model and commercial wines were 205 and 278 ppm, respectively. In contrast, the detection thresholds of (-)-epicatechin were not found for the concentrations tested which went up to 501ppm. Threshold testing was selected as a starting point to understand how individuals perceive these compounds at sub- and suprathreshold levels.

CS and other processing conditions can lead to color compound extraction or must oxidation, resulting in darker colored wines and potentially increasing bitterness intensity. The presence of an association between darker white wine color and bitterness intensity was investigated by having a panel of wine professionals rate the bitterness of color-modified white wine samples. The results did not indicate that wine professionals associate darker white wine color with bitterness.

A final study investigated the possibility of differentiating Riesling clones through the analysis of juice monomeric phenolic and tannin profiles. The results did not show significant differences in the monomeric phenolic and tannin profiles among the clones analyzed. However, preliminary sensory results suggest that wine professionals can differentiate among monoclonal Riesling wines. If true, it may be possible to differentiate among Riesling clones through the analysis of other volatile and non-volatile compounds.

BIOGRAPHICAL SKETCH

Diane M. Schmitt grew up in Scottsville, New York where she attended St. Agnes Elementary School and Wheatland-Chili Junior and High School (Class of 2007). After high school, Diane spent her first year of college at Indiana University studying biology. She then transferred to Cornell University to major in Food Science. At Cornell Diane was involved in various organizations, product development teams, undergraduate research, and worked as an undergraduate student assistant to Kathy Chapman in the Cornell University Food Sensory Analysis Laboratory. She was fortunate to be able to complete undergraduate research with Dr. Joseph Hotchkiss and Kathy Arnink on projects that focused on grape juice and wine, respectively. Diane graduated from Cornell 2011 with a Bachelor's with Distinction in Research for the successful completion of her senior honors thesis work on the inhibitory effects of select monomeric phenolic compounds on wine spoilage yeasts. In August 2011, Diane started graduate school at Cornell in the Food Science department under the direction of Dr. Anna Katharine Mansfield. This body of work represents the culmination of four and a half years of research investigating white wine processing techniques and human bitterness perception.

I dedicate this work to my family.

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CHAPTER 1

INTRODUCTION

"Wine is one of the most civilized things in the world and one of the most natural things of the world that has been brought to the greatest perfection, and it offers a greater range for enjoyment and appreciation than, possibly, any other purely sensory thing."
–Ernest Hemingway

Wine is a dynamic product that can be produced from a large number of grape cultivars using a variety of winemaking processes. The wine tasting experience is shaped by intrinsic product components such as taste, smell, flavor, tactile sensations, and color, and also by extrinsic product components such as bottle and label design, product information, environmental factors, and consumer expectations. The experience of consuming wine is made up of much more than just its chemical components, as wine is not consumed in sensory isolation. Everything that an individual experiences prior to and while tasting a wine can have an effect on their perception of the alcoholic beverage and the overall experience. In this regard, the understanding of how wine production methods affect sensory qualities, and how these qualities are perceived by consumers in a variety of situations, is paramount to winemakers and other wine industry stakeholders.

Part I. Winemaking practices and the chemical composition of grapes, juice, and wine.

In the production of aromatic white wines, a number of optional pre- and post-fermentation practices may be performed to obtain products that exhibit particular desirable characteristics. One pre-fermentation method of particular interest is cold soak (CS), also referred to as maceration or skin contact.

Pre-fermentation cold soak. Various white wine grape cultivars are sometimes cold soaked after crushing and prior to pressing in order to extract aromatic compounds from grape skin and pulp

(Sánchez Palomo et al. 2007; Cabaroglu and Canbas 2002). During traditional white wine making grapes are destemmed, crushed and then immediately pressed to separate the juice from the pulp and skins. In comparison, during CS grape must is held, commonly at temperatures below ambient (20-21°C), in between crushing and pressing in order to allow for additional extraction of flavor compounds from grape skins and pulp into the juice.

CS can be an especially important processing technique to use when attempting to enhance the varietal character of wines (Moreno-Arribas and Polo 2009), since only partial extraction of volatile compounds, which are primary contributors to varietal character, occurs during traditional white wine making processes. Varietal character is the collection of sensory characteristics which distinguish wines made from one grape cultivar from wines produced from other cultivars. Odorants and secondary metabolites present in grape must as well as those produced and modified during fermentation contribute to the distinctive varietal character of a wine (Moreno-Arribas and Polo 2009). For example, monoterpenes, especially terpineol, linalool, and geraniol, have been found to be directly related to varietal aroma of monovarietal wines made from various white and red grapes. (Piñeiro et al. 2006).

Cultivars that may benefit from CS include those with prominent aromatic profiles such as Riesling, Gewürztraminer, Traminette, Valvin Muscat and other Muscat cultivars. Specific CS methods vary greatly, but the process generally involves holding grape must, from ripe grapes that are free of disease, at reduced temperatures (<15°C) for a set period of time prior to pressing (Wilker 2010). Processing aids used during CS may include carbon dioxide to prevent oxidation and enzymes to break down cell walls. The additive sulfur dioxide is also used to prevent microbial growth. CS may last from several hours to several days depending on the cultivar being processed, the desired sensory qualities of the finished wines, and the preferred practices of the winemaker.

In some cases CS has been shown to increase the extraction of volatile compounds, leading to alteration of organoleptic qualities and improved sensory scores in finished wines (Sánchez Palomo et al. 2007). It has also been suggested that skin contact improves fermentation rates due to the increased extraction of amino acids in the juice (Wilker 2010).

While there are many potential benefits of the CS of white wines, there is uncertainty as to the effect of CS on the evolution of undesirable gustatory qualities, such as bitterness and astringency. Bitter and astringent compounds in white grapes include phenolic compounds, including the flavan-3-ols and condensed tannins, which can both be extracted during CS or fermentation on the grape skins. Notable flavan-3-ols found in white grape cultivars include (+)-catechin and (-)-epicatechin, while the condensed tannin group includes compounds such as grape seed tannin and tannic acid. Interestingly, the concentrations of the phenolic compounds (+)-catechin, gallic acid, grape seed tannin, and tannic acid, have been shown to have linear relationships with reported bitter and astringent intensities and aftertaste duration (Robichaud and Noble 1990).

Grape cultivar clones. Grape cultivar clones are a genetically similar group of individuals derived originally from a single individual by asexual propagation (cuttings, grafting, etc.) (Hartman et al. 1990). However, clones do have slight genetic variations which can be distinguished through genetic testing (Regner et al. 2000) and may result in unique chemical and/or sensory characteristics among clones. For these reasons clonal selection is a topic of interest in certain grape cultivars. Furthermore, several clones of the same cultivar are often grown together and/or mixed during processing in an effort to contribute sensory complexity to wine.

Although phenotypic differences are thought to be present among clones, limited work has been performed to differentiate clones through the analysis and profiling of compounds that elicit

sensory responses, such as phenolic compounds (Burin et al. 2011). While many studies have focused on identifying differences across grape cultivars for compounds such as monomeric phenols (Ali et al. 2011; Castillo-Muñoz et al. 2010; de Villiers et al. 2005; Goldberg et al. 2000; Masa et al. 2007; Nelson 2011; Pour Nikfardjam et al., 2007; Soleas et al. 1997), anthocyanins, tannins (Vázquez et al. 2011) and amino acids (Ali et al. 2011; Asensio et al. 2002), intra-cultivar chemical differences have largely been unexplored. Similarly, little is known about the influence of clonal variation on the sensory qualities of juice and wine produced from most grape cultivars. Grape cultivar clones that are known to have varying sensory qualities include Pinot blanc and Pinot gris as well as Chardonnay Musque and Chardonnay. Several clones of Riesling are grown in the New York Finger Lakes region, but it has not been determined whether monoclonal Riesling wines are sensorially different.

Vineyard site and terroir. There is much speculation on the effects that site, and the more encompassing concept of terroir, have on the sensory qualities of wines. In addition to growing site, terroir takes into account regional variations in factors such as soil composition, weather, and climate when accounting for differences in wines made from the same cultivar in different geographic areas. Interestingly, most wine professionals have expanded the definition of terroir to include regional viticulture and enology practices which may result in modifications to soil content, vine nutrition, and irrigation procedures. Various studies have examined the influence of terroir on chemical and sensorial variations within and across grape cultivars grown in different wine producing regions, or within a region but separated by distance and/or site-specific vineyard conditions (Douglas et al. 2001; Fisher et al. 1999; Peña-Neira et al. 2000; Vázquez et al. 2011). Overall, terroir is thought to have a larger impact on variations in juice and wine characteristics within a single cultivar than intrinsic grape genetic factors alone.

The effect of terroir on cultivar clones is poorly understood, and it is difficult to determine the extent to which fruit and wine chemistries and sensory qualities are influenced by terroir versus innate grape clone chemical differences. Given the potential importance of terroir on the development of regional characteristics, such as a strong petrol note in Riesling wines produced in warm climate regions (Marais et al. 1992), a comprehensive analysis of terroir is warranted.

Chemical Components of Wine

An individual's perception of wine is influenced by many factors; historically, the most studied are chemical components that elicit gustatory, olfactory, and tactile responses. Wine components which are thought to impart unique sensory characteristics to wines (Pisarnitskii 2001), include the non-volatile phenolic compounds and organic acids, as well as a number of volatile compounds. Wine volatile compounds are primarily responsible for the development of distinctive varietal characteristics. Volatile compound groups include hydrocarbons, terpenes, alcohols, aldehydes, ketones, acids, esters, acetals, amines, select phenols, and sulfur-containing compounds (Bakker and Clarke 2012).

In aromatic white wines, the major compounds of interest are non-volatile phenolic compounds and the volatile monoterpenes, both of which are thought to be extracted during CS. Specifically, increases in phenolic compounds during CS are thought to increase perceived intensity of bitterness and astringency (Darias-Martín et al. 2000; Gawel et al. 2013; Gómez-Míguez et al. 2007; Gordillo et al. 2010; Singleton et al. 1975; Sokolowsky et al. 2015). Increases in monoterpene concentration are also reported to occur during CS (Overbosch et al. 1986) and can lead to the development of more intense fruity/floral aroma profiles in finished wines (Sánchez Palomo et al. 2007; Cabaroglu and Canbas 2002). Specifically, monoterpenes including linalool, geraniol, nerol, and *cis*-rose oxide are found in high quantities in Riesling, Gewürztraminer,

Traminette (Skinkis et al. 2008) and/or Muscat (Wilson et al. 1986) grapes, where they can contribute to varietal character. An in-depth review on the contribution of terpenes to grape and wine aroma can be found elsewhere (Marais 1983).

Phenolic compounds. Bitter phenolic compounds found in wine grapes include the flavan-3-ols (+)-catechin and (-)-epicatechin, along with the proanthocyanidans and polymeric phenols (Oberholster et al. 2009; Arnold et al. 1980; Kallithraka et al. 1997; Thorngate and Noble 1995). Flavan-3-ols can be found in grape skin hypodermal cells and in the soft parenchyma of the outer seed coat (Adams 2006). In finished wines the total concentration of flavan-3-ols is reported to range from 10-50ppm (Singleton and Esau 1969) with 25ppm as the average concentration present in typical young white wines (Singleton 1988).

Extraction of the flavan-3-ols, (+)-catechin and (-)-epicatechin, has been reported during CS, with longer CS durations showing a positive correlation to flavan-3-ol concentrations (Gómez-Míguez et al. 2007). (+)-Catechin is soluble in aqueous solutions, and its solubility increases with increases in temperature (Cuevas-Valenzuela et al. 2014; Srinivas et al. 2010) and ethanol concentration (Cuevas-Valenzuela et al. 2014). Similarly, the aqueous solubility of the monomeric phenolic compounds gallic acid and protocatechuic acid, which can also be found in substantial quantities in white wine, have been shown to increase exponentially with increases in temperature (Srinivas et al. 2010).

At the same concentration, (-)-epicatechin has been shown to have higher bitterness (Kallithraka et al. 1997; Thorngate and Nobel 1995) and astringency (Ferrer-Gallego et al. 2014) intensities than (+)-catechin. It has been proposed that the configuration of the molecules, specifically the stereochemistry of the C3 hydroxyl group (Figure 1.1) and difference in the bonding site between the two units (C4→C6 versus C4→C8), modifies the way the two flavan-3-

ols bind to taste receptors (Peleg et al. 1999) and thus changes how the compounds are perceived. However, this effect is not universal. Thorngate and Noble (1995) reported that (-)-epicatechin was perceived to be more bitter than (+)-catechin at a concentration of 900ppm, but equally bitter at 500 and 1,200ppm.

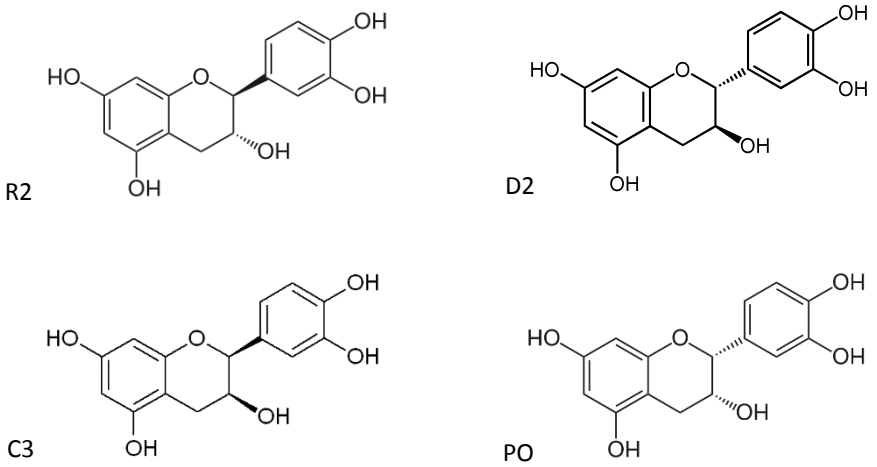


Figure 1.1. Structures of the stereoisomers catechin and epicatechin: (+)-catechin (R2), (-)-catechin (D2), (+)-epicatechin (C3), (-)-epicatechin (PO).

Detection thresholds of (+)-catechin and (-)-epicatechin have been determined in water and model wine (Delcour et al. 1984; Hufnagel and Hoffman 2008), but not in commercial wines. Such simple sample matrices do not take into account the influences other wine components have on stimuli perception (Canals et al. 2005; Fontoin et al. 2008, Gawel et al. 2007; Gawel et al. 2013; Meillon et al. 2010; King et al. 2013). Use of simple sample matrices likely result in detection thresholds that differ from the detection threshold of the same compound in an actual wine. By using a wine matrix, compound detection thresholds can be determined in the presence of other

compounds that may interfere with sensory perception resulting in data that is more relatable to actual wines.

Detection thresholds for specific wine components such as (+)-catechin and (-)-epicatechin may be modified by ethanol concentration (Canals et al. 2005; Fontoin et al. 2008, Gawel et al. 2007; Gawel et al. 2013; Meillon et al. 2010; King et al. 2013), total acidity, pH (Gawel et al. 2013), sugar content, and other components present in wine. Furthermore, wine component interactions, including those among phenolic compounds, can lead to synergistic effects that cannot be explained by solely assessing the sensory properties of individual compounds (Hufnagel and Hoffman 2008; Ferrer-Gallego et al. 2014). For example, the addition of a white wine-derived fruity aroma extract has been shown to decrease the perceived bitterness and astringency and increase the perceived sweetness of red and white wines (Sáenz-Navajas et al., 2010).

Condensed tannins, which are formed from multiple flavan-3-ol subunits, elicit astringent sensations (Peleg et al. 1999; Robichaud and Noble 1990) and are likely the major contributors to astringency in wines. Monomeric flavan-3-ols and procyanidin dimers and trimers have also been found to contribute to astringency in several studies (Hufnagel and Hofmann 2008; Kallithraka et al., 1997; Peleg et al. 1999; Robichaud and Noble 1990; Thorngate and Noble 1995). The concentration at which humans perceive flavan-3-ols as bitter is reported to be in the range of 58 to 261ppm (Hufnagel and Hofmann 2008). However, the total flavan-3-ol concentrations usually present in white wines (10-50ppm) (Singleton and Esau 1969) are substantially lower than the concentrations at which these compounds are perceived to be bitter. Hydroxybenzoic and hydroxycinnamic acids have also been reported to contribute to astringent puckering and velvety sensations (Hufnagel and Hofmann 2008). In the absence of proanthocyanins, coutaric acid and

quercetin-3-O-rutinoside have been shown to become significant contributors to wine astringency (Gonzalo-Diago et al. 2014).

The sensory impact of condensed tannins is dictated by molecular weight, with higher numbers of flavan-3-ol subunits correlating to increased astringency and decreased bitterness intensities (Peleg et al. 1999; Robichaud and Noble 1990). Compounds with a wide range of molecular weights can be perceived to be astringent. Bate-Smith et al. (1962) reported that a molecular weight between 500 and 3000 is required for a phenolic compound to elicit astringent sensations, while Lea et al. (1990) stated that condensed tannins with molecular weights greater than ~2,100 have limited solubility in wine and thus cannot interact with proteins in the oral cavity to elicit an astringent sensation. However, more recent findings demonstrate that condensed tannins with a mean molecular weight of ~21,000 are perceived to be astringent (Vidal et al. 2003) and suggest that astringency intensity increases with molecular weight without a compound size limit (Cheynier et al. 2006).

It has been proposed that flavan-3-ols elicit astringency through direct interactions with trigeminal receptors, as compared to high molecular weight tannins, which interact with proteins to form unprecipitated complexes (Schöbel et al.. 2014). Specifically, simple phenols, including the flavan-3-ols, (+)-catechin and (-)-epicatechin, can elicit astringent sensations through the cross-linking of 1,2 dihydroxy or 1,2,3 trihydroxy groups with proteins (McManus et al. 1981). Furthermore, condensed tannin molecular size is reported to have a more influential effect on bitterness and astringency perception than flavan-3-ol subunit stereochemistry variations (Peleg et al. 1999).

Monoterpenes. Monoterpenes contribute to the varietal aroma profiles of several wines, including Riesling, Gewürztraminer, and Traminette. The major monoterpenes in these three grape cultivars are linalool, geraniol, nerol, and (+)-*cis*-rose oxide (Figure 1.2).

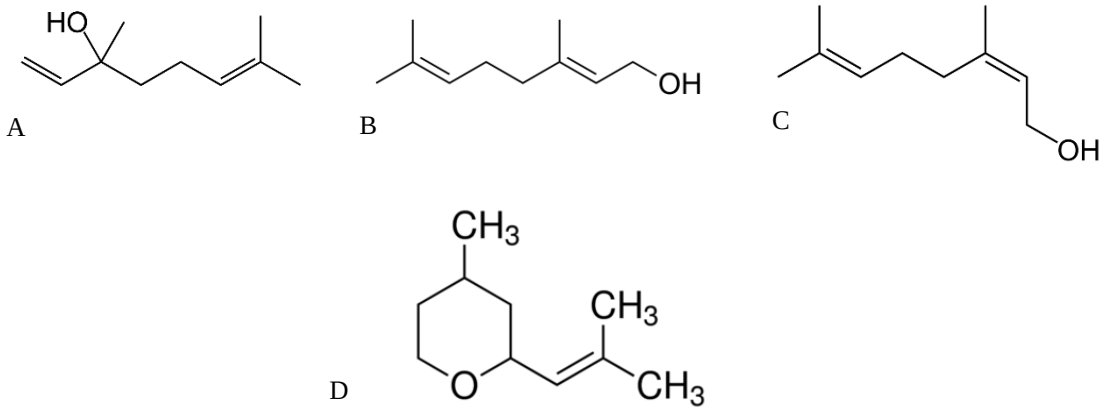


Figure 1.2. Structures of the monoterpenes linalool (A), geraniol (B), nerol (C), and (+)-*cis* rose oxide (D).

Monoterpenes are present in free and glycosidically-bound forms and are distributed throughout grape skin, juice, and mesocarp (Wilson et al. 1986). At the time of harvest, Park et al. (1991) reported that 4.6% and 5.9% of the major monoterpenes of Muscat of Alexandria grapes were present as free terpenes in the skin and mesocarp, respectively. In comparison, 31% and 59% of total terpenes were glycosidically bound in the skins and mesocarp, respectively (Park et al. 1991). Techniques such as enzymatic hydrolysis may be effective in cleaving the glycosidic bonds and as a result potentially increase the quantity of volatile compounds in juice and finished wines. (For a review of this topic refer to Maicas and Mateo 2005).

Final Thoughts

Based on the current scientific literature, the need for CS to enhance volatile extraction during wine production is uncertain. It is understood that the effect of this processing technique on

volatile and gustatory compound content is dependent on grape cultivar (Moreno-Pérez et al. 2013). Different cultivars may contain varying compositions and concentrations of volatile and gustatory compounds within grape pulp, skins, and seeds. As such the CS conditions used during the production of wine from one grape cultivar may extract a more diverse set of compounds and/or higher concentrations of compounds than from a different grape cultivar that has less compound diversity and/or lower compound concentrations to begin with. For this reason, CS may be more appropriate to use for some cultivars over others. In addition, some researchers theorize that the need for CS may be less when grapes are machine harvested. Grapes invariably sustain some cell wall damage during machine harvesting, effectively subjecting them to skin contact or "cold" soaking in transit prior to entering the winery (Boulton et al. 1996). It is possible that the volatile components of these faux cold soak grapes may be fully extracted prior to processing, rendering CS unnecessary. Much research needs to be done to determine volatile and gustatory compound extraction kinetics during CS and to determine the effects of CS on a wide range of grape cultivars.

In order to develop optimized CS practices for individual and groups of grape cultivars, a substantial number of cultivars will need to be studied within and across regions over multiple years to construct robust, cultivar-specific pre-fermentation practice recommendations. The multi-year aspect of the study is especially important as grape properties can be substantially modified by yearly variations caused by environmental, geographical, or agronomical conditions. The resulting CS recommendations will greatly benefit winemakers who may use the information during harvest to assess the benefits and disadvantages of using CS based on cultivar, year-to-date weather conditions, and terroir.

Part II. Sensory Perception: The wine tasting experience

Many people view wine not just as a consumable, but also as an experience. If this is true, then what humans perceive as wine is not just the summation of the physical properties of the beverage. While the perception of wine is formed partially through gustatory and olfactory signals sent to the brain, it is greatly impacted by the intrinsic and extrinsic factors that an individual is exposed to. Like many other foodstuffs, the perception of wine is dynamic in nature, and as such is influenced by a variety of factors.

Gustatory, olfactory, and flavor perception are based on sensory stimuli interacting with taste and olfactory receptors, which then transmit signals through afferent neurons to the brain. Taste receptor cells are located within tastebuds, which in turn are housed in three types of papillae on the tongue: circumvallate, foliate, and fungiform (Goldstein 2013). In addition to taste receptors, pain and touch receptors can also be present in taste buds, and respond to stimuli such as heat from ethanol and food texture, respectively. Olfactory receptors are located in the olfactory epithelium and are attached to olfactory cilia that extend into the mucosal membrane and bind with volatile compounds (Goldstein 2013).

Olfactory perception can be formed through two mechanisms, orthonasal and retronasal perception. Orthonasal perception is formed when olfactory stimuli interact directly with olfactory cilia in the mucosal membrane. In comparison, retronasal perception starts with the introduction of olfactory stimuli into the oral cavity. From there the stimuli is transferred to the olfactory epithelium, where the volatile compounds interact with olfactory receptors and the resulting nerve signals are sent up to higher brain regions for further processing and formation of a smell and/or flavor percept (Goldstein 2013).

An individual's perception of a sensory stimulus can be influenced by both bottom-up and top-down processing and from the integration of sensory information from multiple sensory systems. The use of sensory receptor signals transmitted to the brain for perception formation is known as bottom-up processing. In comparison, when an individual is presented with cognitive information about a wine, such as brand or country of origin, she may form an expectation (D'Alessandro and Pecotich 2013) of what the wine will taste and smell like or whether or not she will like it. These expectations can cause modifications to perceptual constructs that have been formed based on gustatory and olfactory stimuli during what is known as top-down processing (Goldstein 2013). Finally, an individual's perception of a wine can be influenced by crossmodal interactions between sensory systems including the visual and olfactory systems and systems involved in somatosensory sensations. Of these, visual-taste and visual-smell interactions are likely the most influential in the formation of wine perception (Goldstein 2013). Bottom-up and top-down processing, as well as crossmodal effects, all contribute to an individual's perception of a wine and to the overall wine tasting experience.

Sensory Perception: Taste and Olfaction

Olfaction. Olfaction is the ability to detect and discriminate between low molecular weight compounds known as odorants; humans are thought to be able to discriminate at least one trillion olfactory stimuli (Bushdid et al. 2014). The genetic code for olfactory receptors varies greatly; in a study of 189 test subjects, almost every individual had a unique combination of intact and pseudogene olfactory receptor (OR) alleles (Menashe et al., 2003). Olfactory ability can also be modified through several disorders, generally caused by non-genetic factors such as head trauma or genetic-environmental factors such as allergies (McNeill et al., 2007; Nordin and Bramerson, 2008), and in rare cases by inherited genetic factors alone. Such disorders, which may be temporary

or permanent, include anosmia, the inability to smell; hyposmia, the decreased ability to smell; and hyperosmia, the heightening of olfactory abilities (Goldstein 2013). The temporary occurrence of hyperosmia has been anecdotally reported to occur during pregnancy although support for this phenomenon is currently lacking in the literature (Cameron 2014). In addition, specific anosmia, the inability to smell a specific odorant, and specific hyperosmia, the increase in sensitivity to a specific odorant, can occur (Goldstein 2013).

The knowledge of one's olfactory abilities, particularly the occurrence of a specific anosmia or hyperosmia, is highly desirable for individuals working in the food and beverage industry. In the case of wine evaluation, wine professionals should be aware of any specific anosmia or other olfactory disorders that they possess in order to prevent themselves from making inaccurate judgments, especially during wine competitions. The ability to detect and discriminate between odorants can be improved through repeated and/or prolonged exposure, which, in turn, leads to learning-induced changes in odor response profiles in the piriform cortex and the orbitofrontal cortex (Li et al. 2006). As a result, wine professionals with olfactory disorders should be aware of their limitations, and learn to work around them.

Gustation. Gustation, or taste, is the ability to perceive bitter, sweet, sour, salty, and umami compounds in food and other matter put into contact with taste receptors located in the oral cavity. The function of taste has three dimensions: sensory/discrimination, affective response, and physiologic reflex, such as salivation (Mennella et al. 2013). The current work focuses on the perception of the gustatory component bitterness due to the extraction of various bitter compounds during CS (See Part I, above).

Bitter taste receptors. Bitter compounds in the oral cavity interact with G-protein coupled taste receptors (T2Rs) as the first step of the process of bitter perception, which is outlined in Figure

1.3. Briefly, the T2Rs are a family of over 40 different receptors (Adler et al. 2000; Bachmanov and Beauchamp 2007; Matsunami et al. 2000) located in taste buds that interact with a large and diverse group of compounds. Tastants may selectively bind with one or with many T2Rs, initiating a number of signaling events at the molecular level within taste receptor cells that results in neurotransmitter release from the taste receptor cell and subsequent activation of an afferent nerve fiber. This fiber transmits the signal through the facial, glossopharyngeal, and vagal cranial nerves to the brain, where taste signal information is transmitted to circuits that subserve various functions, such as oromotor and physiological reflexes, discriminative perception, and affective processing (Mennella 2012). While much is still unknown about how T2Rs and compounds interact, studies looking at differences in the bitterness intensities of flavan-3-ol stereoisomers, (+)-catechin and (-)-epicatechin, give some limited insight on the effects of structural changes on the perceived bitterness of a compound. The bitterness intensities of (+)-catechin and (-)-epicatechin differ with the latter having a significantly higher and longer persistence of bitterness than the former (Robichaud and Noble 1990, Noble 1994, Thorngate and Noble 1995, Kallithraka et al. 1997a, Peleg et al. 1999). This difference may be due to the more planar conformation of the (-)-epicatechin C ring, which results in increased lipophilicity and aids attachment to the T2R receptor (Haslam 1982).

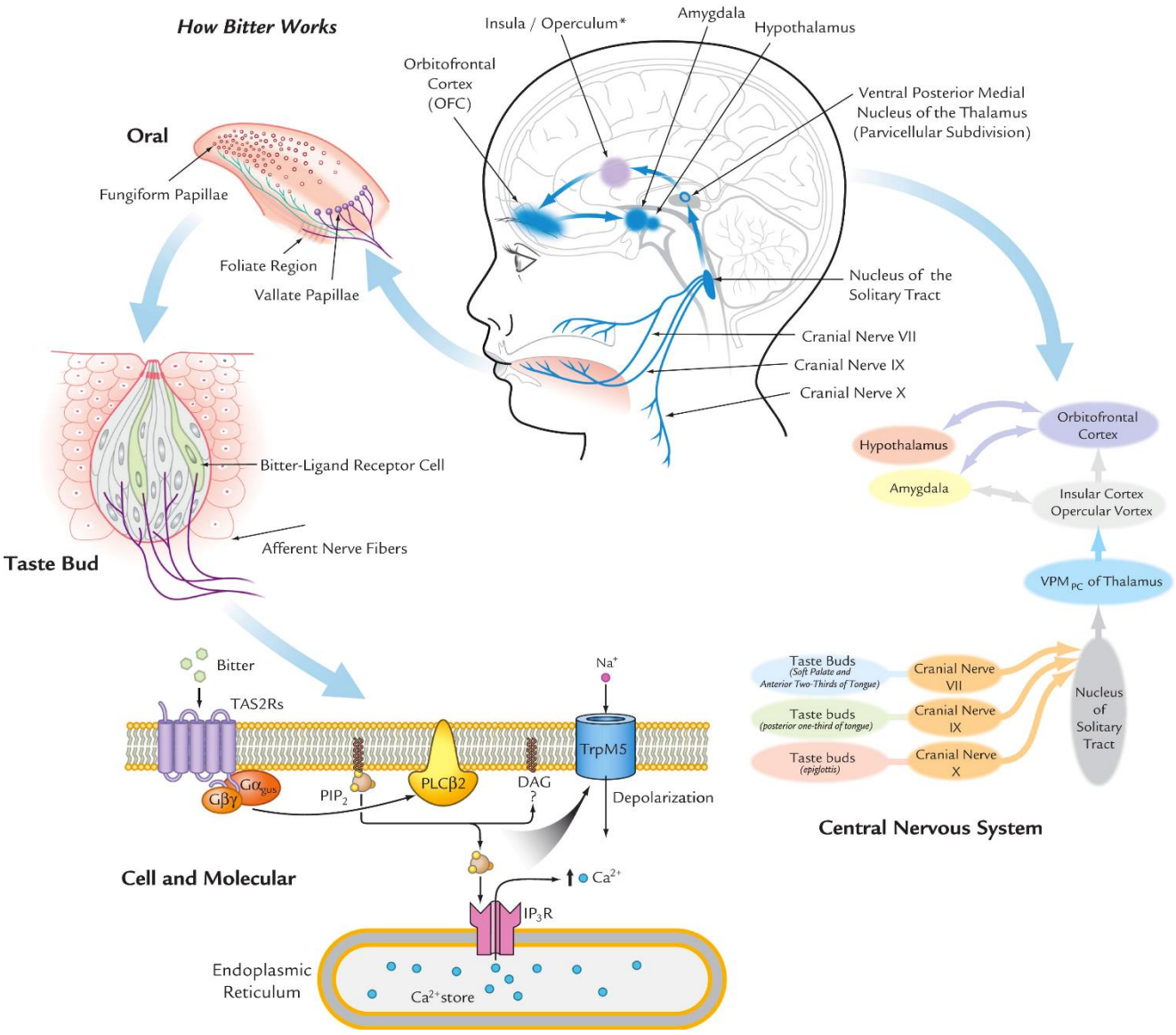


Figure 1.3. How bitter works: the process of bitter perception.

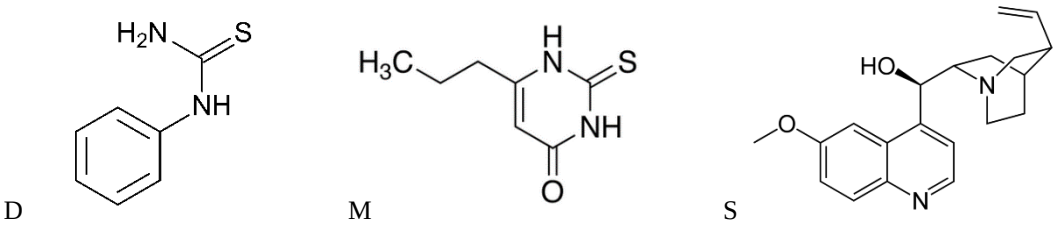
Oral: A bitter compound enters the oral cavity through ingestion and binds to a taste G protein-coupled receptor (T2R) found in taste buds on the tongue. (T2Rs also exist in pharyngeal and epithelia areas.)

Cell and Molecular: Binding of the bitter compound to the T2R starts a cascade of signaling events, leading to the release of neurotransmitter.

Central Nervous System: Released neurotransmitter activates an afferent nerve fiber that transmits the signal via the facial, glossopharyngeal, and vagal cranial nerves to the brain. Once in the brain, the taste signal information is transmitted to multiple structures and used for various functions including discriminative perception and affective processing among others.

(Reprinted with permission from Elsevier. Mennella JA, Spector AC, Reed DR and Coldwell SE. © 2012. The bad taste of medicines: overview of basic research on bitter taste. Clinical Therapeutics 35(8):1225-1246.

Bitter taste sensitivity. Bitterness is often a difficult taste to study, as sensitivity to some bitter compounds substantially varies among the population due to genetic factors. Bitter compounds that are known to elicit these variable responses include phenylthiocarbamide (PTC), 6-*n*-propylthiouracil (PROP), and quinine (Figure 1.4). Of these compounds quinine is commonly used during sensory tests as a bitter standard. In a genome-wide association study of 1457 twins and their siblings, 49.5% of PROP trait variation was contributed by the T2R38 gene. T2R38 allelic variations include PAV (proline-alanine-valine), which corresponds to PROP tasting (medium and high/supertaster), and AVI (alanine-valine-isoleucine), which corresponds to PROP non-tasting; other variants are rare. Consequently, among the general population the most common genotypes expressed are PAV/PAV, PAV/AVI, and AVI/AVI (Kim et al. 2003). Furthermore, the density of fungiform papillae, a taste-bud containing structure on the anterior part of the tongue, was formerly thought to be related to PROP sensitivity, but recent studies have failed to find a correlation between the two (Gameau et al. 2014). Interestingly, there is an overrepresentation of PROP medium tasters and supertasters among wine experts. It is thought that these individuals self-selected professions in which they have a genetic advantage (Hayes and Pickering 2012).



D M S

Figure 1.4. Structures of phenylthiocarbamide (PTC) (D), 6-n-propylthiouracil (PROP) (M), and quinine (S).

Variation in quinine tasting ability can be partially contributed to the T2R19 (formerly known as T2R48) gene in conjunction with adjacent salivary protein genes (Reed et al. 2010). It is proposed that, in lieu of a single genetic variant that controls for quinine trait variation (Fischer

and Griffin 1963; Reed et al. 2010; Smith and Davies 1973), there are likely several alleles of bitter receptors or salivary proline-rich protein genes on chromosome 12 that together determine this variation (Reed et al. 2010). Variations in taste sensitivity to caffeine and sucrose octaacetate are also thought to exist (Hansen et al. 2006), but no single-nucleotide polymorphisms responsible for these trait variations have been found (Reed et al. 2010).

The clear contribution of the T2R38 gene to variations in PROP sensitivity make this compound useful for population studies. PROP, however, is part of a large and structurally diverse family of bitterants, and as such information obtained from its study cannot be universally extrapolated. Current mainstream usage of the term 'supertaster' implies such extrapolation, and further incorrectly implies that a PROP taster is more sensitive to all gustatory sensations. This was not demonstrated, however, in a study of 159 women where genetic sensitivity to PROP was not predictive of sweetness intensity ratings (Drewnowski et al. 1997). Others have found that PROP taster status is not predictive of the bitterness intensity scores of compounds such as the flavan-3-ols (+)-catechin and (-)-epicatechin (Thorngate and Noble 1995). Much research on bitter compounds and their interactions with the T2Rs is required before generalizations about bitter taste mechanisms can be made.

Sensory Perception: everything is in our head

"Alice laughed: "There's no use trying," she said; "one can't believe impossible things."
"I daresay you haven't had much practice," said the Queen. "When I was younger, I always did it for half an hour a day. Why, sometimes I've believed as many as six impossible things before breakfast."
–Lewis Carroll, Alice's Adventures in in Wonderland

"Reality is merely an illusion, albeit a very persistent one."
-Albert Einstein

Sensory perceptions of foods are not just the sum of their parts. An individual rarely consumes foods and beverages in a setting devoid of all sensory stimuli except those emulating from the food being consumed. Thus an individual is subject to a multitude of sensory stimuli from each of the five basic sensory systems, taste, smell, audition, vision, and touch. Sensory stimuli are processed in the brain and the stimuli information of one sensory system may be integrated and/or influence the perception of stimuli in another system. Taste and smell perception is especially susceptible to these cross sensory systems or crossmodal influences.

Sources of sensory stimuli that can influence perception include extrinsic product cues, such as color, as well as intrinsic and extrinsic conditions of an individual, such as health status and the environment/setting that an individual is in. Although some of these factors, such as product cues and environment, are external to an individual, the processing of extrinsic sensory stimuli signals occurs within the brain where sensory stimuli information from multiple sensory systems, including olfaction, taste, and vision, is further processed and integrated. Crossmodal interactions facilitate the modulatory effects of intrinsic or extrinsic factors on taste and smell perception and as an extension flavor percepts. Thus taste, smell, and flavor perception formation is developed through crossmodal interactions of the senses along with the influence of top-down processing factors such as an individual's prior experience and expectations.

Crossmodal interactions. Crossmodal interactions of the senses have been well documented in the literature (Deadwyler et al. 1987; Gottfried et al. 2003; Jadauji et al. 2012; Small 2002). Taste and olfactory stimuli signals interact with each and both, especially olfaction, are known to be highly influenced by the visual, auditory, and tactile sensations. Of these, vision most likely has the largest influence on taste and smell.

Perhaps unsurprisingly, the olfactory, visual, and auditory systems converge in the brain. There is some evidence that the hippocampus, a structure accessible either directly or indirectly by all sensory modalities (Deadwyler et al. 1987; Small 2002), mediates reactivation of crossmodal semantic sensations, such as the pairing of an image with an odor, independent of explicit memory processing. Gottfried et al. (2003) reported that vision-olfactory system interaction elicited the strongest enhancements in olfactory perception when images and odors are presented in logical combinations, such as red and cherry compared to blue and cherry, making them semantically congruent. This enhancement was associated with increased activity in the anterior hippocampus and the rostromedial orbitofrontal cortex (Gottfried et al. 2003). Furthermore, in a study by Demattè et al. (2011) an individual's awareness of odorants in the environment as measured by the Odor Awareness Scale (Smeets et al. 2008) was found to not be predictive of her ability to correctly identify odors in an odor identification task. However, when color cues were present aroma identification accuracy increased, at least when the color was congruent with the aroma (Demattè et al. 2011). In addition, studies inducing transcranial magnetic stimulation of the visual cortex improved individuals' ability to identify odors during an odor discrimination task, indicating a causal contribution of visual processing to olfactory perception (Jadauji et al. 2012).

The synaptic separation between the odor receptor neurons in the nasal mucosa, projecting via the olfactory bulb and the entorhinal cortex to the hippocampus, has been shown to be as few as three synapses (Schwerdtfeger et al. 1990). In macaque monkeys, the orbitalfrontal cortex (OFC) receives afferent input from the olfactory (piriform) cortex and visual processing areas (Carmichael and Price 1995), and individual olfactoresponsive neurons within OFC have been found in primates that respond to olfactory and visual stimulation separately or together (Rolls and Baylis 1994). The OFC is one of the most activated structures in olfactory neuroimaging

experiments (Zatorre et al. 1992; Zald and Pardo 1997; Sobel et al. 2000) and has been shown to have a role in olfactory-visual associative learning (Gottfried et al. 2002). Crossmodal interactions, especially those between the taste, smell, and visual systems, are critical factors in the formation of sensory percepts such as flavor.

Flavor. Flavor is a multi-modal sensory perception that is a combination of gustatory, oral-somatosensory, and retronasal olfactory signals originating in the oral cavity (Small 2012). In addition, flavor perception is also influenced by ancillary factors such as the visual (DuBose et al. 1980; Garber et al. 2015; Levitan et al. 2008; Oram et al. 1995) and auditory (Spence 2012; Spence 2015) systems. Sensory signals from each of these systems are integrated in the anterior ventral insula to form a core flavor percept (Small 2012). This percept is then conveyed to both upstream and downstream brain regions for further unimodal signal modifications, which may include top-down processing influences (Grabenhorst et al. 2008), that result in conscious perception of a flavor (Small 2012).

Part III: Sensory Analysis Methods

"A person with increasing knowledge and sensory education may derive infinite enjoyment from wine."
- Ernest Hemingway

Food sensory analysis, the assessment of sensory attributes by panelists, is ultimately required to confirm sensory variations in wines. Sensory data can be obtained from a range of methods, including difference testing of wines, intensity rating of a stimulus such as bitterness, and detection threshold determination. Of these, intensity rating of specific sensory stimuli can be performed using a number of analysis techniques, although variants of the magnitude estimation method are mostly commonly used.

Labeled Magnitude Scale

An optimal magnitude estimation scale is one in which all sensory stimuli can be measured and compared, and which allows for the collection of data that can be reliably compared across the population. This idealized scale would allow for the comparison of PROP intensity to the brightness of a light or the intensity of a pain. Of course, sensory tests are riddled with potential sources of variation that make the realization of this optimal crossmodal scale difficult. Within taste, smell, and related sensory research there are multiple methods than can be used to determine the intensity of a sensory attribute, one of which is the labeled magnitude scale (LMS).

The LMS is a modified log-based line scale, with anchor words commonly used to describe sensory stimuli intensities (barely detectable, weak, moderate, strong, very strong, and strongest imaginable) placed on the scale at intervals corresponding to how humans perceive differences in stimuli intensities (Green et al. 1993). For example, the stimuli intensities 'weak' and 'moderate' are thought to be closer together than the intensities 'very strong' and 'strongest imaginable,' although in both cases the anchor words are located next to each other on the line scale. When used correctly the LMS is reported to give better results than category rating and magnitude estimation. In addition, the LMS yields ratio-level data that can be compared across the population and is not subject to ceiling effects (Green et al. 1993; Lim et al. 2009). A variation to the LMS, the hedonic general LMS (gLMS), has been found suitable for comparisons across groups, and provides more accurate data than traditional 9-point hedonic scales (Kalva et al. 2014). These and other magnitude estimation scales can all be used for inter-panelist comparisons of responses to different samples, and/or to evaluate panelist reproducibility.

The efficacy of the LMS has been shown in a number of studies comparing the scale to other methods. Galindo et al. (2009) reported that intensity ratings, such as those obtained when

using a LMS and the staircase threshold method, are more reliable than intensity rating data obtained from the Just Noticeable Difference technique and the Harris-Kalums threshold test when separating individuals into sodium chloride and PROP taster groups. The LMS and staircase threshold tests provide reliable predictions on individuals phenotypic information, which can be then be used in further genotype-phenotype studies. Finally, ratio-level based scales have also been used to demonstrate synergism between the tastants monosodium glutamate and 5'-guanylate (Rifkin and Bartoshuk 1980).

Despite its efficacy, the LMS is subject to method weaknesses that should be considered. Schifferstein (2012) critically reviewed the use of LMS and concluded that, while the results obtained from this scale are similar to those obtained from magnitude estimation, it seems unlikely that they actually represent ratio-level data. Additionally, the inability to compare LMS data between individuals and groups due to the susceptibility of the results to context effects was cited as a critical reason for the abandonment of the use of the LMS to measure stimuli intensities. However, much can be done to reduce variability among panelists within a group and across groups, especially through the development of standardized training techniques and consistent interpretation and use of the scale during sensory studies. When used properly the LMS is a valuable tool for the measurement of sensory attributes such as bitterness in wine.

Challenges of Working with Human Subjects

"People, people! Oh, no! What scurvy luck!"
–Bear, Bedknobs and Broomsticks

The effects of crossmodal interactions on olfactory and gustatory processing present many challenges when conducting food sensory testing. Due to the occurrence of these interactions it is necessary to determine which factors must be controlled during sensory testing to ensure the

attainment of valid results. When sensory tests are aimed at producing results that can be directly related back to "real life" wine consumers and wine consumption experiences, it is especially important to balance variable control and semblance of a "natural" wine consumption occasion. Most wine consumers do not drink wine in an isolated and highly controlled setting while being asked to concentrate on performing specific evaluation tasks. The challenge is determining how much experimental variability can be controlled before the data no longer represents a natural human response.

Inter-panelist variability. Among wine professionals a distribution of detection thresholds of greater than 3 $\log_{10}$ units has been reported for odorants (Tempere et al., 2011) and tastants. It is possible that, as reviewed by Spence (2011), differences in salivary flow among individuals may affect gustatory responses, particularly when astringency intensity and persistence is being assessed. Furthermore, genetic variation among the population in oral cavity enzymes capable of in vivo degradation such as amylases (Perry et al. 2007) capable of releasing sugar may also contribute to inter-panelist variability.

Intra-panelist variability. Taste and olfactory sensitivity measurements, such as detection threshold determination, only provide a snapshot of the gustatory abilities of an individual at the time of the sensory evaluation session. Sensitivity to specific non-volatile or volatile compounds may change from day to day or within a day based on an individual's levels of alertness, attention, fatigue and changes in events occurring at the molecular level and health status (ASTM International, E1432-04). Training (Tempere et al. 2012), repeat exposure to a stimulus (Tempere et al. 2011), and age (Fusari and Ballesteros 2008) are also know to effect taste and olfactory sensitivity over time.

Final Thoughts

The use of highly variable human subjects for food sensory testing warrants agreement within the industry on the level of variability considered acceptable for each type of data collected and test performed. There is also a great need for the standardization of certain sensory practices, in addition to standards already set by ASTM International, to obtain results that can be compared across studies. In the case of LMS data, much of the variability is likely due to methods used to train panelists which can vary from one investigator to another. LMS training, scale interpretation, and sources of variability that should and should not be controlled for need be standardized. For each sensory test performed it is essential to consider the type of test, type of data to be obtained, sample and other method variables, and human variables, to determine what variables are critical to control for and which do not need to be considered.

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CHAPTER 2

INFLUENCE OF PRE-FERMENTATION SKIN CONTACT AND ON-SKIN FERMENTATION ON THE CHEMICAL AND SENSORY CHARACTERISTICS OF WHITE WINES

Abstract

The effects of pre-fermentation cold soak (CS) and on-skin fermentation (FS) treatments on the extraction of select phenolic and monoterpene compounds in the *Vitis vinifera* grapes Riesling and Gewürztraminer, and the hybrid grapes Traminette, Valvin Muscat, Frontenac gris, and La Crescent, were explored in a three-year study. The effects of these processing treatments on the perceived bitterness intensities of the Riesling, Gewürztraminer, and Traminette wines was also investigated. Juice and wine monomeric phenolics and monoterpenes were measured using solid phase extraction techniques, followed by either high performance liquid chromatography or gas-chromatography mass-spectrometry analysis, respectively. Wine bitterness intensity ratings were determined by a trained panel of wine consumers using a labeled magnitude estimation scale. No relationship between duration of CS or FS of white grape cultivars and the extraction of either monomeric phenolic or monoterpene compounds was found, with the exception of the flavan-3-ols (+)-catechin and (-)-epicatechin. The total monomeric phenolic concentration of the wines, however, did tend to increase with increasing CS duration for all cultivars, but the differences were not always significant. The most notable differences in total monomeric phenolic concentrations being between the CS treatments and the FS treatment. Sensory analysis of the Riesling, Gewürztraminer, and Traminette wines failed to find a relationship between CS duration and perceived bitterness intensity.

Introduction

White wine grapes of various cultivars are sometimes cold soaked (CS) prior to pressing and fermentation to extract aromatic compounds from grape skin and pulp (Sánchez Palomo et al. 2007; Cabaroglu and Canbas 2002). The extraction of volatile compounds during this process has been shown to alter organoleptic qualities and improve sensory scores in finished wines (Sánchez Palomo et al. 2007). However, there is some evidence that compounds eliciting undesirable gustatory qualities, such as bitterness and astringency, are also extracted during CS of white grapes and are retained in finished wines (Darias-Martín et al. 2000; Gawel et al. 2013; Gómez-Míguez et al. 2007; Gordillo et al. 2010; Singleton et al. 1975; Sokolowsky et al. 2015). The effect of CS on volatile and gustatory compound concentrations is dependent on grape cultivar and is highly subject to yearly variation caused by environmental, geographical, and agronomical conditions (Heymann and Cantu 2013; Jackson and Lombard 1993).

Cold soak methods. CS is commonly performed in an effort to enhance the varietal character of white wines through the extraction of volatile compounds from grape pulp and skin into juice (Moreno-Arribas and Polo 2009). CS is thought to aid in the extraction or release of monoterpenes and other volatile compounds into juice through prolonged contact with fruit solids, degradation of cell walls, and changes in juice chemistry (Lund and Bohlmann 2006). In the current work, CS is defined as holding must at or near refrigeration temperatures (4°C) for a set period of time prior to fermentation. Holding must at temperatures above 4°C prior to fermentation is considered here as pre-fermentation skin contact and not CS, although others do consider must being held at or less than 15°C to be CS (Wilker 2010).

White grape cultivars that may benefit from CS include Riesling, Gewürztraminer, Traminette, Valvin Muscat and other Muscat cultivars due to their prominent aromatic varietal

characteristics. CS durations can last for several hours to several days depending on the cultivar being processed, the desired sensory qualities of the finished wines, and the preferred practices of the winemaker.

Volatile compounds. Monoterpenes constitute one group of volatile compounds that may be extracted during CS (Overbosch et al. 1986). The monoterpenes linalool, geraniol, nerol, and *cis*-rose oxide are found in high concentrations in Riesling, Gewürztraminer, and Traminette wines, where they contribute to varietal character. All three cultivars have similar monoterpene constituents, with fifteen monoterpenes common among them, and an additional two present in Gewürztraminer and Traminette. However, the dominant monoterpenes and monoterpene concentrations are different for each cultivar, as can be expected from the wines' distinct aroma profiles.

Specifically, Traminette grapes have been reported to have twice the concentration of bound and free monoterpenes as Riesling and Gewürztraminer when grown in the same vineyard (Skinkis et al. 2008). In addition, *cis*-rose oxide is an impact odorant of Gewürztraminer and Traminette grapes and wine, and accounts for 13 to 35% of the total monoterpene content of these wines, respectively. In comparison, the aromatic profile of Riesling grapes is dominated by norisoprenoids although monoterpenes are also present. The most important norisoprenoids being 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), a characteristic aroma of Riesling wines (Skinkis et al. 2008).

Color and gustatory compounds. In addition to the extraction of volatile compounds, CS of white grape cultivars can lead to the extraction of various phenolic compounds from grape skin, seeds, and pulp (Gómez-Míguez et al. 2007; Gordillo et al. 2010; Sokolowsky et al. 2015; Darias-Martín et al. 2000). During red wine production, the extraction of phenolic compounds like anthocyanins,

condensed tannins, and co-pigmentation factors during CS is desirable. Anthocyanins can be extracted from cell vacuoles or anthocyanic vacuolar inclusions (AVIs) through losses in cell wall integrity (Conn et al. 2010) resulting from grape crushing, among other mechanisms. Interestingly, the structure of purified *Vitis vinifera* AVIs have been shown to contain long-chain tannins (Conn et al. 2010).

CS of white grape musts can result in color changes due to enzymatic and non-enzymatic browning reactions that result in darker colored juices, and wines with increased color intensity in the yellow spectral region (Lopez-Toledano et al. 2004). Specifically, reactions involving the phenolic compounds *t*-caftaric acid and GRP (grape reaction product; 2-S-glutathionyl caftaric acid) are known to contribute to the oxidative browning of wines. Must undergoing CS is more susceptible to these reactions due to the increased holding time prior to pressing and fermentation compared to must that is pressed immediately after destemming and crushing.

Reactions leading to color changes in white wine can also affect wine gustatory attributes. For example, enzymatic browning during winemaking is known to have negative effects on sensory attributes as a result of losses in color, flavor, and aroma, and increases in astringency (Escudero et al. 2002; Ferreira et al. 1997; Schneider 2001; Silva-Ferreira et al. 2002). In addition to CS alone, the effects of both CS and oxidation/hyperoxidation on phenolic composition has been studied in Airén wines (Cejudo-Bastante et al. 2011), Chardonnay wines (Cheynier et al. 1989), white port wines (Ho et al. 1999) and in red wine made from Grenache grapes (Cheynier et al. 1989; Ricardo-da-Silva et al. 1993).

In addition to color changes, phenolic compounds extracted during CS may have positive, negative, or no effects on wine gustatory qualities. Undesirable phenolic compounds potentially extracted during the CS process include those that elicit bitter and astringent sensations in finished

wines. Bitter phenolic compounds found in wine grapes include the flavan-3-ols, (+)-catechin and (-)-epicatechin, as well as proanthocyanidan and polymeric phenols (Oberholster et al. 2009; Arnold et al. 1980; Kallithraka et al. 1997; Thorngate and Noble 1995). Astringent compounds found in wines include hydroxybenzoic and hydroxycinnamic acids, which are reported to contribute to puckering and velvety sensations (Hufnagel and Hofmann 2008). Many studies have reported that the monomeric flavan-3-ols and procyanidin dimers and trimers also contribute to astringency (Kallithraka et al., 1997; Peleg et al. 1999; Robichaud and Noble 1990; Thorngate and Noble 1995), though other studies disagree (Hufnagel and Hofmann 2008). Finally, in the absence of proanthocyanins, coutaric and quercetin-3-O-rutinoside become significant contributors to wine astringency (Gonzalo-Diago et al. 2014).

The relationship between phenolic compound extraction and changes in the sensory characteristics of white wines is unclear. Some studies suggest that the extraction of phenolic compounds impacts the bitterness and/or astringency of wine (Gawel et al. 2013; Singleton et al. 1975). For example, Oberholster et al. (2009) reported that anthocyanins contributed to astringency when added to white grape juice with or without added white grape skins. However, this effect was not seen for high-purity anthocyanins evaluated in model systems (Vidal et al. 2004). Other studies report that the effects of phenolic compounds are highly influenced by intrinsic wine components such as pH and ethanol (Gawel et al. 2013). In addition, pH (Gawel et al. 2013) and ethanol (Gawel et al. 2007; Fontoin et al. 2008; Canals et al. 2005) can affect bitterness and astringency in the absence of phenolics.

Finally, a relationship exists between the intensity of bitterness and astringency with condensed tannin flavan-3-ol chain length. Compounds with fewer flavan-3-ol subunits are perceived as being more intensely bitter, with monomer flavan-3-ols being the most bitter. As

chain length increases through the addition of more flavan-3-ol subunits the compounds become less bitter and more astringent (Peleg et al. 1999).

When evaluating consumer perception of bitterness in a food or beverage such as wine, does it matter if some individuals are more sensitive to bitter compounds than others? There are certain bitter compounds for which the general population can be divided into non-tasters, medium tasters, and supertasters. These compounds include phenylthiocarbamide (PTC), 6-*n*-propylthiouracil (PROP), and quinine (Reed et al. 2010).

The phenolic and monoterpene content and bitterness intensity of a wine can vary based on cultivar, growing conditions, and processing techniques, such as CS. The current study aims to determine the effect of CS duration on the extraction of select phenolic and monoterpene compounds present in the *Vitis vinifera* grapes Riesling and Gewürztraminer, and interspecific hybrid grapes Traminette, Valvin Muscat, Frontenac gris, and La Crescent. The study also investigates the effects of CS on the perceived bitterness intensities of Riesling, Gewürztraminer, and Traminette wines.

Materials and Methods

Fruit. *Vitis vinifera* grapes Riesling and Gewürztraminer and hybrid grapes Traminette (Joannes Seyve 23.416 x Gewürztraminer), Valvin Muscat (Couderc 299-35 [Muscat du Moulin] x Muscat Ottonel), Frontenac gris (single bud mutation of the hybrid Frontenac [*Vitis riparia* 89 x Landot 4511]), and La Crescent (St. Pepin x ES 6-8-25 [*Vitis riparia* x Muscat Hamburg]), used for this study were harvested at commercial ripeness as dictated by grape sampling analyses and the harvesting schedules at the places of origin (Table 2.1).

Table 2.1-Grape sources, harvest information, and pre-processing parameters.

Grape	Location[1]	Year	Harvest Date	Picking Method	Pre-Processing Storage Length (h)	Storage Temperature
Riesling	Penn Yan	2011	10/05/2011	Machine	12	Ambient[2]
		2012	10/02/2012	Machine	0	Ambient
		2013	10/15/2013	Hand	24	4°C
Gewürztraminer	Lodi	2011	09/28/2011	Hand	0	Ambient
		2012	09/20/2012	Hand	72-96	4°C
		2013	09/30/2013	Hand	24	4°C
Traminette	Branchport	2011	10/10/2011	Hand	24-48	4°C
		2012	10/05/2012	Hand	24-48	4°C
		2013	09/27/2013	Hand	24	4°C
Valvin Muscat	Romulus	2011	09/20/2011	Hand	24-48	4°C
	Penn Yan	2012	09/17/2012	Machine	0	Ambient
La Crescent	Trumansburg	2011	09/26/2011 09/27/2011	Hand	24-48	4°C
Frontenac gris	Trumansburg	2011	09/26/2011	Hand	24-48	4°C

[1]All grapes were sourced from vineyards and wineries located in the New York State Finger Lakes region.
[2]Ambient temperature varied based on weather conditions.

Treatments. Grapes were subjected either to various durations of pre-fermentation cold soaking (CS) followed by fermentation or on-skin fermentation (FS) for 7-14d (Table 2.2).

Table 2.2- Processing treatments of aromatic white grape cultivars

Cultivar	Cold Soak Treatments[1]	Skin Fermentation
Gewürztraminer	2h, 4h, 24h, 24hE[2], 48h	7d
Riesling	2h, 4h, 24h, 24hE, 48h	7d
Traminette	2h, 4h, 24h, 24hE, 48h	7d
Valvin Muscat	2h, 24h, 48h	14d
Frontenac gris	2h, 24h, 48h	7d
La Crescent	2h, 24h, 48h	7d

[1]Pre-fermentation cold soak treatments were held at approximately 4°C during cold soaking.
[2]24hE: 24h cold soak with added enzyme. Lallzyme C (Lallemand, Montréal, CA) was added at a rate of 0.03g/L in 2011 and Cuvee blanc (Scott Laboratories, Inc., Pickering, ON, Canada), was added at a rate of 0.03-0.04g/L in 2012 and 2013.

Grape, must, and juice processing. All grapes were destemmed (Model Eno-15S; Midwest Supplies, St. Louis Park, MN) and divided into equivalent treatment lots by weight. Sulfur dioxide (SO_2), in the form of sodium metabisulfate, was added at a rate of 100ppm to the must for all treatments, with the exception of the 2h CS treatment, which received an addition of 60ppm of SO_2 after pressing. The must was then either CS or inoculated with yeast and fermented on the skins (14-15°C) in stainless steel vessels (Table 2.2). Additions to the 24hE CS treatments were

made after crushing. The commercial pectinase enzyme blends used were Lallzyme C (Lallemand, Montréal, CA) added at a rate of 0.03g/L in 2011 and Cuvee blanc (Scott Laboratories, Inc., Pickering, ON, Canada) added at the manufacture's recommended rate of 0.03-0.04g/L in 2012 and 2013.

After CS the musts were pressed (Mori press type PZ.82, Impianti Enoligici, Italy) at 200psi, the resulting juice was settled in glass carboys overnight, racked, and separated into replicate treatments of equal weight. In 2012, Scottszyme KS (Scott Laboratories, Inc., Pickering, ON, Canada), a blend of polygalacturonase, pectin esterase, pectin lyase, cellulose, and protease, was added to the juice to help prevent the development of off-aromas that were observed in the 2011 wines. This addition was not repeated in 2013.

For skin fermentation treatments, 30kg lots of crushed grapes were placed in replicate stainless steel vessels, chaptalized as needed, fermented, and then hand pressed after seven days, with the exception of the 2011 Valvin Muscat FS treatment, which was hand pressed after 14d. Wines were then transferred to glass carboys and allowed to complete fermentation, if necessary. It should be noted that the 2011 Valvin Muscat was the first grape cultivar to undergo the FS treatment, and FS duration was reduced to 7d for all further experimental wines produced.

Fermentation processing. All CS treatments were chaptalized as needed to reach 20-21°Brix. Must was then inoculated with rehydrated yeast strain R2 (Lallemand, Montréal, Canada), following manufacture's recommendations, at a rate of 1g/gal; 0.3g/L GoFerm yeast nutrient (Lallemand, Montréal, Canada) was added to the rehydrated yeast solution. On-skin fermentation treatments were chaptalized and inoculated 24h after crushing. Yeast strain R2 was selected due to its temperature range of 5-32.2°C, moderate fermentation speed, competitive factors against other microorganisms, 16% alcohol tolerance, moderate nitrogen needs, and recommended use for

Gewürztraminer, Riesling, and dry whites in general (Scott Laboratories, Inc. 2010). Nitrogen in the form of diammonium phosphate (Presque Isle Wine Cellars, North East, PA, USA) and Fermaid K (Lallemand, Montréal, Canada) was added as needed to treatments 24h after inoculation to bring the total yeast assimilable nitrogen (YAN) concentration up to the recommended level of 200mg N/L (Scott Laboratories, Inc. 2010). Fermentations were carried out at 14-15°C.

Post fermentation processing. When fermentations reached <0.2% residual sugar as indicated by Clinitest tablet measurements, wines were racked, received 60ppm SO_2 additions, and held at 5°C. Cold stability was monitored through titratable acidity (TA), pH, and organic acid analysis. Wine acidity adjustments were made as needed to reach target acidity levels, with TA at bottling ranging from 5.5-10.0g/L depending on the grape cultivar, treatment, and vintage. After cold stabilization, wines were bottled in 750mL brown glass bottles with screw-cap closures (Saint Gobain, La Défense and Courbevoie, France) using a single-head vacuum pump bottle filler (Enolmatic®; Tenco S.N.C., Avegno, Italy), and stored in a climate controlled storage facility at 4°C until needed for analysis.

Juice and Wine Sampling. Samples for chemical analysis (40mL) were collected in duplicate at crushing, pressing, and at the end of alcoholic fermentation of each treatment and frozen for future analysis. Ascorbic acid (0.1%) was added to juice samples prior to freezing to limit oxidation reactions upon thawing.

Analyses

The analyses performed on the grape cultivars processed in 2011-2013 are shown in Table 2.3.

Table 2.3. Analyses performed on the 2011-2013 Riesling, Gewürztraminer, Traminette, Valvin Muscat, Frontenac gris, and La Crescent juices and wines.

Parameter	Method[1]	Cultivar[2]	Sample/ Treatment[3]
Yeast Assimilable Nitrogen	Enzymatic Analysis	All grape cultivars	Juice (Crushing)
Organic Acids	HPLC[4]	All grape cultivars	PAF[6], all treatments
Monoterpenes	GC-MS[5]	Riesling Gewürztraminer Traminette	2h CS 48h CS
Monomeric Phenolics	HPLC	All grape cultivars	PAF, all treatments
		Riesling Gewürztraminer Traminette	Sensory analysis, 2011-2013[7]
Color	CIELAB	Riesling Gewürztraminer Traminette	Sensory analysis, 2013

[1]Methods for each analysis technique are described in the text under the section Analyses.
[2]Cultivars used in 2011-2013: Riesling, Gewürztraminer, Traminette, Valvin Muscat (2011-2012 only), Frontenac gris (2011 only), and La Crescent (2011 only)
[3]Treatments: 2h, 4h, 24h, 24h with added enzyme, and 48h pre-fermentation cold soak (CS); 7d on-skin fermentation (FS). Treatments varied among cultivars.
[4]HPLC: High Performance Liquid Chromatography
[5]GC-MS: Gas Chromatography- Mass Spectrometry
[6]PAF: Post Alcoholic Fermentation
[7]Sensory analysis treatments: 2h, 24h, 24h with added enzyme (2011-2012 only), and 48h CS; 7d FS.

Yeast Assimilable Nitrogen (YAN). YAN measurements were determined using a Chemwell 2910 multi-analyzer equipped with Software Version 6.3 (Awareness Technology, Palm City, FL, USA) and enzymatic analysis kits for primary amino nitrogen compounds (Primary Amino Nitrogen UniTab Reagent Kit) and ammonium (Ammonia UniTab Reagent Kit) (Unitech Scientific, Hawaiian Garden, CA, USA).

High-Performance Liquid Chromatography (HPLC)

Reagents. HPLC grade methanol, hydrochloric acid, acetonitrile, neat formic acid, ethyl acetate, phloroglucinol, and phosphoric acid used for high performance liquid chromatography analysis and solid phase extraction (SPE) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Organic acids. Tartaric, malic, acetic, and citric acids were quantified in wine samples via HPLC using a method by Castellari et al. (2000). Analysis was performed on a 300mm x 7.8mm Aminex

HPX-87H Ion Exchange column fitted with an inline cation refill guard cartridge (Bio-Rad Laboratories, Hercules, CA, USA). Samples were prepared by filtering about 1.0mL of wine through a 13 mm, 0.2 µm polyethersulfone (PES) syringe filter (Krackeler Scientific, Inc., Albany, NY, USA) immediately prior to HPLC analysis.

Phenolic compounds. Phenolic compound analysis was performed using an Agilent 1260 Infinity series HPLC (Agilent Technologies, Santa Clara, CA, USA) containing a diode array detector fitted with a 10mm path, 1µl volume Max-light cartridge flow cell. All sample fractions were analyzed on Kinetx ® core-shell columns (Phenomenex, Torrance, CA, USA) fitted with Krudkatcher™ guard filters (Phenomenex, Torrance, CA, USA). Specifically, the monomeric phenolic and tannin fractions were analyzed on a 100 x 4.6mm C_{18} column packed with 2.6µm diameter particles with a 100Å pore size. The instrument was controlled using Agilent Chemstation software version B.04.03, service pack 2 with spectral software module.

Samples were either filtered and then directly injected into the HPLC or separated into monomeric phenolic and tannin fractions using the method developed by Jeffery et al. (2008a) and modified by Manns and Mansfield (2012). Briefly, frozen samples were thawed and then centrifuged at 10,000x g for 5 min. Samples were then mixed with an equal amount of 0.01 N HCl in water and loaded onto a $3cm^3$, 60mg Oasis HLB solid phase extraction cartridge (Waters, Milford, MA, USA). Samples that were analyzed on the HPLC by direct injection were centrifuged as previously described and filtered through a 0.22µm PES membrane immediately prior to sample analysis.

Gas Chromatography-Mass Spectrometry (GC-MS)

Reagents. Linalool, geraniol, nerol, and 2-octanol of >97% purity were obtained from Sigma-Aldrich (St. Louis, MO). Tartaric acid was sourced from Presque Isle Wine Cellars (North East,

PA). HPLC grade methanol, ethanol, and dichloromethane were sourced from Fisher Scientific (Waltham, MA). Model wine samples were prepared with 12% (v/v) ethanol and 5 g/L tartaric acid. Model wine pH was adjusted to 3.5 using sodium hydroxide.

Monoterpenes were isolated from wine using a solid-phase extraction (SPE) protocol adopted from conditions used in previous studies (Kwasniewski et al. 2010; Lopez et al. 2002) and validated by Sacks et al. (2012). Wine samples (50mL) received 25μL of the internal standard (2-octanol, 0.5 g/L in acetonitrile), and were loaded onto SPE cartridges (Merck, Darmstadt, Germany) containing 200 mg of LiChrolut EN sorbent and preconditioned with 4 mL each of dichloromethane, methanol, and model wine. At the completion of column loading, the cartridge was allowed to dry under nitrogen for 20 min. Analytes were then eluted with 1.3 mL of dichloromethane.

Analysis was conducted on an Agilent 6890 gas chromatograph with a split−splitless injector (Santa Clara, CA) coupled to an Agilent HP 5973 Mass Selective Detector. Separation was performed using an Agilent DB-5ms column (30m x 0.25 mm i.d., 0.25μm film thickness). An initial oven temperature of 35 °C was held for 3 min, then ramped to 200 °C at 6 °C/min, then to 240 °C at 30 °C/min, and held at 240 °C for 3 min. The GC was operated at a constant flow rate of 1 mL/min. One microliter of extract was injected in splitless mode at 250 °C with a purge time of 1.00 min (purge flow 50ml/min; inlet pressure 68.9kPa). The auxiliary channel, set point quadruple, set point source, and detector temperatures were 280, 150, 230, and 250°C, respectively. Helium was used as the carrier gas.

Data processing and quantification were performed using Agilent Enhanced MSD ChemStation software (G170EA E.02.00.493). Peak identifications for linalool, geraniol, nerol, *cis*-linalool, and nerol oxide were confirmed by comparison with the retention times of standards

and with a mass spectra library. The response ratio of the analyte peak area to the 2-octanol standard (SIM m/z 41, 43, 45, 55) peak area was calculated and converted to a concentration via the calibration curve of the nerol standard. The nerol calibration standard was prepared in triplicate in model wine over the range 5-309 μg/L and had had a calibration curve R^2 value of 0.99.

Sensory Analysis.

Wines. The perceived bitterness intensity of the 2011-2013 Riesling, Gewürztraminer, and Traminette 2h, 24h, 24hE (2011 and 2012 only), 48h CS treatments as well as the 7d FS treatment were analyzed in duplicate (2011 and 2012) or triplicate (2013) in winter 2013 and 2014, and fall 2015, respectively.

Panelists. For all sensory tests, panelists were recruited via email and selected based on their frequency of wine consumption (at least several times a month), non-smoking status, age, and availability to attend all of the required sensory training and product evaluation sessions. Panelists gave informed consent to participate in these Cornell University Internal Review Board approved studies at the start of the first training session. Panels consisting of fifteen (8 female; ages 23 to 73 yrs, mean age 42), twenty-six (14 female; ages 24 to 80yrs; mean age of 45yrs), and twenty-eight (25 female; ages 21 to 55 yrs; mean age of 25yrs) individuals rated the 2011, 2012, 2013 wines, respectively.

Training sessions. Panelists attended two training sessions for the analysis of the 2011 wines and four training sessions for the analysis of the 2012 and 2013 wines. Panelists were trained and tested on their ability to differentiate between bitter and astringent wine reference samples, instructed on use of the labeled magnitude scale (LMS) (Green et. al. 1993), and screened for their ability to taste a 0.56mM solution of PROP (Sigma-Aldrich, St. Louis, MO, USA). Panelists were asked to

rate wine bitterness intensity using the entire LMS scale to prevent overuse of anchor word positions.

Reference Samples. Quinine sulfate (Fisher Scientific, Pittsburgh, PA, USA) and aluminum sulfate (Sigma-Aldrich, St. Louis, MO, USA) were used as bitter and astringent reference samples, respectively, during training and evaluation sessions. Standards were mixed in both distilled water and a commercial white wine (Heritage Mountain Chablis, Almaden Vineyards®, Madera, California, USA; Packaged in 5L bag-in-box containers.) Bitter reference samples were produced using 0.025, 0.0375 (2013 only), and 0.05g/L of quinine sulfate, and astringent reference samples using concentrations of either 20g/L (2011), 5g/L (2012), or 9g/L (2013) aluminum sulfate. All reference samples were prepared the same day as the sensory sessions, poured into 22mL plastic cups approximately one hour prior to each session, and held at room temperature (21°C). These samples were intended for use as references for sensations of general bitterness and astringency, rather than the specific bitter and astringent characteristics of wine.

Sample evaluation sessions. During sensory evaluation sessions wine samples (30mL) were served in 215mL wine tasting glasses (ISO 3591:1977), labeled with random three-digit numbers, and were poured approximately one hour prior to sensory analysis and held at room temperature (21°C). Samples were presented under red lighting to mask any differences in appearance. Panelists were asked to evaluate the perceived bitterness of each individual sample using the LMS. Tasting breaks were enforced throughout the evaluation sessions to reduce bitterness or astringency carryover effects (four minutes after each set of three wines for the 2011 samples, 15 min after every fourth 2012 sample, and 5 min after every fifth 2013 sample). All samples were presented in a randomized service order using a Latin Square design. In addition, panelists were

required to wear nose clips, expectorate all samples, were given water and bread or rice cakes as palate cleansers, and were instructed to take short breaks in between samples.

Statistical Analysis. Statistical analysis of HPLC, GC-MS, and sensory data was performed on JMP Pro 10 (SAS Institute, Inc., Cary, NC, USA) using an analysis of variance (ANOVA) fit model and a least squares means Tukey Honestly Significance Difference (HSD) test using $p<0.05$ as a minimum for significance.

Color measurements. 2013 Riesling, Gewürztraminer, and Traminette sample colors were quantified using the International Commission on Illumination L*a*b* (CIElab) color space/ scale which utilizes an Adams-Nickerson cube root formula to measure small color differences among samples. In this scale L* represents lightness (white to black), a* represents redness (positive values)/greenness (negative values), and b* represents yellowness (positive values)/blueness (negative values). Color scale measurements were made using a Hunter Lab Ultra Scan VIS equipped with Easy MatchQC software (Hunter Associates Laboratory, Inc., Reston, VA, USA) in TTRAN total transmission mode. The total color change of a sample from the control (ΔE) can be determined using the following equation:

$$\Delta E=\sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

Where ΔL*, Δa*, Δb* are the differences in lightness, redness/greenness, and yellowness/blueness, respectively, between the treatment and control samples.

Results

Juice and wine chemistry. Standard juice and wine chemical parameter data for grapes processed in 2011, 2012, and 2013 are shown in Tables 2.4-2.7, respectively. Average pH and titratable acidity measurements taken throughout processing for each cultivar (2011-2013) are shown in Table 2.8.

Table 2.4. Riesling juice and wine parameters, 2011-2013.

Year	Treatment	JUICE ANALYSIS						WINE ANALYSIS Pre Cold Stabilization		WINE ANALYSIS Post Cold Stabilization; Pre TA Adjustments						WINE ANALYSIS Bottling	
		Brix	pH	TA[1] (g/L)	AMM[2] (mg N/L)	PAN[3] (mg N/L)	YAN[4] (mg N/L)	pH	TA[1] (g/L)	pH	TA[1] (g/L)	Tartrate (g/L)	Malate (g/L)	Lactate (g/L)	Citric (g/L)	pH	TA[1] (g/L)
2011	2h	17.1	3.18	7.7	59.3	57.0	116	3.17 ±0.00	9.3 ±0.04	3.05 ±0.010	7.4 ±0.2	3.1 ±0.2	2.9 ±0.2	0.20 ±0.01	0.30 ±0.02	3.05 ±0.010	7.4 ±0.2
	4h							3.16 ±0.01	9.1 ±0.01	3.03 ±0.010	7.5 ±0.1	3.0 ±0.2	2.8 ±0.1	0.20 ±0.0	0.30 ±0.01	3.03 ±0.010	7.5 ±0.1
	24h							3.20 ±0.00	9.3 ±0.04	3.09 ±0.010	7.2 ±0.08	2.9 ±0.01	2.7 ±0.1	0.20 ±0.01	0.20 ±0.0	3.09 ±0.010	7.2 ±0.08
	24h E							3.23 ±0.04	9.5 ±0.2	3.15 ±0.00	7.6 ±0.4	2.9 ±0.09	2.2 ±0.02	nd	0.10 ±0.02	3.15 ±0.00	7.6 ±0.4
	48h							3.22 ±0.00	9.4 ±0.5	3.07 ±0.00	7.2 ±0.07	3.1 ±0.08	3.0 ±0.05	0.30 ±0.0	0.30 ±0.02	3.07 ±0.00	7.2 ±0.07
	7d							3.11 ±0.00	9.0 ±0.03	3.05 ±0.00	7.2 ±0.2	2.7 ±0.1	3.1 ±0.3	0.20 ±0.02	0.40 ±0.06	3.05 ±0.00	7.2 ±0.2
2012	2h	18.1	3.16	7.4	64.3	66.0	130	3.09 ±0.02	8.6 ±0.1	3.01 ±0.030	8.3 ±0.4	3.72 ±0.01	2.2 ±0.03	0.23 ±0.0	0.24 ±0.01	3.01 ±0.030	7.2 ±0.07
	4h							3.12 ±0.00	7.7 ±0.10	3.05 ±0.030	7.4 ±0.02	3.24 ±0.00	2.4 ±0.05	0.18 ±0.0	0.22 ±0.0	3.05 ±0.030	7.4 ±0.07
	24h							3.23 ±0.01	7.8 ±0.2	3.13 ±0.00	7.1 ±0.1	3.0 ±0.1	2.3 ±0.02	0.17 ±0.01	0.24 ±0.0	3.13 ±0.00	7.1 ±0.1
	24h E							3.22 ±0.00	8.1 ±0.05	3.10 ±0.020	7.4 ±0.01	3.1 ±0.06	2.3 ±0.01	0.19 ±0.0	0.25 ±0.01	3.10 ±0.020	7.4 ±0.00
	48h							3.26 ±0.00	7.7 ±0.04	3.14 ±0.010	7.2 ±0.01	2.9 ±0.02	2.4 ±0.01	0.21 ±0.01	0.26 ±0.0	3.14 ±0.010	7.2 ±0.00
	7d							3.14 ±0.01	10.6±0.5	3.19 ±0.010	7.2 ±0.02	2.5 ±0.05	2.5 ±0.02	0.24 ±0.0	0.34 ±0.01	3.25 ±0.090	7.3 ±0.07
2013	2h	19.0a	3.03a	9.1a	38.7a	50.0a	89.0a	2.95e ±0.00	10cd ±0.04	2.85i ±0.00	9.9ab ±0.02	3.7 ±0.03	2.1fg ±0.04	0.40cd ±0.0	0.20e ±0.0	2.85k ±0.00	9.9a ±0.02
	4h							2.98e ±0.02	9.4cde ±0.2	2.89hi ±0.00	9.2bcd ±0.07	3.5bcd ±0.03	2.4ef ±0.04	0.40cd ±0.0	0.20de ±0.0	2.89jk ±0.00	9.2b ±0.07
	24h							3.06d ±0.00	9.0 ±0.3	2.94gh ±0.050	7.7e ±0.04	3.0 ±0.1	2.4e ±0.04	0.50c ±0.0	0.20de ±0.0	2.94gh ±0.050	7.7e ±0.04
	24h E							3.07d ±0.01	9.1cde ±0.1	3.00fg ±0.00	8.7de ±0.06	3.5ef ±0.05	2.2efg ±0.08	0.40cd ±0.0	0.20e ±0.0	3.00fg ±0.00	8.7bc ±0.06
	48h							3.11d ±0.00	8.6ef ±0.03	2.87i ±0.00	7.9e ±0.06	3.2de ±0.00	2.5e ±0.05	0.50 ±0.01	0.30cde ±0.0	2.87efg ±0.00	7.9de ±0.06
	7d									3.00fg	8.9cd ±0.2	3.3cde ±0.10	2.1gh ±0.04	0.70 ±0.07	0.20cde ±0.0	3.00ij ±0.020	8.9bc ±0.2

[1]TA: Titratable acidity in tartaric acid equivalents (TAE)
[2]AMM: Ammonium ions; Measured by enzymatic analysis.
[3]PAN: Primary amino nitrogen; Measured by enzymatic analysis.
[4]YAN: Yeast assimilable nitrogen; Measured by enzymatic analysis.

Table 2.5. Gewürztraminer juice and wine parameters, 2011-2013.

Year	Treatment	JUICE ANALYSIS						WINE ANALYSIS Pre Cold Stabilization		WINE ANALYSIS Post Cold Stabilization; Pre TA Adjustments						WINE ANALYSIS) Bottling	
		Brix	pH	TA[1] (g/L)	AMM[2] (mg N/L)	PAN[3] (mg N/L)	YAN[4] (mg N/L)	pH	TA[1] (g/L)	pH	TA[1] (g/L)	Tartrate (g/L)	Malate (g/L)	Lactate (g/L)	Citric (g/L)	pH	TA[1] (g/L)
2011	2h	19.8	3.23	5.1	33.0	67.0	100	3.17 ±0.030	7.6 ±0.0	3.13 ±0.00	6.5 ±0.2	4.8 ±0.0	5.4 ±0.0	0.50 ±0.0	0.40 ±0.0	3.13 ±0.00	6.5 ±0.2
	4h							3.26 ±0.090	7.6 ±0.3	3.19 ±0.00	6.3 ±0.01	2.3 ±0.06	1.9 ±0.02	0.20 ±0.03	0.10 ±0.0	3.19 ±0.00	6.3 ±0.01
	24h							3.43 ±0.00	7.2 ±0.3	3.33 ±0.00	5.9 ±0.3	1.9 ±0.01	2.0 ±0.07	0.10 ±0.02	0.10 ±0.0	3.33 ±0.00	5.9 ±0.3
	24h E							3.46 ±0.00	6.9 ±0.08	3.37 ±0.00	5.8 ±0.07	2.1 ±0.05	1.7 ±0.03	0.20 ±0.0	0.10 ±0.0	3.37 ±0.00	5.8 ±0.07
	48h							3.49 ±0.010	6.6 ±0.2	3.35 ±0.020	5.6 ±0.07	1.8 ±0.03	2.0 ±0.1	0.20 ±0.04	0.10 ±0.0	3.35 ±0.020	5.5 ±0.07
	7d							3.36 ±0.00	10. ±0.2	3.50 ±0.030	5.9 ±0.09	2.4 ±0.01	2.3 ±0.05	0.60 ±0.02	0.40 ±0.03	3.50 ±0.030	5.9 ±0.09
2012	2h	23.4	3.56	4.6			178	3.70 ±0.050	6.0 ±0.07	3.74 ±0.040	4.0 ±0.1	1.5 ±0.07	1.3 ±0.0	0.27 ±0.0	0.22 ±0.0	3.24 ±0.010	7.0 ±0.1
	4h							3.67 ±0.00	5.4 ±0.1	3.70 ±0.00	4.0 ±0.07	1.6 ±0.04	1.3 ±0.01	0.29 ±0.0	0.23 ±0.0	3.22 ±0.020	7.2 ±0.07
	24h							3.75 ±0.010	5.2 ±0.09	3.84 ±0.020	3.7 ±0.08	1.5 ±0.02	1.4 ±0.04	0.29 ±0.02	0.25 ±0.0	3.25 ±0.00	6.8 ±0.07
	24h E							3.80 ±0.00	4.9 ±0.2	3.90 ±0.00	3.5 ±0.04	1.5 ±0.02	1.5 ±0.01	nd	0.26 ±0.0	3.27 ±0.00	6.8 ±0.07
	48h							3.80 ±0.020	5.2 ±0.3	3.93 ±0.00	3.6 ±0.06	1.5 ±0.02	1.5 ±0.01	0.31 ±0.03	0.27 ±0.0	3.28 ±0.010	6.4 ±0.0
	7d							3.57 ±0.00	7.9 ±0.3	3.81 ±0.00	4.5 ±0.1	1.4 ±0.01	1.9 ±0.07	0.52 ±0.0	0.41 ±0.01	3.61 ±0.00	6.2 ±0.42
2013	2h	22.7b	3.40b	6.2b	55.2b	96.0b	151b	3.48b ±0.00	5.6h ±0.3	3.39d ±0.00	6.6f ±0.06	1.8hi ±0.04	1.7i ±0.0	0.40cd ±0.0	0.20de ±0.0	3.39cde ±0.00	6.6fg ±0.06
	4h							3.43b ±0.00	5.7h ±0.06	3.34d ±0.00	6.6f ±0.1	1.8hi ±0.05	2.1gh ±0.01	0.40cd ±0.01	0.20cde ±0.0	3.34def ±0.00	6.6f ±0.1
	24h							3.59a ±0.00	5.6gh ±0.04	3.52c ±0.010	6.0f ±0.05	1.5ij ±0.07	1.7i ±0.0	0.30cd ±0.0	0.20cde ±0.0	3.52bcd ±0.010	6.0fg ±0.05
	24h E							3.59a ±0.00	5.6h ±0.04	3.52e ±0.010	6.0f ±0.05	1.5jk ±0.07	1.7i ±0.0	0.30de ±0.0	0.20de ±0.0	3.52b ±0.010	6.0fg ±0.05
	48h							3.61a ±0.00	6.4gh ±0.3	3.50bc ±0.00	6.1f ±0.2	1.6ij ±0.1	1.9hi ±0.02	0.40cd ±0.0	0.30cde ±0.0	3.50bc ±0.00	6.1fg ±0.2
	7d							3.56a ±0.010	7.7fg ±0.07	3.74a ±0.00	5.9f ±0.04	1.3k ±0.0	2.1gh ±0.01	0.10 ±0.01	0.40a ±0.01	3.74a ±0.00	5.9g ±0.04

[1]TA: Titratable acidity in tartaric acid equivalents (TAE)
[2]AMM: Ammonium ions; Measured by enzymatic analysis.
[3]PAN: Primary amino nitrogen; Measured by enzymatic analysis.
[4]YAN: Yeast assimilable nitrogen; Measured by enzymatic analysis.

Table 2.6. Traminette juice and wine parameters, 2011-2013.

Year	Treatment	JUICE ANALYSIS						WINE ANALYSIS Pre Cold Stabilization		WINE ANALYSIS Post Cold Stabilization; Pre TA Adjustments						WINE ANALYSIS) Bottling	
		Brix	pH	TA[1]	AMM[2]	PAN[3]	YAN[4]	pH	TA[1]	pH	TA[1]	Tartrate[1]	Malate[1]	Lactate[1]	Citric[1]	pH	TA[1]
2011	2h	21.2	3.00	7.7	2.50	40.0	42.0	3.03 ±0.030	11 ±0.2	2.92 ±0.00	8.5 ±0.3	4.2 ±0.02	2.8 ±0.0	0.20 ±0.02	0.40 ±0.08	2.92 ±0.00	8.5 ±0.3
	4h							3.04 ±0.070	11 ±0.5	2.91 ±0.00	8.6 ±0.09	4.1 ±0.02	2.8	0.30 ±0.04	0.40 ±0.01	2.91 ±0.00	8.6 ±0.09
	24h							3.19 ±0.00	8.5 ±0.7	3.00 ±0.00	7.7 ±0.0	3.2 ±0.1	2.9 ±0.1	0.20 ±0.04	0.40 ±0.04	3.00±0.00	7.7 ±0.0
	24h E							3.21 ±0.020	7.9 ±0.4	3.03 ±0.00	7.9 ±0.0	3.5 ±0.3	2.9 ±0.03	0.20 ±0.0	0.40 ±0.01	3.03 ±0.00	7.9 ±0.0
	48h							3.21 ±0.00	7.3 ±0.04	3.05 ±0.00	7.2 ±0.2	3.3 ±0.2	2.9 ±0.04	0.20 ±0.02	0.40 ±0.0	3.05 ±0.00	7.2 ±0.2
	7d							3.13 ±0.030	11 ±0.1	3.26 ±0.020	7.0 ±0.07	1.9 ±0.3	1.0	1.8 ±0.09	0.10 ±0.02	3.26 ±0.020	7.0 ±0.07
2012	2h	22.4	3.04	7.4	5.80	109	115	3.14 ±0.00	8.9 ±0.4	3.02 ±0.020	3.5 ±0.05	3.5 ±0.05	2.1 ±0.02	0.22 ±0.0	0.29 ±0.02	3.20 ±0.14	7.8 ±0.4
	4h							3.15 ±0.00	8.4 ±0.2	3.04 ±0.020	5.5 ±3.	3.2 ±0.0	2.2 ±0.06	0.21 ±0.02	0.29 ±0.02	3.04 ±0.020	7.8 ±0.07
	24h							3.26 ±0.020	8.0 ±0.07	3.19 ±0.020	7.5 ±0.2	2.9 ±0.1	2.4 ±0.0	0.16 ±0.01	0.38 ±0.0	3.19 ±0.020	7.5 ±0.2
	24h E							3.23 ±0.010	8.6 ±0.3	3.11 ±0.00	8.0 ±0.4	3.1 ±0.04	2.4 ±0.01	0.17 ±0.0	0.33 ±0.0	3.11 ±0.00	8.0 ±0.4
	48h							3.24 ±0.00	8.9 ±1.	3.18 ±0.030	7.5 ±0.05	2.9 ±0.01	2.4 ±0.02	0.19 ±0.0	0.37 ±0.0	3.18 ±0.030	7.5 ±0.07
	7d							3.23 ±0.020	9.4 ±0.6	3.27 ±0.00	7.4 ±0.02	2.1 ±0.05	2.8 ±0.01	0.25 ±0.0	0.41 ±0.0	3.27 ±0.00	7.5 ±0.07
2013	2h	20.8g	3.01c	7.4c	4.90c	64.0c	69.0c	3.06d ±0.00	12b ±0.3	2.90hi ±0.00	11a ±0.4	3.6 ±0.1	3.8 ±0.02	0.40cd ±0.01	0.40a ±0.07	3.22fgh ±0.030	8.7bc ±0.07
	4h							3.11d ±0.040	10cd ±0.7	2.90hi ±0.00	9.6abc ±0.4	3.3cd ±0.06	3.1d ±0.01	0.30d ±0.07	0.30bcd ±0.0	3.10hi ±0.040	8.6bcd ±0.07
	24h							3.22 ±0.020	9.8cde ±0.05	2.99fg ±0.00	9.4bcd ±0.2	3.0ef ±0.0	3.2cd ±0.0	0.30de ±0.05	0.30abc ±0.0	3.16gh ±0.020	8.7bc ±0.07
	24h E							3.27c ±0.00	10bc ±0.06	2.99fg ±0.00	9.9ab ±0.09	2.9f ±0.0	3.4bc ±0.0	0.20e ±0.0	0.30abc ±0.0	3.25fg ±0.010	8.8bc ±0.1
	48h							3.21c ±0.010	9.7cde ±0.4	3.05f ±0.00	9.5bcd ±0.4	2.5g ±0.02	3.6 ±0.08	0.30d ±0.07	0.30ab ±0.0	3.27efg ±0.050	8.4cd ±0.0
	7d							3.12d ±0.00	15a ±0.9	3.19e ±0.010	9.5bcd ±0.2	2.0h ±0.0	3.8 ±0.1	0.40cd ±0.01	0.40a ±0.02	3.38cde ±0.080	8.6bc ±0.1

[1]TA: Titratable acidity in tartaric acid equivalents (TAE)
[2]AMM: Ammonium ions; Measured by enzymatic analysis.
[3]PAN: Primary amino nitrogen; Measured by enzymatic analysis.
[4]YAN: Yeast assimilable nitrogen; Measured by enzymatic analysis.

Table 2.7. Valvin Muscat, Frontenac gris, and La Crescent juice and wine parameters, 2011-2013.

Grape	Year	Treatment	JUICE ANALYSIS						WINE ANALYSIS Pre Cold Stabilization		WINE ANALYSIS Post Cold Stabilization; Pre TA Adjustments						WINE ANALYSIS) Bottling	
			Brix	pH	TA[1] (g/L)	AMM[2] (mg N/L)	PAN[3] (mg N/L)	YAN[4] (mg N/L)	pH	TA[1] (g/L)	pH	TA[1] (g/L)	Tartrate (g/L)	Malate (g/L)	Lactate (g/L)	Citric (g/L)	pH	TA[1] (g/L)
Valvin Muscat	2011	2h	16.1	3.15	9.7	51.9	85.0	137	3.23 ±0.00	12 ±0.2	3.10 ±0.00	9.1 ±0.07	2.4 ±0.02	4.9 ±0.07	0.30 ±0.02	0.20 ±0.0	3.10 ±0.00	9.1 ±0.07
		6h							3.29 ±0.040	11 ±0.3	3.15 ±0.040	8.5 ±0.2	2.0 ±0.3	4.9 ±0.1	0.30 ±0.05	0.20 ±0.0	3.15 ±0.040	8.5 ±0.2
		24h							3.32 ±0.00	11 ±0.1	3.19 ±0.00	8.2 ±0.2	1.8 ±0.04	4.8 ±0.03	0.30 ±0.02	0.20 ±0.01	3.19 ±0.00	8.2 ±0.2
		48h							3.36 ±0.020	10 ±0.0	3.24 ±0.00	8.1 ±0.07	1.8 ±0.07	5.0 ±0.1	0.30 ±0.0	0.20 ±0.0	3.24 ±0.00	8.1 ±0.07
		14d							3.48 ±0.20	7.5 ±1	3.51 ±0.16	7.5 ±1	1.5 ±0.2	3.8 ±2	1.8 ±2	0.30 ±0.06	3.51 ±0.16	7.5 ±1
Valvin Muscat	2012	2h	20.7	3.48	5.6	57.7	108	166	3.54 ±0.00	7.4 ±0.4	3.52 ±0.00	5.1 ±0.01	1.9 ±0.2	2.6 ±0.2	0.28 ±0.0	0.23 ±0.02	3.31 ±0.010	7.7 ±0.07
		24h							3.63 ±0.00	7.1 ±0.1	3.63 ±0.00	4.8 ±0.05	1.6 ±0.01	2.5 ±0.0	0.26 ±0.0	0.22 ±0.0	3.33 ±0.00	7.4 ±0.0
		48h							3.62 ±0.00	7.4 ±0.02	3.63 ±0.00	5.0 ±0.03	1.4 ±0.2	2.6 ±0.02	0.29 ±0.01	0.25 ±0.0	3.34 ±0.020	7.5 ±0.07
		7d							3.59 ±0.020	7.6 ±0.09	3.77 ±0.020	5.6 ±0.02	1.2 ±0.06	3.1 ±0.2	0.57 ±0.0	0.33 ±0.01	3.71 ±0.020	7.5 ±0.0
Frontenac gris	2011	2h	21.2	2.95	16	32.1	360	392	2.94 ±0.020	15 ±0.4	2.92 ±0.00	13 ±0.3	5.5 ±0.1	6.4 ±0.01	0.40 ±0.02	0.40 ±0.0	3.31 ±0.040	9.8 ±0.4
		24h							2.94 ±0.010	14 ±0.2	2.91 ±0.020	13 ±0.02	5.0 ±0.09	6.7 ±0.05	0.30 ±0.0	0.40 ±0.0	3.30 ±0.00	9.8 ±0.1
		7d							2.93 ±0.00	15 ±0.3	2.96 ±0.00	13 ±0.1	4.4 ±0.0	5.0 ±0.0	0.50 ±0.0	0.40 ±0.0	3.37 ±0.00	9.6 ±0.09
La Cresent	2011	2h	19.9	3.01	14	6.60	197	204	3.17 ±0.00	13 ±0.2	3.11 ±0.00	11 ±0.1	3.3 ±0.0	6.3 ±0.05	0.40 ±0.01	0.30 ±0.01	3.42 ±0.040	9.2 ±0.07
		24h							3.39 ±0.010	11 ±0.02	3.23 ±0.00	9.5 ±0.7	2.7 ±0.0	6.3 ±0.06	0.40 ±0.0	0.40 ±0.04	3.43 ±0.040	8.9 ±0.3
		7d							3.18 ±0.00	13 ±1	3.23 ±0.010	9.9 ±0.07	2.5 ±0.3	5.7 ±0.05	0.50 ±0.04	0.40 ±0.0	3.45 ±0.00	9.2 ±0.07

[1]TA: Titratable acidity in tartaric acid equivalents (TAE)
[2]AMM: Ammonium ions; Measured by enzymatic analysis.
[3]PAN: Primary amino nitrogen; Measured by enzymatic analysis.
[4]YAN: Yeast assimilable nitrogen; Measured by enzymatic analysis.

Table 2.8. Average pH and titratable acidity (TA) values during winemaking for wine grape cultivars and pre-fermentation cold soak treatment across all production years[1].

Grape	Treatment	pH				TA (g/L)			
		Crushing[2]	End of Alcoholic Fermentation	Post Cold Stabilization	Bottling	Crushing	End of Alcoholic Fermentation	Post Cold Stabilization	Bottling
Riesling	2h	3.12abc ±0.070	3.07abcd ±0.090	2.97f ±0.090	2.97f±0.090	8.1b ±0.8	9.3a ±0.6	8.5b ±1	8.2b ±1
	4h		3.09abcd ±0.080	2.99f ±0.070	2.99f ±0.070		8.7a ±0.8	8.0b ±0.8	8.0b ±0.8
	24h		3.16ab ±0.080	3.05def ±0.090	3.05def ±0.090		8.7a ±0.7	7.4b ±0.3	7.4b ±0.3
	24h E		3.17a ±0.080	3.08bcde ±0.060	3.08bcde ±0.060		8.9a ±0.6	7.9b ±0.6	7.9b ±0.6
	48h		3.19ab ±0.060	3.03ef ±0.12	3.03ef ±0.12		8.6a ±0.8	7.5b ±0.3	7.4b ±0.3
	7d		3.11cdef ±0.020	3.08f ±0.080	3.10f ±0.12		9.8a ±0.7	7.8b ±0.8	7.8b ±0.8
Gewürztraminer	2h	3.40ef ±0.14	3.45fg ±0.24	3.42g ±0.27	3.25g ±0.11	5.3h ±0.7	6.4b ±0.9	5.7de ±1	6.7de ±0.2
	4h		3.45de ±0.18	3.41efg ±0.23	3.25efg ±0.070		6.2b ±1	5.6def ±1	6.7def ±0.3
	24h		3.58ab ±0.14	3.54cd ±0.23	3.34cd ±0.090		6.3bc ±0.9	5.3efg ±1	6.3efg ±0.4
	24h E		3.61a ±0.15	3.60bc ±0.24	3.39bc ±0.11		5.8cd ±0.9	5.1fg ±1	6.2fg ±0.4
	48h		3.63a ±0.14	3.59c ±0.26	3.38c ±0.10		6.1cd ±0.7	5.1gh ±1	6.0gh ±0.4
	7d		3.50bc ±0.10	3.68a ±0.14	3.61a ±0.10		8.5a ±1	5.4efg ±0.7	6.0efg ±0.2
Traminette	2h	3.02def ±0.010	3.08cde ±0.050	2.95ef ±0.050	3.11ef ±0.16	7.5b ±0.1	10a ±1	7.5b ±3	8.3b ±0.4
	4h		3.10cd ±0.060	2.95f ±0.060	3.01f ±0.090		9.6a ±1	7.9b ±2	8.3b ±0.4
	24h		3.22ab ±0.030	3.06def ±0.090	3.11def ±0.090		8.8b ±0.8	8.2b ±0.9	7.9b ±0.5
	24h E		3.23ab ±0.020	3.04cde ±0.050	3.13cde ±0.10		9.0b ±1	8.6b ±1	8.2b ±0.4
	48h		3.22ab ±0.010	3.09cd ±0.060	3.16cd ±0.10		8.6b ±1	8.1b ±1	7.7b ±0.5
	7d		3.16bc ±0.050	3.24a ±0.040	3.30a ±0.070		12a ±2	8.0b ±1	7.7b ±0.7
Valvin Muscat	2h	3.32e ±0.19	3.38cde ±0.18	3.31e ±0.24	3.21e ±0.12	7.7bc ±2	9.4a ±2	7.1cd ±2	8.4cd ±0.8
	24h		3.47bcd ±0.17	3.41de ±0.25	3.26de ±0.080		9.0a ±2	6.5de ±2	7.8de ±0.4
	48h		3.49abc ±0.15	3.43cde ±0.22	3.29cde ±0.050		8.9ab ±2	6.5de ±2	7.8de ±0.3
	14d		3.49ab ±0.12	3.64e ±0.18	3.61a ±0.15		7.5e ±0.5	6.5e ±1	7.5e ±0.5
Frontenac gris	2h	2.95c ±0.00	2.94c ±0.020	2.92c ±0.00	3.31ab ±0.040	15.6a ±0.0	15a ±0.3	13bc ±0.2	9.8d ±0.4
	24h		2.94c ±0.010	2.91c ±0.020	3.30b ±0.00		14b ±0.1	13c ±0.0	9.8d ±0.1
	7d		2.93c ±0.00	2.96c ±0.00	3.37a ±0.00		15a ±0.2	13c ±0.1	9.6d ±0.0
La Crescent	2h	3.01e ±0.00	3.17c ±0.00	3.11d ±0.00	3.42a ±0.040	13.5a ±0.0	13a ±0.2	11c ±0.1	9.2d ±0.0
	24h		3.29b ±0.010	3.23bc ±0.00	3.43a ±0.040		11bc ±0.0	9.5d ±0.6	8.9d ±0.2
	7d		3.18cd ±0.00	3.23bc ±0.010	3.45a ±0.00		13ab ±1	9.9cd ±0.0	9.2d ±0.0

[1] Values with the same letter are not significantly different. ANOVA with Tukey's HSD test was performed within each cultivar across production years ($p>0.05$).
[2]Crush samples were collected in duplicate immediately following destemming and crushing and prior to segregation of grape must into treatment lots.

Monomeric phenolic composition of wines.

Riesling, Gewürztraminer, and Traminette. The monomeric phenolic compositions of the 2011-2013 Riesling, Gewürztraminer, and Traminette wines can be found in Tables 2.9-2.11, respectively. *Trans*-caftaric acid was generally present at concentrations ten times higher than other monomeric phenolics. Concentrations of the flavan-3-ols (+)-catechin and (-)-epicatechin tended to increase with increases in CS duration with the differences being the most pronounced between the CS and FS samples. However, a clear relationship between CS duration and phenolic compound concentration was not found when looking at the results of all of the phenolic compounds together.

Valvin Muscat, Frontenac gris, La Crescent. Monomeric phenolic compositions of Valvin Muscat, Frontenac gris, and La Crescent wines produced in 2011, and Valvin Muscat wines produced in 2012, are shown in Table 2.12. Total monomeric phenolic concentrations for Valvin Muscat and Frontenac gris wines increased with increasing CS duration, but this trend was not observed in La Crescent wines. Notable significant increases in individual compound concentrations between the CS and FS treatments were seen for gallic acid, (+)-catechin, and (-)-epicatechin in the 2011 and 2012 Valvin Muscat wines (Table 2.12). The differences were especially pronounced for (+)-catechin and (-)-epicatechin, with the concentrations of these compounds increasing by a factor of ten or more as a result of FS. Significant increases in *c*-coutaric acid and *t*-coutaric acid concentrations among the Valvin Muscat samples were also seen, although the differences were only seen in one year for each of the compounds. For Frontenac gris wines significant increases in gallic acid, (+)-catechin, (-)-epicatechin, *c*-coutaric acid, *t*-fertaric acid, and ferulic acid concentrations were seen between CS and FS treatments. Significant differences in compound concentrations were not seen for the La Crescent wines (Table 2.12).

Changes in t-caftaric acid and GRP. There were some differences among the *t*-caftaric acid concentrations of C and FS treatments of the same cultivar and vintage collected at the end of alcoholic fermentation. However, there were no differences in GRP concentrations among treatments with the exception of the 2011 Riesling 24 E CS treatment, which was significantly different than the 4h CS, 48h CS, and 7d FS treatments. The 2012 Traminette 24h E CS and 2012 Valvin Muscat 24h CS treatments were also different than the 7d FS treatment from the same vintage and cultivar (Tables 2.9-2.12.)

Table 2.9. Monomeric phenolic compound profiles of the post alcoholic fermentation samples of the 2011-2013 Riesling pre-fermentation cold soak and on-skin fermentation treatments[1].

Year	Treatment	[Individual Monomeric Phenolic] (ppm)																	[Total Monomeric Phenolic] (ppm)
		Gallic Acid	Protocatechuic Acid	Catechin	Epicatechin	Cinnamic Acid	GRP	t-Caftaric Acid	Caffeic Acid	c-Coutaric Acid	t-Coutaric Acid	t-Fertaric	Coumaric Acid	Ferulic Acid	Caffeic Acid Ethyl Ester	Coumaric Acid Ethyl Ester	Quercetin-3-Galactoside	Quercetin-3-Glucoside	Quercetin-3-Rhamnoside	
2011	2h	nd	0.690e ±0.020	1.72g ±0.51	1.27cd	nd	0.627de ±0.050	35.2def ±4.2	nd	3.37de ±0.28	5.63fgh ±0.82	2.78bcd ±0.080	nd	nd	0.125fg ±0.00	nd	nd	0.0940b ±0.020	nd	50.5b ±6.4
	4h	0.0400c ±0.030	0.711e ±0.010	2.89efg ±0.72	nd	nd	0.659de ±0.080	37.0def ±3.1	nd	3.38de ±0.13	6.07efg ±0.58	2.14cd ±1.4	nd	nd	nd	nd	nd	0.133b ±0.020	nd	53.1b ±3.7
	24h	0.570c ±0.020	0.844e ±0.020	4.69defg ±0.44	1.83d ±0.00	nd	0.870cde ±0.070	40.9cd ±0.30	nd	5.90ab ±0.020	6.91de ±0.050	2.85bcd ±0.020	nd	nd	0.119fg ±0.00	nd	nd	0.172b ±0.010	nd	64.7b ±1.51
	24h E	0.767c ±0.070	0.661e ±0.14	7.66def ±1.0	3.12cd ±0.56	nd	1.80bc ±0.81	54.1a ±7.6	nd	5.31abc ±0.93	8.17bc ±0.14	3.29bc ±0.27	nd	nd	nd	nd	0.112b	0.836ab ±0.60	nd	85.0ab ±10.
	48h	0.483c ±0.020	0.751e ±0.030	6.28defg ±0.17	2.09d ±0.070	nd	0.661de ±0.040	36.8def ±0.31	4.99a ±0.030	2.94de ±1.6	6.53def5 ±0.060	2.58bcd ±0.040	nd	0.174a ±0.020	0.0760fg ±0.00	nd	nd	0.166b ±0.020	nd	62.0b ±1.7
	7d	3.70a ±0.48	1.31cde ±0.17	24.6a ±3.2	13.2b ±1.8	nd	0.489e ±0.040	28.4fg ±3.6	nd	6.49a ±0.76	5.12gh ±0.59	2.37bcd ±0.33	1.24a ±0.28	0.763b ±0.030	0.283e ±0.040	0.0610a ±0.00	nd	0.254b ±0.060	nd	87.3ab ±11
2012	2h	0.534c ±0.67	0.786de ±0.060	nd	nd	0.183b ±0.00	1.83abcde ±0.38	19.4g ±1.5	6.59abcde	nd	1.10k	1.25d ±0.030	0.259b ±0.00	nd	1.57d ±0.080	0.428c ±0.020	nd	nd	nd	30.1b ±3.0
	4h	0.208c ±0.00	1.85abc ±0.34	1.86fg ±0.00	0.574a	0.227ab ±0.00	1.07cde ±0.070	28.6efg ±0.21	nd	1.61e	2.38jk ±0.010	6.60a	0.560b ±0.030	nd	1.70cd ±0.050	0.568d ±0.00	nd	nd	nd	44.7a ±2.4
	24h	1.01bc	1.36bcde ±0.59	2.82defg ±0.020	0.973cd	0.201ab ±0.00	0.955cde ±0.060	30.6defg ±0.70	5.58ab ±0.050	1.90e ±0.030	2.78ijk ±0.050	3.37bcd ±0.060	0.484b ±0.030	nd	1.83bc ±0.00	0.650bd ±0.00	nd	nd	nd	53.5ab ±1.5
	24h E	0.759bc ±0.10	2.30ab ±0.010	5.43defg ±3.5	1.11d ±0.00	0.212ab ±0.010	1.91abcd ±0.38	35.6def ±1.2	5.66ab ±0.16	2.18e ±0.090	3.32ij ±0.030	3.66abcd	0.495b ±0.010	0.193a	1.95b ±0.060	0.699bf ±0.010	nd	0.0390b ±0.00	nd	63.6ab ±4.5
	48h	0.340c ±0.040	2.32a ±0.020	3.34defg ±2.2	1.19cd	0.209ab ±0.040	1.07cde ±0.14	33.1def ±0.61	6.82b ±0.040	1.40e ±0.050	3.17ij ±0.050	2.33bcd ±1.4	0.479ab	nd	1.90b ±0.010	0.683bf ±0.00	nd	0.0370b ±0.010	nd	57.6ab ±2.7
	7d	2.28b ±0.14	0.791de ±1.0	18.7ab ±1.3	6.57cd ±1.8	0.277a ±0.040	1.68abcde ±0.020	41.7bcde ±1.6	3.62c ±0.36	3.25cde ±0.16	7.14cde ±0.37	3.79abc ±0.040	0.830ab ±0.030	1.14c ±0.050	0.204ef	nd	0.221a ±0.010	2.09a ±0.030	nd	94.2ab ±6.3
2013	2h	nd	nd	nd	1.00cd	nd	2.01abcde ±0.42	36.3cdef ±0.42	1.32d ±0.81	1.21e ±0.43	4.39ghi ±0.16	2.97bcd ±0.040	nd	nd	0.0520fg	nd	nd	nd	nd	48.7ab ±2.0
	4h	0.537c ±0.11	1.18abcde	7.44defg	nd	nd	2.62abc	49.7abc ±4.2	1.14d	2.34e ±0.27	7.18bcde ±0.89	3.32bcd	nd	nd	0.105efg	nd	nd	nd	nd	67.6ab ±0.20
	24h	0.663bc ±0.010	1.44abcde ±0.00	9.51cd ±0.020	nd	0.132b	2.62abc	53.2ab ±0.79	0.814d	2.55de ±0.020	7.89bcd ±0.090	3.44bcd ±0.060	nd	nd	0.111fg ±0.00	nd	nd	nd	nd	80.5ab ±0.18
	24h E	0.727bc ±0.00	1.71abc ±0.050	5.40b	0.788d ±0.34	0.140b ±0.00	1.30abc	49.4abc ±0.44	8.97e ±0.041	1.91e ±0.017	4.35hi ±0.049	3.53bcd ±0.045	1.02ab	0.101a	3.01a ±0.050	1.18e ±0.040	nd	nd	nd	83.6ab ±5.3
	48h	0.963bc ±0.22	1.28cde ±0.020	8.77def ±5.5	1.33d ±0.040	0.141b	2.82ab ±0.040	57.9a ±0.29	1.31d ±0.040	2.81de ±0.00	8.85b ±0.010	3.30bcd ±0.27	nd	nd	nd	nd	nd	nd	nd	89.4ab ±5.7
	7d	2.04b ±1.1	0.914de ±0.010	15.6bc ±1.5	8.38bc ±4.2	0.175b ±0.00	2.95a ±0.81	57.4a ±2.2	1.67d ±0.47	4.61bcd ±0.060	11.6a ±0.17	3.91b ±0.030	nd	0.833b ±0.00	nd	0.771f	0.111b ±0.00	0.899ab ±0.90	1.71 ±0.040	106.ab ±9.1

[1] Values with the same letter are not statistically significant. ANOVA with Tukey's HSD test were performed for each grape cultivar across all years (p>0.05).

Table 2.10. Monomeric phenolic compound profiles of the post alcoholic fermentation samples of the 2011-2013 Gewürztraminer pre-fermentation cold soak and on-skin fermentation treatments[1].

Year	Treatment	[Individual Monomeric Phenolic] (ppm)																		[Total Monomeric Phenolic] (ppm)
		Gallic Acid	Protocatechuic Acid	Catechin	Epicatechin	Cinnamic Acid	GRP	t-Caftaric Acid	Caffeic Acid	c-Coutaric Acid	t-Coutaric Acid	t-Fertaric	Coumaric Acid	Ferulic Acid	Caffeic Acid Ethyl Ester	Coumaric Acid Ethyl Ester	Quercetin-3-Galactoside	Quercetin-3-Glucoside	Quercetin-3-Rhamnoside	
2011	2h	nd	1.08de ±0.00	2.86b ±0.24	nd	nd	3.71a ±0.11	5.10bcde ±0.020	nd	1.26bcd ±0.00	0.755bcd ±0.00	0.574a ±0.34	nd	nd	nd	nd	nd	nd	nd	7.81d ±8.9
	4h	1.44a ±2.0	1.36de ±0.070	5.02b ±2.8	4.45ab	nd	6.36a ±6.0	6.82bcde ±0.30	nd	1.61bcd ±0.16	1.05bcd ±0.37	0.973a ±0.43	0.161a	nd	nd	nd	nd	nd	nd	23.0cd ±9.6
	24h	0.470a ±0.010	2.20cde ±0.070	7.58b ±0.33	3.82b ±0.25	nd	2.97a ±0.17	13.5abc ±0.73	nd	2.13bcd ±0.040	2.93bcd ±0.16	0.881a ±0.030	nd	nd	nd	nd	nd	0.227a ±0.030	nd	36.7abc ±1.3
	24h E	0.337a ±0.00	2.01cde ±0.040	6.79b ±0.13	3.84b ±0.20	nd	2.75a ±0.090	8.47bcde ±0.060	nd	3.59a ±0.040	0.743d ±0.010	0.861a ±0.010	0.579a	nd	1.09ab ±0.030	0.447a ±0.00	nd	0.0430a ±0.00	0.0490abc	30.1bcd ±1.4
	48h	0.409a ±0.010	2.19cde ±0.050	7.39b ±0.060	4.10b ±0.18	nd	2.29a ±0.31	13.9ab ±0.12	nd	2.43abc ±0.090	3.11bc ±0.040	0.860a ±0.010	0.238a ±0.080	0.428a	0.206c ±0.060	0.0960b ±0.010	nd	0.171a ±0.11	nd	37.4abc ±0.24
	7d	1.99a ±0.71	1.78cde ±0.41	19.8a ±6.9	11.9a ±4.3	nd	2.97a ±0.13	12.1abcde ±0.72	nd	2.63ab ±0.46	1.84bcd ±0.29	1.16a ±0.070	0.134a	1.18a ±0.10	nd	nd	0.356a ±0.11	1.72a ±0.40	0.381abd	58.4a ±15
2012	2h	0.117a ±0.00	2.71cd ±0.28	6.94b ±1.0	nd	0.180a	4.09a ±0.62	0.545e ±0.15	2.45ab ±0.19	nd	0.529cd ±0.16	nd	nd	0.910a ±0.070	0.731bc ±0.060	0.143b	nd	nd	nd	19.2bcd ±0.24
	4h	0.215a ±0.00	3.41c ±0.00	7.39b ±0.44	nd	0.173a	3.72a ±0.020	1.44de ±0.53	3.88ab ±0.23	nd	0.778bcd ±0.030	nd	0.889a	0.947a ±0.070	1.48ab ±0.090	0.307d ±0.00	nd	nd	nd	24.1bcd ±0.91
	24h	0.879a ±0.70	5.48b ±1.6	10.6ab ±0.50	1.52ab	0.208a ±0.00	2.86a ±1.3	1.48de ±0.16	4.98a ±0.090	0.886bcd	0.579bcd ±0.56	1.06a	2.11a ±0.19	0.902a ±0.93	1.80a ±0.00	0.637c ±0.00	nd	nd	nd	34.3abcd ±1.9
	24h E	1.37a ±0.040	6.72ab ±0.32	11.4ab ±0.41	1.86b ±0.46	0.197a ±0.010	3.12a	1.02bcde	nd	1.51bcd ±0.040	0.108bcd	5.71a ±6.4	1.75a ±0.00	1.61a ±0.00	0.656bc ±0.050	0.441a ±0.00	nd	nd	nd	35.4abcd ±4.1
	48h	1.06a ±0.83	7.51a ±0.35	12.2ab ±0.010	1.63b ±0.060	0.195a ±0.010	2.16a ±0.090	1.70cde ±0.34	2.75ab ±2.5	0.736d ±0.81	2.50bcd ±3.1	1.24a ±0.010	1.28a ±1.5	1.00a ±1.0	1.78abc ±0.080	0.726c ±0.010	nd	nd	0.0750ac ±0.00	38.6abcd ±1.2
	7d	2.01a ±0.74	1.74cde ±0.73	10.4ab ±2.7	9.56ab ±2.4	0.202a ±0.00	1.71a ±0.39	12.4abcde ±3.0	nd	1.71bcd ±0.16	3.01abcd ±0.31	1.66a ±0.90	0.619a	1.61a	nd	nd	0.417a ±0.12	1.80a ±0.27	0.419bd ±0.080	48.1abc ±10
2013	2h	0.778a	1.04cde	nd	nd	0.185a ±0.00	1.93a ±0.94	2.81bcde ±0.51	0.474ab ±0.050	0.596cd ±0.050	0.134bcd ±0.020	0.625a ±0.00	nd	nd	0.00100c ±0.010	nd	nd	nd	nd	7.67bcd ±1.4
	4h	0.540a ±0.040	2.14cde ±0.020	7.00b ±0.26	1.18b ±0.010	0.216a ±0.00	3.17a ±0.55	10.2abcde ±0.42	0.826b ±0.030	0.731d ±0.020	1.28bcd ±0.00	0.694a ±0.040	0.100a	nd	nd	nd	nd	nd	nd	28.0abcd ±1.3
	24h	0.440a ±0.32	2.14cde ±0.060	8.99ab	1.61b ±0.030	0.226a	2.80a	14.9abcd ±0.82	1.29ab ±0.00	1.07bcd ±0.090	2.12bcd ±0.22	0.748a ±0.00	0.129a	nd	0.151c ±0.12	0.0590b ±0.00	nd	nd	nd	30.6abcd ±10
	24h E	0.932a ±0.95	2.90cd ±0.86	7.85b ±3.1	1.48b ±0.13	0.142a	3.94a ±1.5	3.25bcde ±3.8	0.776ab	0.630d ±0.19	3.67ab ±2.2	0.798a ±0.00	0.679a	0.352a	1.39ab ±0.83	0.316d ±0.070	nd	nd	nd	28.1abcd ±0.12
	48h	0.756a ±0.73	2.11cde ±0.030	9.62ab ±0.32	1.69b ±0.030	0.214a ±0.00	2.58a ±0.22	13.9abcde ±0.090	1.63ab ±0.080	0.871cd ±0.010	1.77bcd ±0.010	0.749a ±0.00	0.189a	nd	0.367c ±0.020	0.0810b ±0.00	nd	nd	nd	36.5abcd ±0.92
	7d	0.0820a ±0.00	0.685e ±0.99	11.5ab ±5.7	6.59ab ±4.9	1.21a ±2.0	2.51a ±0.97	20.4a ±9.4	1.13b ±0.68	2.36abc ±1.1	5.46a ±0.10	1.45a	0.661a ±0.87	0.819a ±0.58	0.0880c	0.0210b	2.25b ±2.6	2.85b ±2.4	0.530d ±0.060	51.0ab ±23

[1] Values with the same letter are not statistically significant. ANOVA with Tukey's HSD test were performed for each grape cultivar across all years ($p>0.05$).

Table 2.11. Monomeric phenolic compound profiles of the post alcoholic fermentation samples of the 2011-2013 Traminette pre-fermentation cold soak and on-skin fermentation treatments[1].

Year	Treatment	[Individual Monomeric Phenolic] (ppm)																	[Total Monomeric Phenolic] (ppm)
		Gallic Acid	Protocatechuic Acid	Catechin	Epicatechin	Cinnamic Acid	GRP	t-Caftaric Acid	Caffeic Acid	c-Coutaric Acid	t-Coutaric Acid	t-Fertaric	Coumaric Acid	Ferulic Acid	Caffeic Acid Ethyl Ester	Coumaric Acid Ethyl Ester	Quercetin-3-Galactoside	Quercetin-3-Glucoside	Quercetin-3-Rhamnoside	
2011	2h	nd	0.854f ±0.21	1.92h ±0.13	0.745d	nd	1.42b ±0.18	21.1ghi ±0.76	0.471a	2.92de ±0.040	4.40hij ±0.020	0.738cde ±0.050	nd	nd	nd	nd	nd	0.165b ±0.12	nd	33.6h ±0.65
	4h	nd	0.895f ±0.13	2.23g ±0.51	nd	nd	1.32b ±0.040	19.1i ±0.70	nd	3.40cde ±0.30	4.64hi ±0.15	0.798bcde ±0.030	nd	nd	nd	nd	nd	0.0930b ±0.00	nd	32.5h ±1.7
	24h	0.150b ±0.010	1.52bcde ±0.00	4.61efgh ±0.43	1.39d ±0.030	nd	1.30b ±0.050	26.5ef ±0.10	nd	5.36abc ±0.34	8.73cde ±0.10	0.931abc ±0.030	nd	nd	nd	nd	nd	0.302b ±0.020	nd	50.0efg ±0.88
	24h E	0.154b ±0.010	1.19cdf ±0.13	4.01fgh ±0.94	1.66d ±0.23	nd	1.28b ±0.13	25.8f ±2.4	nd	5.12bcd ±0.43	7.73ef ±0.69	0.875abcd ±0.080	0.301ab ±0.040	nd	nd	0.328a ±0.020	nd	0.236b ±0.040	nd	48.2fg ±5.2
	48h	0.401b ±0.10	1.38bcdef ±0.070	3.78fgh ±0.24	1.93d ±0.070	nd	1.39b	26.2ef ±2.4	nd	5.05bcd ±0.41	8.77cde ±0.75	1.04ab ±0.11	nd	nd	nd	nd	0.479a ±0.040	0.395b	nd	48.9efg ±5.0
	7d	1.65a ±0.11	0.984df ±0.28	11.1b ±0.57	4.63b ±0.32	nd	nd	24.0fg ±1.5	4.19bd	3.06cde ±4.2	11.1b ±0.65	0.854abcd ±0.040	1.68a ±0.020	nd	nd	0.106b ±0.00	0.250b ±0.040	2.90a ±0.19	0.230a ±0.030	61.2bcde ±7.8
2012	2h	0.0710ab ±0.00	1.83abcdef ±0.22	4.07defgh	nd	0.244bc ±0.00	2.22ab ±0.74	11.1jk ±0.17	3.51bd ±0.030	2.02cde ±0.11	0.910k ±0.030	0.712bcde ±0.00	0.306ab ±0.010	nd	0.693a ±0.38	0.503e ±0.00	nd	nd	nd	26.1h ±1.2
	4h	0.0470b ±0.00	1.92abe ±0.040	4.95defgh ±0.080	1.12d ±0.19	0.193c ±0.10	2.71ab ±0.36	24.7fg ±0.16	4.71b ±0.060	2.48cde ±0.010	4.17hij ±0.050	0.870abcde ±0.00	0.442ab	nd	1.26a ±0.00	0.994g ±0.00	nd	nd	nd	50.4defg ±0.88
	24h	0.456ab ±0.070	2.56a ±0.27	8.57bcde ±0.00	1.44d ±0.010	0.338abc	2.75ab ±0.39	23.9fgh ±0.62	6.73c ±0.010	3.46bcde ±0.00	4.36hij ±0.070	0.912abcd ±0.00	0.754ab	0.906a	2.10a ±0.020	1.84c ±0.050	nd	0.0530b ±0.020	nd	60.1bcdef ±0.76
	24h E	0.0780ab	1.84abce ±0.26	7.81bcdef ±0.080	1.44d ±0.00	0.327ab ±0.010	2.38b ±0.33	19.3hi ±0.11	7.26c ±0.56	1.79e ±0.47	2.91jk ±0.010	0.781bcde ±0.00	0.881ab ±0.13	nd	7.27a ±7.1	1.69d ±0.020	nd	nd	nd	55.7cdef ±6.9
	48h	0.335ab ±0.29	1.76abce ±0.68	9.64bcd ±1.0	1.88cd ±0.29	0.323abc ±0.00	2.49ab ±0.22	24.4fgh ±0.30	4.78bd	3.45bcde ±0.030	5.57gh ±0.010	0.843abcde ±0.00	0.635ab ±0.020	nd	1.46a ±0.00	1.33f ±0.060	nd	0.133b ±0.020	nd	56.7cdef ±1.7
	7d	0.767ab ±0.51	1.04cdf ±0.040	6.49cdefg ±0.59	3.86bc ±0.28	0.356ab ±0.00	4.56a ±0.23	35.8bc ±2.0	nd	6.52ab ±0.57	9.35cd ±0.96	0.970abcd	1.82a ±0.24	nd	nd	nd	0.308b ±0.030	2.82a ±0.10	0.195a	74.3b ±5.4
2013	2h	0.662ab ±0.080	nd	3.93defgh	nd	0.268abc ±0.020	2.49ab ±1.7	17.0ghij ±2.4	nd	2.10cde ±0.33	2.78ijk ±0.21	0.563cde ±0.050	0.0390ab	0.043a	nd	nd	nd	nd	nd	27.8gh ±4.2
	4h	0.629ab ±0.040	1.12cdef ±0.080	6.78bcdef ±0.17	0.945d ±0.040	0.319abc ±0.010	2.43b ±1.4	31.9cd ±0.00	nd	2.61cde ±0.010	6.61fg ±0.070	0.734bcde ±0.030	nd	0.163a	0.0580a	0.0140b	nd	nd	nd	54.2cdef ±1.8
	24h	0.302ab	1.54bcdef ±0.050	6.00cdefgh ±3.4	1.43d ±0.14	0.350ab ±0.00	2.22b ±1.2	34.3bcd ±0.33	nd	3.25bcde ±0.040	7.96def ±0.18	0.738bcde ±0.00	nd	0.184a	nd	nd	nd	nd	nd	58.0bcdef ±4.1
	24h E	0.567ab ±0.14	2.04ab ±0.18	9.47bcd ±0.16	1.50d ±0.020	0.390ab ±0.00	2.42b ±0.60	30.6de ±1.0	6.70c ±0.13	2.65cde ±0.070	4.77hi ±0.00	0.795bcde ±0.030	1.74ab	0.572a	1.84a ±0.030	1.17h ±0.010	nd	nd	nd	66.0bcd ±1.7
	48h	0.544ab ±0.14	1.79abce ±0.030	10.3bc ±0.70	1.79d ±0.44	0.358ab ±0.00	2.69ab ±0.060	38.5ab ±0.40	1.32a	3.97bcde ±0.00	9.86bc ±0.17	0.544e ±0.32	0.147b ±0.00	nd	nd	nd	nd	nd	nd	71.1b ±2.3
	7d	0.919ab ±0.84	1.03cdf ±0.040	15.9a ±3.2	8.69a ±1.2	0.421a ±0.00	2.33b ±0.84	42.8a ±0.43	3.57d ±0.070	7.63a ±0.040	16.4a ±0.12	1.12a ±0.010	1.12ab ±0.60	nd	nd	0.0880b ±0.00	0.185b ±0.020	3.05a ±0.22	0.231a ±0.05	104.a ±6.2

[1] Values with the same letter are not statistically significant. ANOVA with Tukey's HSD test were performed for each grape cultivar across all years (p>0.05).

Table 2.12 Monomeric phenolic compound profiles of the post alcoholic fermentation samples of the Valvin Muscat (2011-2012), Frontenac gris (2011) and La Crescent (2011) pre-fermentation cold soak and on-skin fermentation treatments[1].

Grape	Year	Treatment	[Individual Monomeric Phenolic] (ppm)																	[Total Monomeric Phenolic] (ppm)
			Gallic Acid	Protocatechuic Acid	Catechin	Epicatechin	Cinnamic Acid	GRP	t-Caftaric Acid	Caffeic Acid	c-Coutaric Acid	t-Coutaric Acid	t-Fertaric	Coumaric Acid	Ferulic Acid	Caffeic Acid Ethyl Ester	Coumaric Acid Ethyl Ester	Quercetin-3-Galactoside	Quercetin-3-Glucoside	Quercetin-3-Rhamnoside	
Valvin Muscat	2011	2h	0.208e ±0.010	0.710c ±0.33	3.57e ±0.25	0.945cd	nd	4.36abc ±0.48	11.3bcd ±0.35	nd	1.80a ±0.070	1.95c ±0.040	0.862a ±0.040	nd	nd	nd	nd	nd	nd	nd	24.0ef ±3.6
		24h	0.641d ±0.030	0.315c ±0.060	8.48cd ±0.62	2.07cd ±0.15	nd	3.68bc ±0.50	15.4ab ±0.57	nd	2.20c ±0.070	3.36a ±0.10	0.789a ±0.030	nd	nd	nd	nd	nd	nd	nd	36.9cd ±5.0
		48h	0.848d ±0.00	0.406c ±0.00	11.2c ±0.19	2.85c ±0.050	nd	3.15c ±0.20	16.3a ±0.47	nd	2.43d ±0.040	3.62a ±0.080	0.845a ±0.020	nd	nd	nd	nd	nd	nd	nd	41.7c ±5.4
		14d	15.0a ±0.090	1.51b ±0.28	94.7a ±1.5	46.1a ±0.37	nd	2.65c ±0.50	9.90de ±0.37	nd	2.92e ±0.060	2.93b ±0.16	0.867a ±0.020	0.321a	nd	nd	nd	nd	0.660a ±0.020	nd	178a ±29
Valvin Muscat	2012	2h	1.05cd ±0.010	1.45b ±0.00	4.54de ±3.8	nd	0.324a ±0.030	4.46abc ±0.62	1.61f ±0.090	2.63a ±0.030	nd	0.907d ±0.00	nd	nd	0.178	0.671a ±0.020	0.111a	nd	nd	nd	18.1f ±1.8
		24h	1.52c ±0.010	2.20a ±0.040	7.95cde ±0.22	1.81cd ±0.030	0.353a ±0.00	3.48bc ±0.30	2.53f ±0.19	4.78b ±0.15	nd	1.03d ±0.040	nd	0.697a ±0.00	nd	1.47b ±0.010	0.211b ±0.00	nd	nd	nd	28.2def ±2.2
		48h	1.64c ±0.010	2.30a ±0.16	9.07cd ±0.27	2.80cd ±1.2	0.313a	5.47ab ±2.5	4.34ef ±0.59	4.44b ±0.020	nd	1.19d ±0.10	nd	nd	nd	1.39b ±0.020	0.241b ±0.00	nd	nd	nd	33.0cde ±2.7
		7d	6.15b ±0.54	2.00ab	41.8b ±4.2	20.4b ±1.7	0.481b ±0.00	6.21a ±0.37	9.87cde ±1.3	1.75c ±0.25	2.66f ±0.060	2.09c ±0.44	nd	nd	nd	0.127c	nd	1.28 ±0.030	5.77b ±0.10	0.280 ±0.020	100b ±11
Frontenac gris	2011	2h	2.02a ±0.54	1.13a ±0.19	3.92a ±1.4	1.52a ±0.80	nd	nd	1.60a ±1.9		1.27a ±0.28	0.464a ±0.17	1.15a ±0.36	nd	0.176a ±0.12	nd	nd	nd	nd	nd	10.63a ±1.1
		24h	2.98b ±0.21	2.92b ±0.25	7.39a ±0.95	3.38a ±0.17	nd	nd	8.06b ±0.35		1.83ab ±0.14	1.37b ±0.070	1.34ab ±0.060	nd	0.143a ±0.010	nd	nd	nd	nd	nd	27.8b ±2.8
		7d	7.01b ±0.66	1.20a ±0.11	17.4b ±3.0	8.12b ±1.2	nd	0.0190 ±0.010	4.71ab ±2.4		2.10b ±0.36	0.446a ±0.17	1.58b ±0.030	nd	0.354b ±0.060	0.0460 ±0.020	nd	0.808 ±0.030	4.44 ±0.31	nd	49.5c ±4.8
La Crescent	2011	2h	0.443a	0.613a ±0.16	2.91a ±2.1	1.72a	nd	4.55a ±1.0	3.07a	nd	nd	nd	0.255a ±0.090	0.0900a	0.426a	nd	nd	nd	0.467a	nd	12.3a ±1.6
		24h	0.638a ±0.14	0.793a ±0.25	3.42a ±1.7	1.22a ±0.18	nd	2.74a ±0.92	nd	nd	0.779a	nd	0.184a ±0.010	0.188a ±0.070	nd	nd	nd	nd	nd	nd	8.70a ±1.5
		7d	2.26a ±1.3	0.495a ±0.34	6.48a ±3.9	4.36a ±1.9	nd	2.54a ±0.56	nd	nd	0.916a ±0.65	nd	0.197a ±0.020	nd	0.429a ±0.090	0.0170	nd	nd	0.0640b ±0.00	nd	14.8a ±2.2

[1] Values with the same letter are not statistically significant. ANOVA with Tukey's HSD test were performed for each grape cultivar across all years ($p>0.05$).

Volatile compounds. Differences in monoterpene concentrations between the 2h and 48h CS treatments varied by cultivar or vintage. Notable differences were observed in nerol oxide and nerol in the 2011 Riesling, *cis*-linalool oxide in the 2012 Riesling, nerol in the 2013 Gewürztraminer, and nerol and geraniol in the 2013 Traminette (Table 2.13).

Table 2.13. Monoterpene profiles of the 2011-2013 Riesling, Gewürztraminer, and Traminette 2h and 48h pre-fermentation cold soak treatments[1].

Grape	Year	Treatment	Compound				
			cis-linalool oxide	Linalool	Nerol oxide	Nerol	Geraniol
Riesling	2011	2h	6.390 ±0.260 b	20.50 ±2.43 b	nd	262.3 ±83.9def	nd
		48h	9.740 ±1.21ab	24.70 ±0.130ab	58.20 ±2.30ab	nd	nd
	2012	2h	nd	23.28 ±0.190b	nd	201.3 ±13.5ef	nd
		48h	6.660 ±0.250b	25.80 ±1.13b	nd	299.3 ±15.6bcde	nd
	2013	2h	nd	21.29 ±0.780b	nd	142.0 ±27.2f	nd
		48h	nd	24.13 ±3.69b	nd	262.9 ±40.7cdef	nd
Gewürztraminer	2011	2h	115.4a	62.41a	34.40a	423.0 ±110abcd	250.4 ±250a
		48h	146.8 ±27.5a	71.09 ±2.84a	77.12 ±5.68a	537.5 ±96.1ab	79.13 ±2.84a
	2012	2h	128.0 ±34.6a	71.09 ±6.75a	90.93 ±11.5a	592.4 ±45.1a	101.3 ±8.91a
		48h	132.3 ±4.31a	73.16 ±5.76a	79.41 ±3.23a	614.4 ±81.2a	51.20a
	2013	2h	145.6 ±13.7a	68.80 ±11.9a	62.94 ±8.64a	nd	36.89 ±20.2a
		48h	222.3 ±3.16a	83.92 ±9.47a	88.39 ±3.16a	677.4 ±30.5a	83.92 ±3.16a
Traminette	2011	2h	158.5 ±10.9a	69.08 ±0.000a	308.2 ±332a	566.0abc	331.6 ±299a
		48h	152.4a	80.81 ±12.7a	97.16 ±14.7a	597.0ab	107.8 ±25.5a
	2012	2h	141.4 ±9.66a	69.29 ±1.72a	63.60 ±22.3a	427.1 ±53.0abcd	137.2 ±46.7a
		48h	142.7 ±11.5a	67.78 ±3.84a	84.94 ±0.640a	501.9 ±1.31ab	117.9 ±2.95a
	2013	2h	146.3 ±14.2a	75.89 ±5.89a	75.89 ±0.000a	639.5 ±5.40a	65.83 ±8.34a
		48h	134.1 ±21.6a	70.83 ±4.77a	75.50 ±16.4a	230.6 ±32.0def	nd

[1]Values with the same letter are not statistically significant. ANOVA with Tukey's HSD test was performed across all grape cultivars, pre-fermentation cold soak treatments, and years ($p>0.05$).

Bitterness intensity ratings. Sensory analysis did not reveal differences in perceived bitterness intensity among CS treatments within a cultivar (Figures 2.1-2.3). Similarly, analysis of monomeric phenolics in the wines at the time of sensory evaluation (Tables 2.15-2.17 in the Appendix) failed to show a correlation between total monomeric phenolic concentration and perceived bitterness intensity.

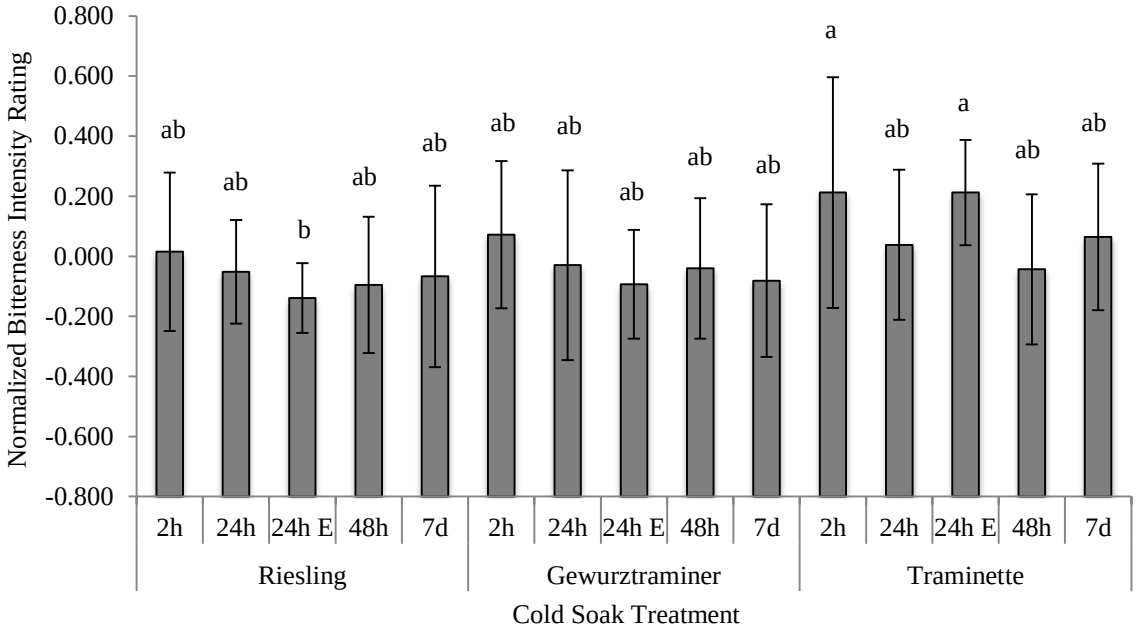

Figure 2.1[1]. Normalized bitterness intensity ratings of the 2011 Riesling, Gewürztraminer, and Traminette 2h, 24h, 24hE, and 48h pre-fermentation cold soak treatments and the 7d on-skin fermentation treatment.
[1]Pre-fermentation cold soak treatments across all cultivars with the same letter are not significantly different. (ANOVA with Tukey's HSD analysis $p>0.05$).

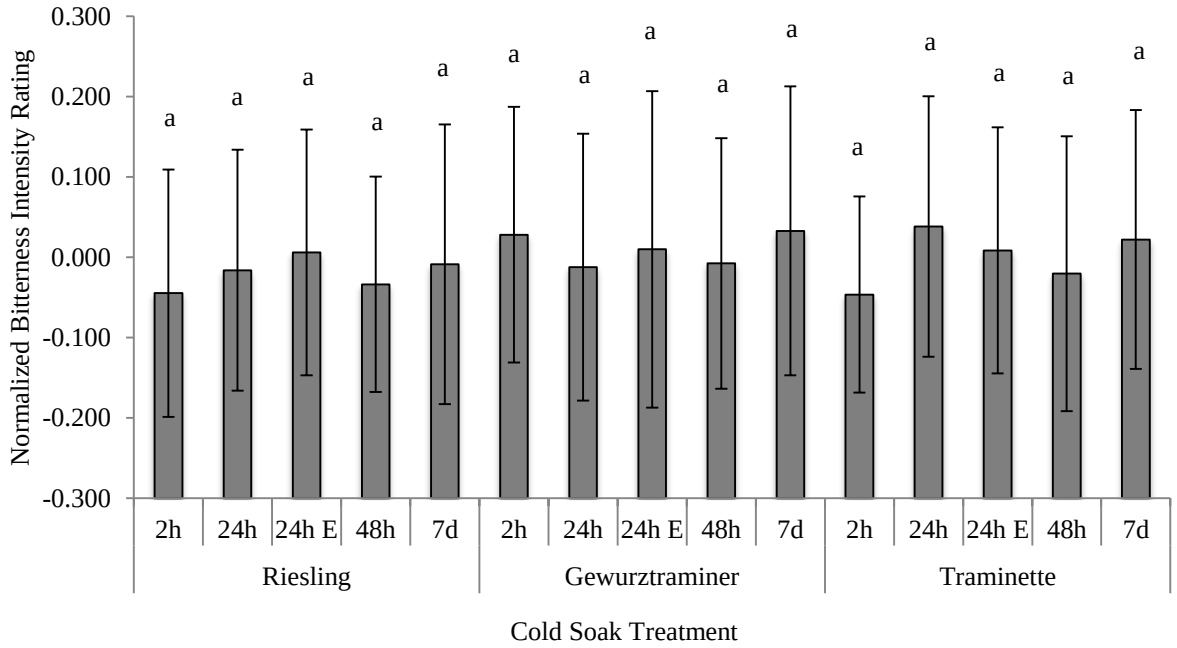

Figure 2.2[1]. Normalized bitterness intensity ratings of the 2012 Riesling, Gewürztraminer, and Traminette 2h, 24h, 24hE and 48h pre-fermentation cold soak treatments and the 7d on-skin fermentation treatment.
[1]Pre-fermentation cold soak treatments across all cultivars with the same letter are not significantly different. (ANOVA with Tukey's HSD analysis $p>0.05$).

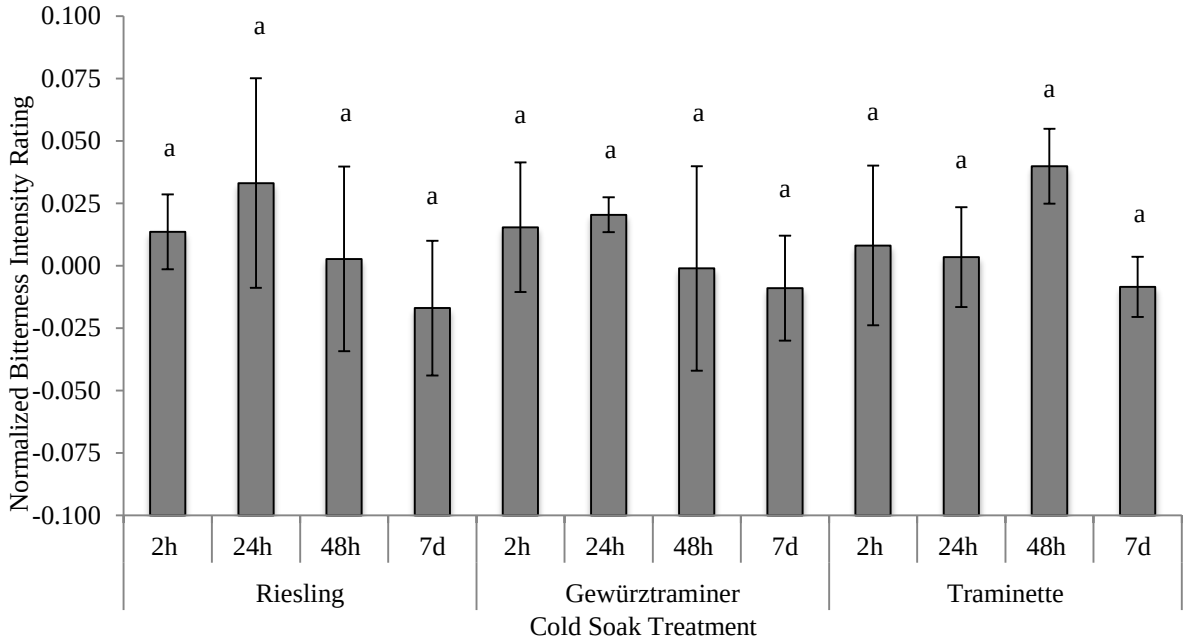

Figure 2.3[1,2]. Normalized bitterness intensity ratings of the 2013 Riesling, Gewürztraminer, and Traminette 2h, 24h, and 48h pre-fermentation cold soak treatments and the 7d on-skin fermentation treatment.
[1]Pre-fermentation cold soak treatments across all cultivars with the same letter are not significantly different. (ANOVA with Tukey's HSD analysis $p>0.05$).

[2]The 24hE pre-fermentation cold soak treatment was not analyzed in 2013

Wine color. The colors of the 2013 Riesling, Gewürztraminer, and Traminette wines at the time of sensory analysis varied with treatment (Table 2.14). Specifically, the a* (redness to greenness) values of Riesling, Gewurztraminer, and Traminette ranged from -1.17 to -1.60, -0.593 to -1.52, and -1.17 to -1.79, respectively. The b* (yellowness to blueness) values ranged from 3.85 to 7.68 for Riesling, 4.14 to 10.4 for Gewurztraminer, and 4.59 to 9.25 for Traminette.

Table 2.14. Color data for the 2013 Riesling, Gewürztraminer, and Traminette wines taken at the time of sensory analysis[1,2].

Grape	Treatment	Color Data[3]			
		L*	a*	b*	ΔE*[4]
Riesling	2h	97.8a ±0.19	-1.37bc ±0.035	4.10d ±0.075	--
	24h	96.6ab ±0.62	-1.14bc ±0.080	3.85de ±0.070	1.19c ±0.62
	48h	97.2abc ±0.26	-1.17b ±0.055	4.38ef ±0.098	0.653c ±0.19
	7d	96.3bc ±0.40	-1.60e ±0.055	7.68b ±0.14	3.88b ±0.26
Gewürztraminer	2h	97.3ab ±0.34	-1.52cd ±0.060	4.90d ±0.12	--
	24h	97.6ab ±0.21	-1.20b ±0.080	4.14ghi ±0.045	0.907c ±0.053
	48h	97.7a ±0.28	-1.24b ±0.072	4.43fg ±0.10	0.749c ±0.17
	7d	95.3c ±0.57	-0.593a ±0.073	10.4a ±0.14	5.97a ±0.16
Traminette	2h	97.7a ±0.45	-1.34bcd ±0.13	4.94hi ±0.12	--
	24h	97.3abc ±0.55	-1.33b ±0.11	4.81i ±0.025	0.575c ±0.35
	48h	96.7ab ±1.1	-1.17b ±0.13	4.59fgh ±0.12	0.938c ±0.53
	7d	96.1abc ±0.39	-1.79de ±0.098	9.25c ±0.11	4.47b ±0.13

[1]Color measurements are based on the CIELAB color scale.
[2] Values with the same letter are not statistically significant. ANOVA with Tukey's HSD test was performed across all cultivars and pre-fermentation cold soak durations ($p>0.05$)
[3]L*: Lightness (white to black); a*: sample redness (positive values)/ greenness (negative values); b*: sample yellowness (positive values)/blueness (negative values); ΔE*: total color difference compared to a standard.
[4]ΔE* for 2h pre-fermentation cold soak treatments cannot be determined as this sample was used as the standard for color analysis.

Discussion

Juice and Wine Chemistries. In all cultivars, CS duration had a larger impact on pH than TA, although both tended to increase with increasing skin contact time (Tables 2.4-2.7). In previous metadata analysis, pH was found to increase with CS duration, but juice and wine TA decreased (Boulton 1980). Wine pH depends on three major factors: the total amount of acid present, the ratio of malic acid to tartaric acid, and the quantity of potassium present (Conde et al. 2007). Wine pH can increase during fermentation through the formation of weak acids such as succinic and lactic acid; increases in the latter result in decreases in both total and titratable acidity (Boulton 1980). During CS the rate of potassium extraction from grape skins increases (Mpelasoka et al. 2003) potentially leading to an increase in overall pH. Specifically, the formation of potassium bitartrate during CS through the exchange of tartaric acid protons and potassium cations leads to a decrease in free acid resulting in an increase in overall pH (Gawel et al. 2000). Tartaric acid precipitation in the form of potassium bitartrate during fermentation or stabilization can lead to decreases in total acidity and titratable acidity and either an increase or decrease in pH depending on wine conditions (Boulton 1980).

In the current study, pH values of the wines at the end of alcoholic fermentation did increase slightly with increasing CS duration (Table 2.8), such that the post fermentation pH values of the Riesling, Gewürztraminer, Traminette, and Valvin Muscat wines, when averaged across all production years, tended to be in agreement with the metadata analysis results reported by Boulton (1980). In general, the 24h, 24hE, and 48h CS treatments of all of the cultivars had the highest pH values. However, the pH of the Riesling and Gewürztraminer 7d FS wines were lower than the pH values of the 24h, 24hE, and 48h CS wines (Table 2.8). This was likely due to the formation and precipitation of potassium bitartrate during fermentation which, due to the low pH of the wine

would result in further decreases in pH. Furthermore, post fermentation pH values were expected to be higher than those of grape must at the time of crushing, as was found in the 24h, 24hE, 48h CS and 7d FS Gewürztraminer and Traminette treatments, and the 2011 Valvin Muscat 24h and 48h CS and 14d FS treatments (Table 2.8).

There were some differences in the concentrations of tartaric acid among the treatments following cold stabilization; with wines subject to longer CS duration having lower tartaric acid levels and higher pH values. The concentrations of malic acid also varied among CS treatments but not in a linear fashion (Tables 2.4-2.7). Changes in tartaric to malic acid ratios may have contributed to observed changes in pH values among wine treatments (Boulton 1980; Gawel et al. 2000).

In contrast to previous results, which included a diverse number of data sets (Boulton, 1980), TA generally did not decrease as CS duration increased. Differences in TA and pH results in the current study compared to those reported by Boulton (1980) may be due to the rapid speed at which K^+/H^+ equilibrium occurred as shown by Harbertson and Harwood (2009) which would prevent further changes from occurring during longer cold soak times. Juice potassium concentrations of Merlot grapes have been shown to be highest after crushing and decrease during a 20-day pomace skin contact time (Harbertson and Harwood 2009). Interestingly, Gómez-Míguez et al. (2007) reported that when CS at low temperatures (5 and 10°C) the total acidity levels of various white wines remained almost constant from fermentation to bottling.

Monomeric phenolic composition. The lack of a clear relationship between CS duration and phenolic compound composition seen in this study (Tables 2.9-2.12) was surprising, as a number of previous studies reported a direct relationship between CS duration and the phenolic compound concentrations of finished wines (Gómez-Míguez et al. 2007; Gordillo et al. 2010; Sokolowsky et

al. 2015; Darias-Martín et al. 2000). Possible sources of variation between the results of the current study and those reported in the literature include differences in CS duration and holding temperature which may affect compound extraction rates from grape skin, seeds, and pulp. Differences in the content and concentrations of phenolic compounds in grape berries due to cultivar, growing location, and other factors that contribute to variation in agricultural commodities such as weather, may also contribute to the variation among the data.

Tannins. A review of the data from a previous tannin analysis of the skins and seeds of the 2011 Riesling, Gewürztraminer, and Traminette fruit suggests that these grapes did contain substantial amounts of tannins, particularly in seeds, that were not extracted during the CS process as determined by HPLC (Figure 2.4) and spectrometric analysis (Data not shown).

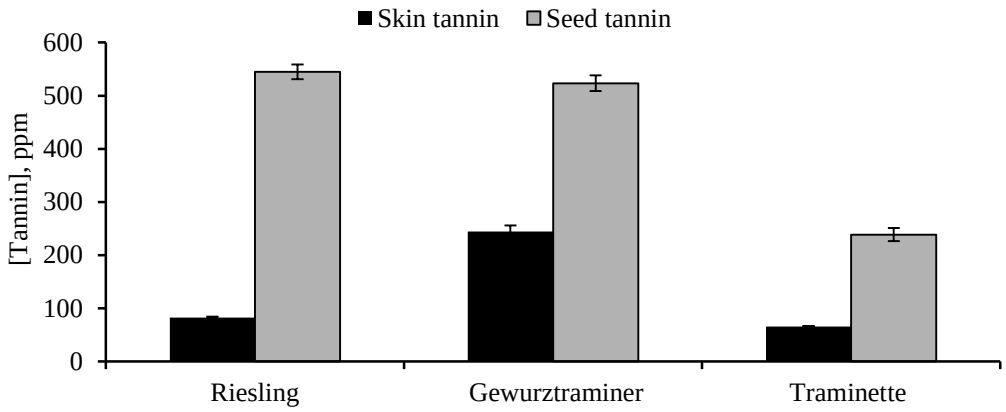

Figure 2.4. Skin and seed tannin content, as measured by High Performance Liquid Chromatography, of select white grape berries grown in the Finger Lakes New York region (Hallberg, Schmitt, Manns, and Mansfield, 2011, Unpublished).

Since there was ample time for extraction during the 7d FS treatments, it is possible that the tannins became bound to other must components during the course of fermentation. Springer and Sacks (2014) have proposed that tannins extracted during red grape skin maceration readily

bind to protein and pectin found in cell walls, especially in hybrid grape cultivars. It is possible that this tannin binding with cell wall protein or pectin could extend to certain white *Vitis vinifera* and hybrid grape cultivars, such as Riesling, Gewürztraminer, and Traminette providing an explanation for the lack of measurable tannin extracted in the current study. Additional studies are warranted to confirm this explanation.

Flavan-3-ols. In contrast to tannin, flavan-3-ol concentrations were positively influenced by CS duration (Tables 2.9-2.12), likely due to the high solubility of these compounds in water. In red wine production, flavan-3-ols are thought to be extracted from grape skins during the first two to five days of maceration (Sun et al. 1999; González-Manzano et al. 2004; Morel-Salmi et al. 2006; Aron and Kennedy 2007; Koyama et al. 2007; Gambuti et al. 2009; González-Manzano et al. 2006). In comparison, full extraction of flavan-3-ols from white cv. Viura grape seeds has been reported to take as long as two to three weeks (González-Manzano et al. 2004). Extraction rates of phenolic compounds increase during FS due to increasing ethanol levels. This effect has been seen for anthocyanins and proanthocyanidins (Canals et al. 2005) as well as glycosylated flavonols (Jeffery et al. 2008b). However, increases in flavan-3-ol extraction during FS compared to CS are most likely due to the extended skin contact time prior to ethanol production as these compounds are highly water soluble.

Changes in *t*-caftaric acid and GRP. Concentrations of *t*-caftaric and coutaric acids are known to decrease during winemaking, due to reactions with glutathione, polyphenoloxidase, and oxygen resulting in the production of Grape Reaction Product (GRP) or 2-S- glutathionyl caftaric acid, an important anti-browning agent in white wines (Singleton et al. 1985). GRP concentrations were relatively low compared to *t*-caftaric acid concentrations in all treatments, independent of cultivar and vintage; the 2012 Gewürztraminer was the only exception to this trend (Table 2.10). This

indicates that the enzymatic conversion of *t*-caftaric acid to GRP was limited. In the case of the 2012 Gewürztraminer wines, concentrations of *t*-caftaric acid ranged from 0.545 ±0.15 ppm in the 2h CS treatment to 12.4 ±9.4 ppm in the 7d FS treatment, while the GRP concentrations ranged from 4.09 ±0.62 ppm for the 2h CS treatment to 1.71 ±0.39 ppm for the 7d FS treatment. In general, the *t*-caftaric acid concentrations for the Gewürztraminer wines were lower than the concentrations of the compound observed in the Riesling and Traminette wines produced during all production years. Overall, differences in *t*-caftaric acid concentration among cultivars and treatments, due to differences in oxygenation during crush, appeared to be random and a clear relationship between CS duration and *t*-caftaric acid concentration was not evident. However, it is interesting to note that the concentration of GRP tended to decrease and the *t*-caftaric acid concentration tended to increase with increasing CS duration of the 2012 wines, although significant differences were not seen for either compound (Table 2.9-2.12). Concentrations of GRP were similar across grape cultivars and vintages.

Wine Color. As a measure of unwanted browning resulting from CS the 2013 wines used for sensory analysis were analyzed using the CIELAB color scale (Table 2.14). In the current study, the 7d FS treatments tended to have significantly different a* and b* values compared to the CS treatments within a grape cultivar (Table 2.13). Gómez-Míguez et al. (2007) reported increases in phenolic compound concentrations and color changes when *Vitis vinifera* cv. Zalema grape musts received skin contact treatments prior to fermentation (5, 10, 20°C) for 2, 4, 6, 8, 12, 18, and 24h. Color measurements show that the Zalema wines had similar a* values (-0.860 to 1.32 units) but much larger changes in b* values (5.40 to 21.3 units) compared to the wines analyzed in the current study.

Based on the CIELAB results, the 2013 CS wine samples used for sensory analysis should appear yellow with a green hue. The lightness of the samples (L*) varied very little among the CS treatments; all had strong positive L* scores, suggesting light color. However, the color of the wines did become more intense with increasing CS duration. The overall color change (ΔE) of the 7d FS treatment was significantly different than the color changes observed for the 24h and 48h CS treatments (Table 2.14). A ΔE value was not assigned to the 2h CS treatment as this treatment was used as the control for ΔE calculations.

Enzyme additions. The use of various enzymes during the winemaking process have been shown to alter the composition of juice and wine compounds (Armada et al. 2010; Bautista-Ortín et al. 2013) Surprisingly, the use of pectinase enzymes during the 24hE CS treatment was ineffective at increasing the concentration of monomeric phenolic compounds (Tables 2.9-2.12). Other enzymes, such as xylanase, cellulase, polygalacturonase and pectinmethylesterase, have been reported to contribute to the degradation of seed cell walls and thereby facilitate the release of proanthocyanidins (Bautista-Ortín et al. 2013). Glycosidase enzyme added during the pre-fermentation skin contact (6h at 8-10°C) or pectinase clarification enzyme added after fermentation of Albariño musts have been shown to result in wines with significantly different aroma characteristics compared to untreated control wines. Furthermore, the addition of glycosidase enzyme at the time of Albariño pre-fermentation skin contact resulted in higher free terpene, C_{13}-norisoprenoid, benzene derivatives, ethyl esters, and phenylethyl acetate concentrations compared to wines with added pectinase clarification enzyme and wines in which both enzymes were added (Armada et al. 2010). Interestingly, the effects of the glycosidase and clarification enzymes appeared to negate each other when used during the production of a single wine (Armada et al. 2010).

It should be noted that the 2012 wines were treated with Scottzyme KS (a blend of polygalacturonase, pectin esterase, pectin lyase, cellulose, and protease) after pressing and before fermentation. Total monomeric phenolic concentrations in the 2012 wines were not significantly different than the same treatments produced in 2011 and 2013 with a few exceptions; specifically, the total monomeric phenolic compound concentrations of the 2012 and 2011 Riesling 24h CS treatments were significantly different (Table 2.9), as were those of the 2012 and 2011 Traminette 2h CS treatments, and the 2012 and 2013 Traminette 7d FS treatments (Table 2.11). These exceptions are most likely due to agricultural product variations rather than enzyme use.

Monoterpenes. The limited differences between monoterpene compositions of Riesling, Gewürztraminer, and Traminette wine treatments (Table 2.13) suggest that skin contact duration does not impact the composition and concentrations of wine monoterpenes. This is in agreement with the findings of Rapp (1990) but contradicts the findings of others (Sánchez Palomo et al. 2007; Selli et al. 2006; Skinkis et al. 2010; Cabaroglu and Canbas 2002).

Free and glycosidically-bound monoterpenes are present in grape berries (Günata, 1985; Williams et al. 1982a; Williams et al. 1982b). As a result, only a portion of the total monoterpene content of grapes can be normally extracted during CS. It has been suggested that increasing must pH to 5.0 will allow for the activation of natural β-glucosidases in the berry and the release of monoterpenes (Rapp, 1990), though this practice is highly impractical for wine production. During the current study, the pH of the Riesling, Gewürztraminer, and Traminette wines at bottling ranged from pH 2.8-3.5 (Table 2.8), with variation occurring across grape cultivars and treatments. The naturally low pH of finished wines, and the subsequent potential for low β-glucosidase activity during CS, may contribute to the lack of variation in monoterpene extraction among the treatments.

In other works, pre-fermentation skin contact, at temperatures above those used for CS, have led to increases in free and bound aroma compound concentrations (Sánchez Palomo et al. 2007; Cabaroglu and Canbas 2002). Sánchez Palomo et al. (2007) reported increases in free and glycosidically bound aroma compounds, especially terpenes, norisoprenoids, and benzene compounds, in white Albillo (*Vitis vinifera)* wines produced with either 15h or 23h of skin contact at 18°C. In addition, the skin contact treatments intensified 'fresh' characteristics in Albillo wines due to increases in C_6 compounds as well as 'fruity' characteristics attributed to C_{13}-norisoprenoids concentrations. (Sánchez Palomo, 2007). Similarly, pre-fermentation skin contact of Muscat of Alexandria musts (7h, 15°C) was reported to increase both free and bound aroma compound levels, resulting in an improvement in overall wine quality due to increases in aroma intensity and quality (Cabaroglu and Canbas, 2002). These studies clearly link pre-fermentation skin contact to increased intensity and diversity of perceived aroma characteristics as well as increases in volatile compound concentrations, the latter of which was not seen in this research (Table 2.13).

The chemical rearrangement of monoterpenes during bottle aging (Ebeler, 2001) may account for some of the lack of variation in monoterpene content. Decreases in the concentrations of volatile compounds in white wines (pH 3.48) during extended storage has been reported in Debina (*Vitis vinifera)* white wine, where storage for up to twenty months resulted in decreases in the concentrations of isoamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, and linalool (Lambropoulos and Roussis 2007). In the current study wines were stored for one to three years between bottling and GC-MS monoterpene analysis allowing ample time for in-bottle acid-catalyzed degradation reactions to occur. Furthermore, the pH values of the CS and FS wines (Tables 2.4-2.8) were less than the pH of the Debina wine used in the Lambropoulos and Roussis

(2007) study indicating the ability of in-bottle acid-catalyzed degradation reactions to occur at the same or a higher rate than observed in the Debina wine.

Interestingly, caffeic acid and gallic acid, when added to Debina wines at a rate of 60ppm, were found to inhibit the decrease of volatile compounds (Lambropoulos and Roussis 2007). A concentration-dependent inhibitory effect of caffeic and gallic acid was also seen in a model wine matrix containing isoamyl acetate, ethyl hexanoate, linalool, and SO_2. Specifically, caffeic acid was most active at 7.5ppm and gallic acid at 15ppm. Furthermore, SO_2 enhanced the inhibitory effect of these compounds in some cases (Lambropoulos and Roussis 2007) by increasing oxygen consumption rates thereby preventing the accumulation of reactive oxygen species that could oxidize linalool in a model system. Concentrations of caffeic and gallic acid in the current study were below the reported optimal inhibitory levels in all CS wines except the 2013 Riesling 24hE treatment, which had a caffeic acid concentration of 8.97 ±0.04ppm. Caffeic acid concentrations of the 2012 2h CS (6.59ppm) and 48h CS (6.82 ± 0.04ppm) Riesling, 2012 Traminette 24h CS (6.73 ±0.010 ppm) and 24hE CS (7.26 ± 0.56ppm), and the 2013 Traminette 24hE CS (6.70 ±0.13 ppm) all came close to the reported optimal inhibitory action level (Tables 2.9-2.12). Interestingly, the caffeic acid concentrations of the 2012 Riesling and Traminette samples taken at the time of sensory analysis ranged from 0.947-13.3ppm and 8.00-17.0ppm, respectively. Caffeic acid was not detected in the 2012 Gewürztraminer wines at the time of sensory analysis, and 2011 and 2013 Riesling, Gewürztraminer, and Traminette wines had caffeic acid concentrations at or below 4.6ppm. The gallic acid concentrations of all wines were well below the concentration at which inhibition of monoterpene degradation is optimized (Tables 2.9-2.12). It should also be noted that CS and FS wines contain many more and higher concentrations of compounds that could participate in monoterpene degradation inhibition redox reactions than were present in the model

wine in the Lambropoulos and Roussis (2007) study making the data not directly transferrable. Finally, olfactory based sensory analysis of the 2h and 48h CS wines was not performed as no differences in monoterpene concentration were found during GC-MS analysis.

Bitterness intensity ratings. Sensory panel findings agree with Sokolowsky et al. (2015), who reported that increases in the total phenolic content of Riesling and Gewurztraminer wines did not have an effect on the perceived bitterness intensities of the wines, although the same group also reported that CS did have an impact on perceived bitterness intensity and other taste, aroma, and tactile characteristics. Interestingly, Skinkis et al. (2010) found that skin contact times of three or eight hours did not affect the overall aroma, bitterness, or astringency intensity of Traminette wines.

Of the monomeric phenolic compounds evaluated, (+)-catechin and (-)-epicatechin were influenced the most by CS duration, especially by the 7d FS treatment. However, the concentrations of these compounds in the Riesling, Gewürztraminer, and Traminette wines were relatively low, with a maximum concentration of 24.6 ±3.2 ppm for (+)-catechin in the 2011 Riesling 7d FS. This concentration is well below reported taste thresholds for (+)-catechin and (-)-epicatechin of 290 and 270ppm, respectively (Hufnagel & Hofmann 2008). Based on these results, it seems unlikely that flavan-3-ols have supra threshold effects on wine sensory qualities. However, phenolic compounds are known to have synergistic effects even at subthreshold concentrations, as mixtures of phenolic compounds have been shown to have higher bitterness and astringency intensities than solutions of a single phenolic compound when evaluated at the same total phenolic concentration. There is also some evidence that the presence of multiple phenolic compounds creates a synergism between bitterness and astringency (Ferrer-Gallego et al., 2014).

Interestingly, 93% of the panel reported being PROP tasters, and a large portion self-reported that PROP elicited a ‘very strong’ or ‘strongest imaginable’ taste. However, the perceived

bitterness intensity scores of the Riesling, Gewürztraminer, and Traminette wines produced in 2011-2013 were not significantly different among the treatments. While many of the panelists were sensitive to PROP, this sensitivity did not transfer to the bitter compounds found in the white wines such as the flavan-3-ols (+)-catechin and (-)-epicatechin or to the quinine sulfate used in the bitter reference samples during the 2013 vintage evaluations. This finding is corroborated by Thorngate and Noble (1995), who reported that panelist perception of bitterness and astringency of (+)-catechin and (-)-epicatechin in a hydroalcoholic solution (0.01g/L ethanol) was not influenced by PROP tasting status.

One point of interest and concern was the lack of a significant difference in the bitterness intensity ratings of the bitter reference samples used in the 2013 sensory test, except for those of the base wine and the lowest concentration bitter reference (0.025g/L quinine sulfate) (Data not shown). During training sessions, many panelists indicated that it was difficult to detect a difference between the base wine and 0.025g/L quinine sulfate samples, but that a difference between the base wine and the 0.0375g/L quinine sulfate reference sample was noticeable. In addition, panelists were able to detect the difference between the 0.0375g/L bitter reference sample and the base wine when completing a series of triangle tests.

The lack of a difference in bitterness intensity ratings within each cultivar and year may be due to low compound concentrations of bitter eliciting compounds in grapes and to compound extraction kinetics. However, other studies have concluded that total phenolic compound concentration is not correlated with perceived bitterness intensity of Riesling and Gewürztraminer wines (Sokolowsky et al. 2015). As similar results were seen in the current study in three separate sensory tests conducted over three years, it seems probable that differences in the bitterness intensity of the treatments are not present.

Conclusions

No clear relationship between CS duration and either monomeric phenolic or monoterpene concentration or perceived bitterness intensity of the wines was observed. However, as CS is associated with increases in bitterness intensity and wine color becomes darker with increases in CS duration, it is possible that a learned association between bitterness and wine color exists. Furthermore, the possible effects of CS duration on astringency perception or phenolic character was not determined in study, and could be the subject of future evaluation. Overall, the lack of a relationship between CS duration and wine bitterness intensity can be seen as a benefit to the wine industry, if aromatic white grapes can be CS to enhance desirable wine characteristics without also extracting undesirable bitter compounds.

Appendix

Table 2.15. Monomeric phenolic compound profile of the 2011 Riesling, Gewürztraminer, and Traminette 2h, 24h, 24hE, and 48h pre-fermentation cold soak treatments and the 7d on-skin fermentation treatment at the time of sensory analysis.

Grape	Treatment	[Compound] (ppm)														Total [Monomeric Phenolic] (ppm)
		Gallic Acid	Protocatechuic Acid	Catechin	Epicatechin	Cinnamic Acid	GRP	*t*-Caftaric Acid	Caffeic Acid	*c*-Coutaric Acid	*t*-Coutaric Acid	*t*-Fertaric Acid	Coumaric Acid	Ferulic Acid	Caffeic Acid Ethyl Ester	Coumaric Acid Ethyl Ester	
Riesling	2h	nd	1.3a	2.9a	nd	0.15a	0.37a	30a	2.0a	1.7a	5.3a	nd	0.81a	0.21a	0.30a	nd	47a
	24h	0.57a	1.4a	3.7a	1.3a	0.080a	nd	42a	1.5a	2.1a	7.7a	3.3a	1.1a	0.34a	0.31a	0.060a	68a
	24h E	0.78a	1.4a	4.8a	1.6a	nd	0.32a	47a	1.5a	2.4a	8.6a	3.3a	1.2a	0.41a	0.34a	0.11a	77a
	48h	0.53a	1.2a	5.8a	nd	0.15a	0.33a	40a	1.6a	2.1a	7.6a	3.2a	1.1a	0.32a	nd	nd	67a
	7d	7.1a	2.1a	20a	7.7a	0.11a	nd	30a	nd	2.1a	5.8a	2.5a	nd	0.63a	0.73a	0.17a	81a
Gewürztraminer	2h	0.040a	1.2a	2.4a	0.66a	0.13a	1.3a	4.8a	nd	0.39a	0.79a	nd	0.28a	0.34a	nd	nd	12a
	24h	0.52a	2.8a	7.6a	1.7a	nd	0.82a	14a	nd	1.0a	3.2a	1.2a	nd	0.53a	0.030a	0.050a	34a
	24h E	0.42a	2.9a	7.1a	1.8a	nd	0.58a	5.4a	1.4a	0.81a	0.21a	1.1a	1.8a	0.68a	2.4a	0.53a	28a
	48h	0.47a	2.9a	7.9a	1.8a	0.13a	0.64a	15a	nd	2.4a	3.4a	1.2a	0.78a	0.67a	0.29a	0.12a	39a
	7d	3.4a	3.9a	16a	5.4a	0.13a	0.19a	11a	nd	1.1a	3.0a	1.0a	nd	0.43a	0.24a	nd	48a
Traminette	2h	nd	0.42a	2.0a	0.61a	0.21a	0.91a	21a	0.53a	1.9a	4.7a	0.96a	0.030a	0.10a	nd	nd	34a
	24h	0.32a	2.0a	3.7a	nd	0.29a	0.84a	26a	0.51a	2.8a	9.3a	1.1a	0.47a	nd	nd	nd	48a
	24h E	0.19a	1.5a	3.5a	1.1a	0.28a	0.83a	23a	nd	2.7a	5.9a	1.0a	1.8a	0.080a	0.91a	1.0a	44a
	48h	0.28a	0.80a	4.0a	1.2a	0.28a	0.68a	25a	0.32a	3.1a	9.1a	1.2a	0.45a	nd	nd	nd	48a
	7d	2.5a	2.8a	8.0a	1.5a	0.36a	nd	24a	nd	5.1a	13a	1.0a	1.2a	nd	0.70a	0.44a	62a

[1] Values with the same letter are not statistically significant.

Table 2.16. Monomeric phenolic compound profile of the 2012 Riesling, Gewürztraminer, and Traminette 2h, 24h, 24hE, and 48h pre-fermentation cold soak treatments and the 7d on-skin fermentation treatment at the time of sensory analysis.

Grape	Treatment	[Compound] (ppm)														Total [Monomeric Phenolic] (ppm)
		Gallic Acid	Protocatechuic Acid	Catechin	Epicatechin	Cinnamic Acid	GRP	*t*-Caftaric Acid	Caffeic Acid	*c*-Coutaric Acid	*t*-Coutaric Acid	*t*-Fertaric Acid	Coumaric Acid	Ferulic Acid	Caffeic Acid Ethyl Ester	Coumaric Acid Ethyl Ester	
Riesling	2h	4.1a	nd	2.2a	nd	0.67a	nd	13a	0.95a	0.35a	4.6a	1.8a	0.67a	4.8a	0.67a	44a	44a
	24h	nd	nd	6.0a	4.8a	0.16a	nd	25a	12a	1.4a	1.1a	4.5a	2.8a	0.57a	5.8a	1.3a	65a
	24h E	nd	nd	nd	5.4a	0.21a	nd	26a	13a	1.3a	1.1a	4.7a	3.0a	0.68a	6.1a	1.4a	63a
	48h	1.1a	nd	nd	5.2a	0.17a	nd	28a	12a	1.4a	1.3a	4.6a	2.8a	0.62a	5.6a	1.3a	64a
	7d	4.3a	nd	21a	8.4a	0.26a	nd	32a	12a	2.2a	3.0a	4.5a	4.1a	1.3a	4.3a	1.4a	98a
Gewürztraminer	2h	4.1a	1.7a	2.2a	nd	0.67a	nd	nd	nd	nd	nd	1.2a	1.5a	1.1a	1.6a	0.26a	14a
	24h	4.1a	nd	3.2a	nd	1.8a	3.8a	nd	nd	0.88a	8.0a	1.5a	3.5a	1.7a	4.7a	0.76a	34a
	24h E	4.7a	nd	7.6a	nd	nd	nd	nd	nd	0.23a	8.0a	1.4a	3.7a	2.0a	3.5a	0.71a	32a
	48h	nd	nd	8.3a	0.86a	0.15a	nd	5.0a	nd	1.2a	8.2a	1.6a	4.0a	2.0a	4.8a	0.85a	37a
	7d	3.8a	nd	24a	3.5a	0.19a	nd	11a	nd	1.8a	0.88a	1.6a	nd	2.2a	2.8a	1.0a	54a
Traminette	2h	nd	nd	7.0a	nd	0.25a	nd	3.3a	8.0a	1.2a	nd	0.82a	2.9a	0.21a	4.1a	0.82a	29a
	24h	0.67a	nd	nd	nd	0.15a	nd	12a	15a	2.3a	nd	0.46a	0.98a	7.0a	8.3a	2.7a	49a
	24h E	1.1a	nd	nd	nd	0.16a	nd	5.5a	17a	1.7a	nd	0.27a	0.84a	6.9a	9.1a	2.0a	45a
	48h	0.69a	nd	nd	nd	0.14a	nd	16a	nd	2.2a	0.79a	1.1a	5.8a	0.24a	6.1a	2.7a	36a
	7d	1.7a	nd	nd	nd	nd	nd	20a	14a	4.6a	2.3a	1.1a	10a	0.34a	5.3a	4.0a	63a

[1] Values with the same letter are not statistically significant.

Table 2.17. Monomeric phenolic compound profile of the 2013 Riesling, Gewürztraminer, and Traminette 2h, 24h, and 48h pre-fermentation cold soak treatments and the 7d on-skin fermentation treatment at the time of sensory analysis.[1,2]

Grape	Treatment	[Compound] (ppm)															Total [Monomeric Phenolic] (ppm)
		Gallic Acid	Protocatechuic Acid	Catechin	Epicatechin	Cinnamic Acid	GRP	*t*-Caftaric Acid	Caffeic Acid	*c*-Coutaric Acid	*t*-Coutaric Acid	*t*-Fertaric Acid	Coumaric Acid	Ferulic Acid	Caffeic Acid Ethyl Ester	Coumaric Acid Ethyl Ester	
Riesling	2h	1.4a ±0.02	2.8ab	7.8b ±1.	nd	0.22a ±0.008	2.2a ±0.006	33abc ±0.1	2.2abc ±0.7	3.0a ±3	4.8ab ±0.08	3.9a	3.3a	0.62a	0.38a ±0.3	0.22a	60c ±6
	24h	0.69a ±0.02	3.8ab ±0.1	11b ±0.4	nd	0.25a ±0.006	1.6abc ±0.05	48ab ±1	3.2abc ±0.09	1.6a ±0.05	7.9ab ±0.2	3.9a ±0.1	0.77b ±0.02	nd	0.23a ±0.2	0.055a	82ab ±2
	48h	0.91a ±0.003	5.1ab ±0.1	16ab ±0.6	nd	0.20a ±0.01	1.5abc ±0.1	53a ±0.1	3.7ab ±0.003	1.7a ±0.01	9.3ab ±0.02	4.0a ±0.008	0.87b ±0.006	0.34a ±0.004	0.23a ±0.003	nd	96a ±1
	7d	0.85a ±0.007	7.3ab ±5.	14ab ±20	nd	0.14a ±0.5	0.91bc ±0.4	36abc ±30	2.9abc ±3	2.1a ±2	7.4ab ±7	2.6a ±2	0.55b ±0.58	0.69a	0.27a ±0.6	-0.24a ±0.6	100a ±10
Gewürztraminer	2h	1.5a ±0.008	4.2ab ±0.02	3.6b ±1	nd	0.15a ±0.009	2.2a ±0.05	3.5c ±0.04	0.67c ±0.003	0.098a	0.42a ±0.0	0.93a ±0.004	0.52b ±0.03	nd	0.05a ±0.003	nd	17d ±0.7
	24h	0.54a ±0.004	4.8ab ±1.	8.4b ±0.2	nd	0.24a ±0.01	1.8ab ±0.03	15bc ±0.1	1.8abc ±0.06	nd	2.3ab ±0.04	nd	0.91b ±0.02	0.52a	0.23a ±0.3	nd	36d ±2
	48h	0.53a ±0.01	5.5ab ±0.1	8.5b ±0.04	nd	0.36a ±0.02	1.4abc ±0.06	14c ±0.4	2.2abc ±0.05	0.53a	1.9ab ±0.05	1.1a	0.93b ±0.02	0.54a ±0.01	0.63a ±0.02	nd	35d ±4
	7d	3.0b ±0.01	15c ±0.07	29a ±10	7.6a ±0.2	0.30a ±0.0	1.1bc ±0.04	24abc ±0.02	2.1abc ±0.02	2.3a ±0.06	6.3ab ±0.02	1.6a ±0.002	1.1b ±0.008	1.1a ±0.005	0.44a ±0.02	0.16a ±0.005	97a ±10
Traminette	2h	0.89a ±0.8	1.4b ±0.008	3.2b ±0.1	nd	0.23a ±0.0	0.98c ±0.6	19c ±0.5	2.3bc ±2	0.81a ±0.5	2.9ab ±2	nd	0.57b ±0.5	nd	nd	0.0010a ±0.002	34d ±2
	24h	0.74a ±0.01	3.9ab ±1	7.0b ±1	nd	0.29a ±0.003	0.94bc ±0.5	35abc ±0.4	2.2abc ±0.02	2.2a ±0.05	9.3ab ±0.1	nd	1.2b ±0.02	nd	0.15a ±0.003	0.054a ±0.001	64bc ±3
	48h	0.79a ±0.007	3.8ab ±2	8.8b ±2	nd	0.22a ±0.01	0.93bc ±0.6	26abc ±21	2.6abc ±0.06	12a ±17	8.3ab ±5	nd	1.1b ±0.7	nd	0.22a ±0.002	0.10a ±0.002	66bc ±13
	7d	0.49a ±1.	7.9a ±2	20ab ±7	4.9b ±0.3	0.32a ±0.08	1.0bc ±0.6	35abc ±0.3	4.6a ±0.03	9.0a ±6	11a ±8	0.53a	1.2b ±0.8	1.0a ±1	0.38a ±0.3	0.55a ±0.4	99a ±8

[1] Values with the same letter are not statistically significant.
[2]The 24h E CS treatment was not evaluated for the 2013 wines.

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CHAPTER 3

(+)-CATECHIN AND (-)-EPICATECHIN THRESHOLDS IN MODEL AND COMMERCIAL AROMATIC WHITE WINES

Abstract

In order to better understand the effects that (+)-catechin and (-)-epicatechin have on the perceived sensory attributes of white wines, the detection thresholds of these compounds in model and commercial wines were assessed. Detection thresholds for (+)-catechin in model and commercial wines were determined to be 205 ppm and 278 ppm, respectively. These values are much higher than the total flavan-3-ol concentrations usually present in white wines, which have been reported to range from 10-50ppm (Singleton and Esau 1969) with 25ppm as the average concentration present in typical young white wines (Singleton 1988). Detection thresholds for (-)-epicatechin were not found for the concentration range tested (25.1-501ppm). Based on this threshold data, it seems unlikely that the levels of (+)-catechin and (-)-epicatechin present in white wines have a direct impact on white wine sensory qualities. However, it is possible that interactions among subthreshold concentrations of (+)-catechin and (-)-epicatechin and other wine components have an effect on wine sensory qualities.

Introduction

White and red wines share many similar sensory characteristics and descriptor categories, however the sources, intensities, and/or global effects of a particular sensory quality are likely to differ between the two. Bitterness and astringency are two of these qualities. While certain levels of bitterness and astringency are desirable in many red wines, they generally are undesirable in white wines. Pre-fermentation cold soak (CS) of white grape musts, typically employed to extract desirable aromatic compounds, is also thought to extract a number of non-volatile compounds that

may elicit undesirable bitter and astringent gustatory stimuli (Gómez-Míguez et al. 2007; Gordillo et al. 2010; Sokolowsky et al. 2015; Darias-Martín et al. 2000). Among these are several classes of phenolic compounds, most notably the flavan-3-ols, (+)-catechin and (-)-epicatechin (Robichaud and Noble, 1990), which are located in grape skin hypodermal cells and in the soft parenchyma of the outer seed coat (Adams 2006).

While reports of (+)-catechin and (-)-epicatechin detection thresholds in actual wine are limited, the detection thresholds of these compounds in water and model wine have been determined (Delcour et al. 1984; Hufnagel and Hofmann 2008). The detection thresholds for (+)-catechin and (-)-epicatechin in acidified water (pH=4.5) have been reported as 290 and 270ppm, respectively (Hufnagel and Hoffman 2008). Similar levels of free flavan-3-ols have been found in Cabernet Sauvignon wines (Casassa et al. 2013). Differences in the perception of bitterness and astringency, and possibly detection threshold levels of (+)-catechin and (-)-epicatechin are thought to be a result of the S or R stereochemistry of the C3 hydroxyl group. The common forms of the two compounds in wine are 3S (+)-catechin and 3R (-)-epicatechin (Peleg at al. 1999). These stereochemistry differences are thought to have the ability to cause variations in detection thresholds of these flavan-3-ols. In comparison to detection threshold reports made by Hufnagel and Hoffman (2008), Delcour et al. (1984) reported a (+)-catechin detection threshold of 46.1ppm in water. Differences in reported threshold concentrations across studies are likely due to the effects of water acidification, which can influence perceived astringency (Fontoin et al. 2008).

Detection thresholds of (+)-catechin and (-)-epicatechin vary by sample matrix. Purified water is an optimal sample delivery medium for threshold studies using isolated pure compounds, as it is essentially tasteless, making any contamination or additions at threshold or suprathreshold levels readily detectable (Wiley 1976). Consequently, any differences perceived in water samples

are directly related to the compound added to the matrix. To determine how a compound affects the sensory qualities of a food or beverage such as wine, the use of a simple water matrix does not provide accurate information, since the impact of other matrix components on sensory perception are not addressed. In wine, ethanol (Canals et al. 2005; Fontoin et al. 2008, Gawel et al. 2007; Gawel et al. 2013; Meillon et al. 2010; King et al. 2013), sugar, pH (Gawel et al. 2013) and volatile aromatic compounds (Sáenz-Navajas et al. 2010), can suppress or enhance gustatory sensations and influence overall sensory perception. Specifically, pH, acidity, and ethanol concentrations have been shown to directly influence the perceptual effects of phenolic compounds (Fontoin et al. 2008; Gawel et al. 2013). In addition, combinations of gustatory and aromatic compounds can have synergistic effects at both sub and suprathreshold concentrations (Hufnagel and Hoffman 2008; Ferrer-Gallego et al. 2014), as subthreshold synergistic effects between (+)-catechin and (-)-epicatechin as well as coumaric acid, caffeic acid, protocatechuic acid and gallic acid are possible (Ferrer-Gallego et al. 2014).

In order to better understand the effect of (+)-catechin and (-)-epicatechin on the perceived sensory attributes of white wines, this research sought to determine the detection thresholds of each compound in a model wine and a commercial white wine. Flavan-3-ol thresholds in model wine were determined to allow for comparison with values previously reported in the scientific literature. In addition, threshold determination in both wine and model wine facilitated the comparison of detection threshold results for each sample system within the same panel.

Materials and Methods

Model and commercial wines. Model wine used for this study consisted of 10% (v/v) ethanol, 100ppm malic acid, and 300ppm potassium bitartrate in distilled water, and was adjusted to pH 3.5 using sodium hydroxide (Sacks et al., 2012). Heritage Mountain Chablis from Almaden

Vineyards® (Madera, California, USA), packaged in 5L bag in box containers, was used for commercial wine trials.

The reagents (+)-catechin hydrate (99.5% purity) and (-)-epicatechin (90.3% purity) (Sigma Aldrich Co®, St Louis, Missouri, USA) were selected for detection threshold determination. (+)-Catechin hydrate will be referred to as (+)-catechin going forward. The model wine, commercial wine, and reagents specified were used for all sensory sessions.

(+)-Catechin and (-)-epicatechin concentrations. For benchtop trials, test concentrations of 0, 5, 30, 40, 50, 100, 175, 230, 300, 400, and 500 ppm of (+)-catechin and (-)-epicatechin were evaluated in model wine. This scale allowed screening of a large range of concentrations and was selected based on previously reported thresholds of (+)-catechin, 46.1ppm and 290ppm (Delcour et al. 1984; Hufnagel and Hofmann 2008), and (-)-epicatechin, 270ppm (Hufnagel and Hofmann 2008). The upper level of the scale included concentrations of these compounds well above their reported thresholds as positive controls.

Based on the results of the benchtop test, six (+)-catechin solutions, 1.58, 3.16, 6.31, 12.6, 25.1, and 50.1ppm, were used for the model and commercial wine threshold tests. Following the Fechner theory of taste perception (Lawless 1980), these concentrations corresponded to the logarithmic steps, 0.2, 0.5, 0.8, 1.1, 1.4, and 1.7 log, respectively. An additional array of (+)-catechin solutions with concentrations of 100, 200, 251, 316, 398, and 501 ppm, corresponding to increasing logarithmic steps of 2.0, 2.3, 2.4, 2.5, 2.6, and 2.7, were also tested after the initial concentrations used failed to provide useful detection threshold data. Concentrations of 25.1, 50.1 100, 200, 251, 316, 398, and 501 ppm were used for the (-)-epicatechin threshold tests in model and commercial wines.

Sample presentation. All samples (10mL) were poured into 22.2 mL plastic cups labeled with random three-digit codes and held at room temperature (21°C) prior to testing. Sample codes were randomized among panelists. All samples were presented in order of increasing concentration.

For the benchtop trials, panelists were supplied with an unadulterated wine control and test samples were presented individually. Panelists were asked determine if the sample tasted different than the control. The test was concluded when a panelist correctly identified a sample as tasting different than the control twice in a row. Panelists were required to take a four-minute break after every fourth sample evaluated.

A forced-choice ascending concentration series method of limits (ASTM International, E679-04) test was used to determine the detection thresholds of (+)-catechin and (-)-epicatechin in model and commercial wines. Panelists performed a triangle test at each concentration level and a sensory session ended when the panelist gave the correct answer twice in a row. Untreated reference samples of model or commercial wine were made available to panelists during sessions. Panelists were required to wear nose-clips, expectorate all samples evaluated, and rinse their mouths with water in between samples.

Panelists. All panelists were at least 21 years of age, non-smokers, and consumed wine at least 2-3 times per month. All provided informed consent as required by the Cornell University Internal Review Board. Panelists were classified as casual observers (ASTM International, E1432-04) and as such were not trained or selected based on specific performance criteria, other than those listed above.

Sensory panels were composed of 12 participants (6 female; mean panelist age 36yrs) for benchtop tests, 21 panelists (7 female; mean panelist age 46yrs) for the (+)-catechin model and commercial wine threshold tests, 30 panelists (16 female; mean panelist age 41yrs) for the (-)-

epicatechin commercial wine threshold test, and 25 panelists (10 female; mean panelist age 44yrs) for the (-)-epicatechin model wine threshold test.

Data analysis. A detection threshold was defined as the concentration of (+)-catechin or (-)-epicatechin in commercial or model wine at which 50% of the population could differentiate from the control, after the proportion of correct answers was adjusted for chance using Abbot's formula (Lawless and Heymann 1999).

$$Adjusted\ Percent\ Correct = (Observed\ Proportion\ Correct) - [2 * (Chance\ of\ Correct\ Answer)]$$

The proportion correct was then estimated for the entire population ($\alpha = 0.05$). At a precision rate of p=0.5, the detection thresholds reported for 20, 25, and 30 panelists will be 22, 20, and 18% accurate. This counterintuitive outcome of obtaining more accurate data with fewer panelists is referred to as the accuracy paradox.

Results

Benchtop test. During the benchtop test, all panelists were able to detect (+)-catechin and (-)-epicatechin in model wine over the concentration range tested, with the exception of one individual who was unable to detect (+)-catechin at any concentration and was barred from further test sessions (Table 3.1).

Table 3.1: Percentage of panelists (n=12) reaching individual (+)-catechin and (-)-epicatechin detection thresholds in model wine at each concentration.

Concentration (ppm)	(+)-Catechin	(-)-Epicatechin
5	(0%	0%
30	25%	67%
40	42%	8%
50	25%	17%
100	0%	8%
175	0%	0%
230	0%	0%
300	0%	0%
400	0%	0%
500	0%	0%
None	8%	0%

Formal threshold sensory test sessions. For the formal detection threshold sensory test sessions, the (+)-catechin concentrations at which 50% of the population (22% accuracy) could detect a difference between the spiked and control samples were 205 ppm and 278 ppm for model and commercial wines, respectively (Figures 3.1A and 3.1B). In contrast, the (-)-epicatechin threshold test data did not yield a population detection threshold at or above the 50% chance level in either model or commercial wines for the for the concentration range tested (Figure 3.1C and 3.1D).

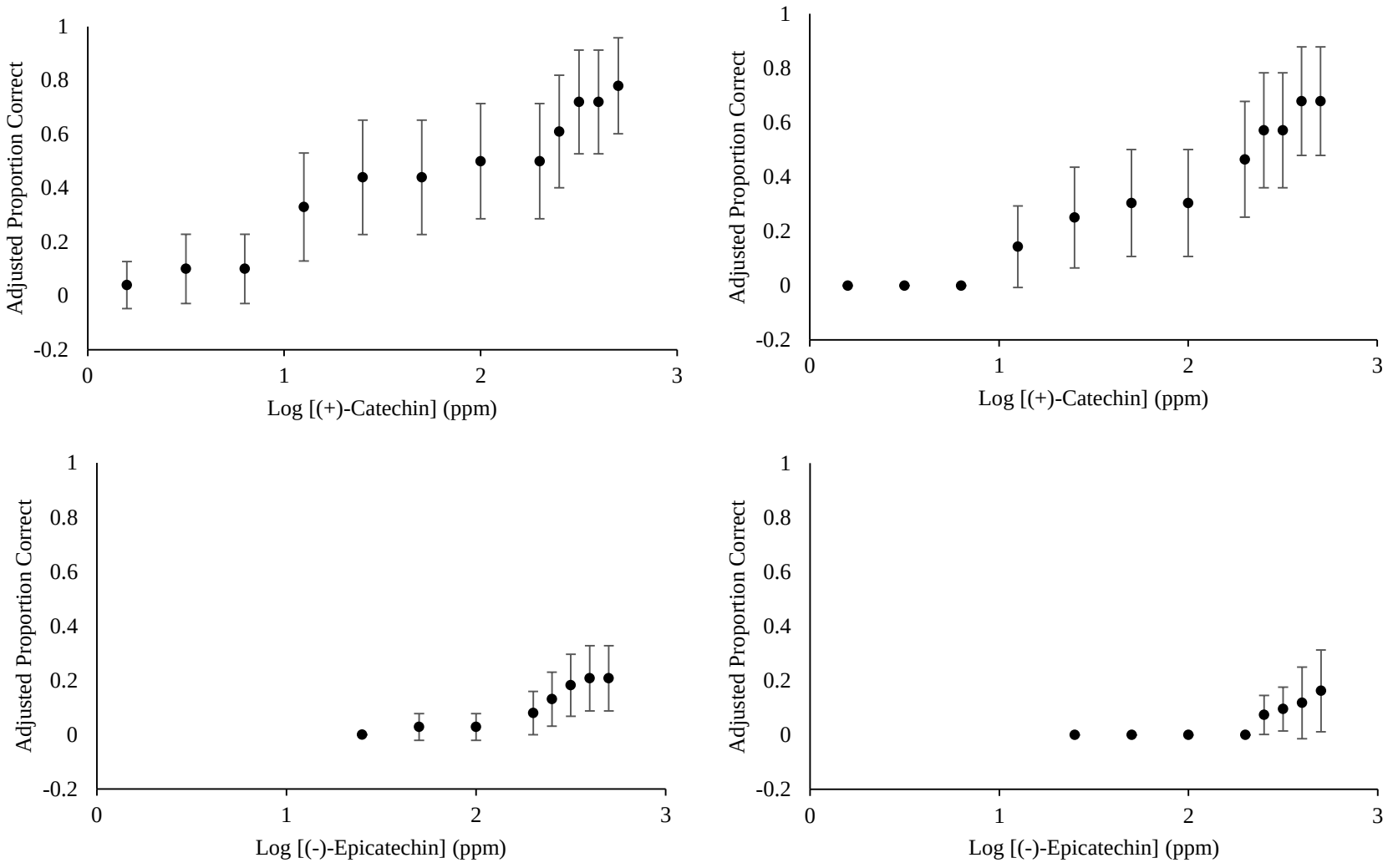

Figure 3.1. (+)-Catechin and (-)-epicatechin threshold testing data. Estimation of the proportion correct for the population after adjustment of the proportion correct in the sample (α=5%) as a function of [(+)-catechin] (ppm) in model wine (n=21) **(A)**, [(+)-catechin] (ppm) in commercial wine (n=21) **(B)**, [(-)-epicatechin] (ppm) in model wine (n=25) **(C)**, and [(-)-epicatechin] (ppm) in commercial wine (n=30) **(D)**.

Discussion

Benchtop test. As shown in Table 3.1, most panelists could detect (+)-catechin and (-)-epicatechin in model wine at 40ppm and 30ppm, respectively. This difference in taste sensitivity between the stereoisomers is aligned with research reporting that the bitterness and astringency intensities of (-)-epicatechin are higher than those of (+)-catechin (Robichaud and Noble 1990, Noble 1994, Thorngate and Noble 1995, Kallithraka and Bakker). Anecdotally, panelists in the benchtop trial commented that model wine solutions containing (-)-epicatechin tasted fruiter than the (+)-catechin model wine solutions. As the perceived sensory qualities of the compounds in model wine may differ, the thresholds of the compounds may be different as well. Based on the benchtop test results, it was deemed unnecessary to have panelists evaluate solutions greater than 200ppm (+)-catechin or (-)-epicatechin during the formal threshold test evaluation sessions.

(+)-Catechin thresholds in model and commercial wines. When 50% of the panelists did not reach detection threshold in either the model or commercial wines with the initial (+)-catechin concentration range used (1.58-50.1ppm), concentrations up to 501ppm were evaluated during additional sensory sessions. The thresholds of (+)-catechin in model wine and commercial wine were determined to be 205 ppm and 278 ppm, respectively (Figures 3.1A and 3.1B.) These results are similar to previously reported (+)-catechin threshold data of 290ppm in acidified water (Hufnagel and Hofmann, 2008). The (+)-catechin threshold concentration was likely higher in commercial wine due to the modulatory effects of sugars and other wine components, including phenolic compounds, which were not added to the model wine.

(-)-Epicatechin thresholds in model and commercial wines. It is unclear why a detection threshold for (-)-epicatechin in model and commercial wines could not be established with the concentrations tested (Figures 1C and 1D). (-)-Epicatechin has been reported to be more bitter

(Robichaud and Noble 1990, Noble 1994, Thorngate and Noble 1995, Kallithraka and Bakker 1997) and astringent (Ferrer-Gallego et al. 2014), and elicit longer durations of bitterness and astringency perception than (+)-catechin, although the significance of the data is disputed (Peleg et al. 1999). These characteristics suggest that the detection threshold of (-)-epicatechin is generally lower than (+)-catechin, and Hufnagel and Hoffman (2008) did report a detection threshold of 270ppm for (-)-epicatechin in acidified water, compared to 290ppm for (+)-catechin. In the current study, (-)-epicatechin detection threshold testing was concluded at a maximum concentration of 501ppm, ~54% higher than the (-)-epicatechin threshold reported by Hufnagel and Hoffmann (2008) and over 50 times greater than reported concentrations of the compound in white wines including Riesling and Gewürztraminer (Goldberg et al. 1999).

Panelist variability. It is likely that issues with individual panelist reproducibility along with cross-panelist differences in tasting abilities are responsible for the variation in threshold data seen (Figure 3.1). Specifically, individual (-)-epicatechin detection threshold results were not conserved across replicates for a portion of the panel. In some cases, intra panelist variations in detection threshold results indicated a large difference in sensitivity to (-)-epicatechin between sessions. This suggests that tests such as detection threshold determination only provide a snapshot of the gustatory abilities of an individual at the time of the test. Such abilities may change due to additional training (Tempere et al. 2012), repeat exposure to a stimulus (Tempere et al. 2011), health status (ASTM International, E1432-04), and/or age (Fusari and Ballesteros 2008). Alertness, attention, fatigue, and events at the molecular level (ASTM International, E1432-04) are also known to effect gustatory and olfactory abilities. When considering all of these factors it is reasonable to assume that panelists had varying levels of wine tasting expertise and sensitivity to the compounds of interest, which would contribute to variation in the dataset. As panelists were

considered to be casual assessors, did not receive training, and were selected based wine consumption but not taste sensitivity to the compounds of interest, it is possible that this level of variation exists in the general wine consuming public.

Conclusion

The detection threshold concentrations of (+)-catechin and (-)-epicatechin in commercial and model white wines far exceed the concentrations of these compounds normally present in white wines. Thus it would appear that the effects these compounds have on overall wine sensory perception, if any, occur at a subthreshold level and are likely due to interactions with other wine components. Detection threshold determination is one step in the multi-stage process of understanding how individual or groups of compounds affect the perceived sensory qualities of a wine. Additional studies beyond threshold testing are required to determine what effect, if any, subthreshold levels of (+)-catechin and (-)-epicatechin have on overall wine sensory qualities.

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CHAPTER 4

THE EFFECT OF COLOR ON EXPERT WINE TASTER PERCEPTION OF BITTERNESS IN WHITE WINE

Abstract

White wines may undergo color changes as a result of oxidation or processing treatments such as pre-fermentation cold soak (CS). Both cold soak (skin contact) time and oxidation result in darker white wine color, and both may also enhance wine bitterness. Since these two effects are often linked, frequent wine tasters may learn through experience to associate the color change with increased bitterness intensity. This study sought to determine the effect of white wine color on bitterness perception in expert wine tasters, who were tasked with rating the bitterness intensity of four white wine solutions of increasingly darker color using a category check box scale. It was expected that a vision-taste association between white wine color and the perceived bitterness intensity ratings of expert wine tasters would be present. However, no association between color and bitterness was found.

Introduction

Taste and smell are fluid constructs, and as such are highly influenced by intrinsic and extrinsic cues. The taste and smell of a wine is a result of the specific chemical components present, the stimuli signals sent from receptors to the taste and olfactory cortices, and ultimately the translation of this information into conscious perception. Consumers rarely, if ever, consume wine in an isolated setting devoid of all sensory stimuli except those of taste and smell. An individual's perception of a food or beverage can be influenced by visual, auditory (Spence 2012), and texture/touch (Spence 2015) cues within the product, as well as by the individual's internal and external environments. A wine taster's external environment, such as the ambiance of the room, lighting (Spence et al. 2014), background noise (Seo et al. 2011; Spence et al. 2014; Woods et al.

2011), and available product information such as brand, country of origin (D'Alessandro and Pecotich 2013), and wine critic ratings (Siegrist and Cousin 2009), can influence an individual's perception of a wine's sensory qualities as well as the overall wine tasting experience.

Visual attributes of wine are especially important because humans really do "eat first with [their] eyes" (Delwiche 2012). When a product is seen, an individual recalls associations with the product and/or packaging and begins to formulate expectations concerning the consumption experience (Delwiche 2012; Okamoto and Dan 2013; Wadhera and Capaldi-Phillips 2014).

The influence of visual attributes on wine taste and smell is a crossmodal effect that is particularly strong for certain learned color-taste and color-odor associations (Demattè et al. 2006; Zellner 2013). These crossmodal effects may be due to perceptual illusion, which can occur during the verbalization phase of odor determination (Morrot et al. 2011). The powerful influence of color on taste perception can be seen in studies examining the effect of color on the perceived sweetness of sucrose solutions. Strawberry-flavored drinks were perceived to be sweeter when colored dark red compared to light red, and when colored light green compared to dark green (Lavin and Lawless 1998). Further, Johnson and Clydesdale (1982) found that darker red-colored solutions were perceived to be 2-10% sweeter than lighter solutions, even when the actual sucrose concentration was 1% less in the former. When performing flavor identification tasks, individuals were less likely to correctly identify the flavor of a food or beverage when the sample was presented as a color not commonly associated with the flavor, such as a lime-flavored drink colored red (DuBose et al. 1980). See Spence et al. (2010) for a comprehensive review on the effects of visual cues on the perception of taste, smell, and flavor.

These crossmodal effects are not reserved for novice wine consumers, as even expert wine tasters can be influenced by visual product cues. For example, wine experts presented with white

wines colored red characterized them using predominantly red wine descriptors, when the same wine presented without color adulteration was described using predominantly white wine descriptors (Morrot et al. 2001). Furthermore, wine experts have been shown to be more influenced by the color of white wines than naïve wine consumers when forming sensory perceptions (Pangborn 1963). The former group rated a pink-colored wine to be sweeter than a non-colored sample of the same wine, while the naïve wine consumers did not show this bias (Pangborn et al. 1963).

One visual-taste interaction that has not been well explored is the effect of white wine color changes, such the darkening of wines due to oxidation or phenolic compound extraction during pre-fermentation cold soak (CS), on expert wine taster's perception of bitterness. In the case of CS, it is widely but falsely assumed that increases in the extraction of certain phenolic compounds leads to increases in white wine bitterness. (Dissertation Chapter 2). However, as CS does lead to changes in finished wine color, it is possible that the changes in perceived bitterness intensity attributed to the extraction of phenolic compounds are actually due to crossmodal interactions between the visual system and either the taste or olfactory systems.

In addition to color changes, oxidation and CS of white wines can result in a number of sensory attribute changes. Negative sensory attribute changes include losses in color, flavor, and aroma, as well as increases in astringency (Escudero et al. 2002; Ferreira et al. 1997; Schneider 2001; Silva Ferreira et al. 2002). Increases in acetaldehyde concentrations leading to increases in perceived bitterness intensity may occur as a result of oxidation (Zhai et al. 2001). Wine is labeled as oxidized when the concentration of acetaldehyde exceeds 50ppm (Zhai et al. 2001). Acetaldehyde also contributes to the formation of flavan-3-ol dimers and trimers (Es-Safi et al. 1999; Fulcrand et al. 1996; Saucier et al. 1997; Timberlake and Bridle 1976). Increases in wine

flavan-3-ol dimer and trimer concentrations can lead to increases in color intensity in the yellow spectral region (Lopez-Toledano et al. 2004).

Non-enzymatic reactions caused by the interaction of iron and copper with wine components can lead to color changes during wine storage. Specifically, the iron- or copper-induced oxidation of (+)-catechin, through a secondary browning pathway that starts with the oxidation of tartaric acid (Clark et al. 2003; Es-Safi et al. 1999; Es-Safi et al. 2000; Es-Safi et al. 2003; Fulcrand et al. 1997), results in the formation of compounds that are either colorless or yellow (Oszmianski et al. 1996).

As the oxidation and CS of white wines leads to changes in color and, often, gustatory attributes, it seems likely that a learned visual-taste interaction, and possibly a visual-taste-aroma interaction, could arise among white wine color, bitterness, and oxidative aromas. These effects may be particularly strong if the occurrence and outcome of oxidation reactions and CS is known prior to wine consumption, as would be the case for expert wine tasters tasked with evaluating the sensory characteristics of a wine. In this case, expert wine tasters may first identify a wine as being oxidized or CS, and then identify sensory attributes resulting in top-down processing that can modify the interpretation of sensory stimuli information in the brain (Goldstein 2013).

The current work focuses on identifying the effects that differences in white wine color have on expert wine tasters' perception of bitterness. It is expected that wines colored to look oxidized and/or subject to CS will be perceived as having higher bitterness intensities, and that this learned visual-taste association will form as a result of repeated exposure to white wines that are both darker in color and have increased bitterness intensities.

Materials and Methods

Wine. Heritage Mountain Chablis from Almaden Vineyards® (Madera, California, USA), packaged in 5L bag in box containers, was used for sample preparation.

Pigments. Red, yellow, blue, and green commercial, food-grade pigments (McCormick®, Sparks, Maryland, USA) were added to the wine in different concentrations to obtain four differently colored white wines forming a color gradient that was representative of authentic white wine colors.

White wine samples. The four white wine samples were base wine (B1), first color intensity (C1), second color intensity (C2), and third color intensity (C3). Sample solutions were prepared each day of testing and stored at 4°C until approximately one hour prior to sensory analysis.

The color of each sample was quantified using the International Commission on Illumination L*a*b* (CIElab) color space/ scale, measured with a Hunter Lab Ultra Scan VIS, equipped with Easy MatchQC software (Hunter Associates Laboratory, Inc., Reston, VA, USA), in TTRAN total transmission mode (Table 4.1).

Table 4.1. Average L*, a*, b*, and ΔE color values of white wine samples.[1]

Sample	Color Data[2]			
	L*	a*	b*	ΔE[3]
Base Wine (B1)	98.0 ± 0.10	-1.30 ± 0.030	4.11 ± 0.010	
First Color Intensity (C1)	96.6 ± 0.29	-1.85 ± 0.12	7.01 ± 0.26	3.28 ± 0.34
Second Color Intensity (C2)	96.6 ± 0.090	-2.58 ± 0.040	9.16 ± 0.13	5.39 ± 0.16
Third Color Intensity (C3)	95.7 ± 0.11	-3.55 ± 0.020	13.9 ± 0.090	10.3 ± 0.11

[1] International Commission on Illumination L*a*b* (CIElab) color space/ scale

[2] L*: Lightness (white to black); a*: sample redness (positive values)/ greenness (negative values); b*: sample yellowness (positive values)/blueness (negative values); ΔE: total color difference from standard

[3] $\Delta E=\sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$; Base wine (B1) was considered to be the control for this analysis.

In addition, sample color stability across and within testing days was confirmed throughout the study (data not shown). Samples were also evaluated prior to formal sensory analysis to ensure

that the pigments used did not contribute noticeable sensory attributes to the wines (data not shown).

Sensory Analysis. A panel of 12 individuals (six female; mean panelist age 48 yrs.), who were non-smokers and at least twenty-one years old, participated in this study. Panelists consisted of expert wine tasters from the wine industry and academia in the New York Finger Lakes region.

For sensory sessions, 30 mL of each solution was poured into 215mL wine tasting glasses (ISO 3591:1977) and held at room temperature (21°C). Glasses were labeled with 3-digit random numbers and the presentation order was randomized using a Latin square design. Panelists were presented with the four differently colored samples at the same time, and were asked to rate the bitterness intensity of each sample according to the following five point check box category scale: not bitter, slightly bitter, moderately bitter, very bitter, and extremely bitter. The test was performed in duplicate under white lighting, and in duplicate again under red lighting to mask color variance.

Statistical Analysis. Analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) test were performed using JMP Pro 10 (SAS Institute, Inc., Cary, NC, USA) to determine difference between treatments at a significance level of $p<0.05$.

Results

In a full factorial analysis of bitterness ratings with lighting, color intensity and panelist as factors, using a partial least squares fit model with Tukey's HSD test (Table 4.2), effects were seen for the panelists ($p<0.023$). Specifically, differences between the bitterness scores of panelist 5 under white lighting, and panelist 10 under red lighting, were seen with the Tukey's HSD test results (data not shown). A significant effect of lighting*color intensity ($p<0.026$) was also seen in the ANOVA results, but not in the subsequent Tukey's HSD test results (data not shown).

Table 4.2. Full model ANOVA results of bitterness intensity rating data with the factors lighting (white and red), solution, and panelist.

Source	N parm	DF	Sum of Squares	F Ratio	Prob > F
Lighting	1	1	4.000	3.675	0.058
Color Intensity	3	3	3.250	0.9950	0.40
Lighting*Color Intensity[1]	3	3	10.50	3.215	0.026*
Panelist[2]	11	11	25.73	2.149	0.023*
Lighting*Panelist	11	11	19.73	1.648	0.098
Color Intensity*Panelist	33	33	20.26	0.5640	0.97
Lighting*Color Intensity*Panelist	33	33	33.09	0.9210	0.59

[1] Subsequent Tukey's HSD test results did not show any significant differences within the lighting*color intensity condition.

[2]Subsequent Tukey's HSD test results showed significant differences between one of the twelve panelists under white lighting and one of the panelists under red lighting.

While the full statistical model (Table 4.2) did not show differences in bitterness scores among the white wine color intensities, an ANOVA analysis of the white lighting condition alone (Table 4.3) did show significant effects. Significant differences in color intensity ($p<0.034$) were seen between solutions B1 and C1 and significant differences in panelists ($p<0.038$) were seen between panelists 5 and 7 when analyzed with Tukey's HSD test (data not shown). Significant differences in bitterness scores for the factors color intensity and panelist were not seen for the red lighting condition (Table 4.4).

Table 4.3. ANOVA results of bitterness intensity rating data with the factors white lighting, color intensity, and panelist.

Source	N parm	DF	Sum of Squares	F Ratio	Prob > F
Color Intensity[1]	3	3	9.375	3.125	0.034*
Panelist[2]	11	11	23.13	2.102	0.038*
Color Intensity*Panelist	33	33	30.17	0.9140	0.60

[1]Subsequent Tukey's HSD test results showed significant differences between first and second color intensity samples.

[2]Subsequent Tukey's HSD test results showed significant differences between two of the twelve participants.

Table 4.4. ANOVA results of bitterness intensity rating data with the factors red lighting, color intensity, and panelist.

Source	N parm	DF	Sum of Squares	F Ratio	Prob > F
Color Intensity	3	3	4.375	1.239	0.31
Panelist	11	11	22.33	1.725	0.096
Color Intensity*Panelist	33	33	23.18	0.5970	0.94

Discussion

White wine color minimally affected experts' perception of bitterness intensity under white lighting. Specifically, the bitterness intensity ratings of solutions B1 and C1, the lightest of the color-adjusted wines, were significantly different, but all other combinations of samples, including those samples that were darker in color, were not. White wine color did not have an effect on experts' perception of bitterness intensity under red lighting. This result is expected, as the red lighting is intended to mask color differences among the wines, and served as a control to determine if differences seen were due to color effects or the taste of the samples. Furthermore, differences in bitterness scores were not seen when the white and red lighting conditions were both included in the model.

The lack of significant effects in the model that included both lighting conditions indicates that the visual-taste effect seen with under white lighting is weak, and that a true effect of color on perceived bitterness intensity is likely not present. Other studies have also reported that color does not have an effect on the perceived tastant or odorant intensities of various beverages (Alley and Alley 1998; Chan and Kane-Martinelli 1997; Frank et al. 1989; Gifford and Clydesdale 1986; Gifford et al. 1987), and still others propose that the effect is more complex than a simple visual-taste or visual-odor interaction (Auvray and Spence 2008; Zampini et al. 2007) could explain. While the association of red color with sweetness is well documented (Johnson and Clydesdale 1982; Lavin and Lawless 1998), the panelists did not evince a learned association between white wine color and bitterness.

Although the test wines were altered in order to present panelists with a color intensity (i.e., darkness) gradient, bitterness intensity ratings were only different for wines B1 and C1. Since C2 and C3 were darker than B1 and C1, their perceived bitterness intensities were expected to be

similar or higher than that of B1 and C1. Thus it is likely that the significant difference observed between solutions B1 and C1 is an artifact of the statistical analysis model used. While the difference is statistically significant, it does not appear to be practically significant, since only the two lightest-colored samples were different, suggesting that the statistical model found a difference when none was present. Further, the lack of significant differences among the solutions in the ANOVA model including both lighting conditions also suggests that this difference is an analysis artifact.

Conclusion

The current study failed to show an appreciable visual-taste association between white wine color and bitter taste. The lack of this specific crossmodal association in white wines can be seen as a benefit to the wine industry. If a color-bitterness association did exist, oxidized and/or CS white wines that are darker in color could be perceived as having higher bitterness intensities than could be contributed by any bitter eliciting compounds present.

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CHAPTER 5

CAN RIESLING CLONES BE DIFFERENTIATED BY ANALYTICAL AND SENSORY METHODS?

Abstract

Several clonal variations of Riesling grapes are grown in the New York State Finger Lakes region, yet little is known about the chemical and sensory characteristics of the individual clones and resulting wines. It is also unclear if clones can be differentiated in the same way as cultivars through sensory analysis or instrumental analysis of specific compounds. For instance, monomeric phenolics and tannins are known to contribute to wine bitterness and/ or astringency (Thorngate and Noble 1995; Robichaud and Noble 1990), and vary in content and concentration among grape cultivars (Goldberg et al. 2000; Pour Nikfardjam et al. 2007). The current study investigated the possibility of differentiating among Riesling clones and vineyard sites based on variations in grape juice monomeric phenolic and total tannin profiles, obtained via High Performance Liquid Chromatography (HPLC) analysis. While monomeric phenolic and tannin concentrations did not vary among the clones and sites studied, preliminary sensory evaluation by a panel of twenty-nine expert wine tasters suggests that there are detectable sensory differences among single vintage monoclonal Riesling wines. Specifically, differences were found in wine fruitiness, mouthfeel/palate weight, and perceived acidity, although there was no panel consensus as to which samples were different. Based on the preliminary sensory test results, it is possible that significant differences in the perceived sensory qualities of wines made from Riesling clones exist, and that these differences are due to compounds other than the monomeric phenolics and tannins measured.

Introduction

Different clones of a single grape cultivar are popularly believed to have unique chemical characteristics and produce wines with noticeably different sensory qualities. One of the best known examples is the cultivar clones Pinot blanc and Pinot gris (Hocquigny et al. 2004), both of which are clones of Pinot noir but produce wines that are sufficiently different that the grapes are given different varietal names. Other polyclonal varieties include Cabernet franc, Cabernet Sauvignon, Traminer, Riesling, Chenin blanc, Grolleau (Pelsy et al. 2010), and Meunier (Franks et al. 2002; Stenkamp et al. 2009). Many studies have sought to determine whether grape cultivars can be differentiated through the analysis of monomeric phenols (Ali et al. 2011; Castillo-Muñoz et al. 2010; de Villiers et al. 2005; Goldberg et al. 2000; Masa et al. 2007; Nelson 2011; Pour Nikfardjam et al., 2007; Soleas et al. 1997), anthocyanins, tannins (Vázquez et al. 2011) and amino acids (Ali et al. 2011; Asensio et al. 2002), as well as through grape morphology assessments (Asensio et al. 2002). Fewer works have investigated whether the clones of a single grape cultivar can be identified through the measurement of specific grape or wine components, such as a phenolic fingerprint (Burin et al. 2011), or through sensory analysis.

In addition to the analysis of chemical characteristics specific to a grape cultivar, the influence of 'terroir' on variations within and across grape cultivars has been investigated (Douglas et al. 2001; Fisher et al. 1999; Peña-Neira et al. 2000; Vázquez et al. 2011). Terroir takes into account grape growing site and regional variations in factors such as soil composition, nutrient levels, water availability, weather, and climate when accounting for differences in wines made from the same cultivar in different geographic areas. Terroir is thought by some to have a larger impact on grape, juice, and wine characteristics than intrinsic grape genetic factors. Grape genomic and genetic studies commonly focus on vintage effects, which can be influenced by environmental,

geographical, or agronomical factors. An interesting historical look at grape origins and genetic diversity can be found elsewhere (This et al. 2006).

While a number of Riesling clones exist, there is a lack of research investigating the possibility of innate chemical and sensory profile differences among the grapes and resulting monoclonal wines, or differences caused by extrinsic factors such as site. The current observational study focuses on determining if (1) individual Riesling clones can be differentiated by their innate or extrinsically modified monomeric phenolic and/or tannin profiles and (2) if monoclonal Riesling wines can be differentiated by expert wine tasters.

Materials and Methods

Monoclonal Riesling grape samples. A total of ten distinct Riesling clone berry samples from the New York State Finger Lakes region were harvested and analyzed during the 2011-2013 growing seasons. Berry samples of Riesling clones 21B, 49, 90, 110, 198, and 239 were obtained in 2011-2013 at the time of harvest from vineyard site 1, located on the east side of Seneca Lake. Riesling clones 49, 198, 239, and HJW were sampled immediately prior to harvest in the fall of 2012 and 2013 from vineyard site 2, located on the east side of Cayuga Lake. Allotments of 200 berries were obtained through random sampling, crushed using a Stomacher® 400 Circulator paddle blender for 30 seconds at 230rpm (Seward Laboratory Systems, West Sussex, UK) and analyzed for soluble solids (°Brix), pH, titratable acidity (TA), and Yeast Assimilable Nitrogen (YAN) content as described below. Duplicate juice samples were then collected, preserved with 0.1% ascorbic acid (Presque Wine Cellars, North East, PA), and frozen at -20°C until monomeric phenolic (2011-2013) and tannin (2012-2013) analysis was performed via the methods described below.

Riesling juice chemistry data from 2008-2015. Juice °Brix, pH, TA, and YAN data was cross-checked with historical data from 2008-2015 reported in Veraison to Harvest (V2H) Newsletters published by the Cornell Enology Extension Laboratory (CEEL) (http://grapesandwine.cals.cornell.edu/newsletters/veraison-harvest). According to CEEL protocols, data in V2H newsletters was generated using randomly-sampled 100-berry lots from vineyard sites in the New York State Finger Lakes, Hudson Valley, and Lake Erie regions. The grapes were crushed using a Stomacher® 400 Circulator paddle blender for 30 seconds at 230rpm (Seward Laboratory Systems, West Sussex, UK) and analyzed for °Brix, pH, TA, and YAN content as described below.

Monoclonal Riesling wine sensory samples. Riesling clones 90, 110, 198, and an unknown Alsatian clone were harvested and processed on October 24, 2014 at the New York State Agricultural Experiment Station Vinification and Brewing Laboratory. The juice was fermented using *Saccharomyces cerevisiae* yeast strain DV10 (Scott Laboratories, Inc., Pickering, ON, Canada). Basic juice and wine chemistry analyses, including °Brix, pH, TA, YAN and organic acid measurements, were conducted throughout the wine making process using the methods described below.

Instrumental Analysis

Titratable acidity (TA), pH, and soluble solids (°Brix). TA was analyzed using a Titrino Plus 848 doser and 869 autosampler (Metrohm USA, Riverview, FL, USA), pH with an Accumet Excel XL25 pH meter (Thermo-Fisher Scientific, Waltham, MA, USA), and soluble solids (°Brix) was determined using a handheld Atago Alpha-PAL refractometer (Atago U.S.A., Inc., Bellevue, WA, USA).

Yeast Assimilable Nitrogen Analysis. YAN measurements of unfermented juice samples were determined using enzymatic analysis (Unitech Scientific, Hawaiian Garden, CA, USA) on a Chemwell 2910 multi-analyzer equipped with Software Version 6.3 (Awareness Technology, Palm City, FL, USA)

High-Performance Liquid Chromatography (HPLC)

Reagents. HPLC grade methanol, hydrochloric acid, acetonitrile, neat formic acid, ethyl acetate, phloroglucinol, and phosphoric acid used for high performance liquid chromatography (HPLC) analysis and solid phase extraction (SPE) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Phenolic compounds. Samples were analyzed using an Agilent 1260 Infinity series HPLC (Agilent Technologies, Santa Clara, CA, USA) containing a diode array detector fitted with a 10mm path, 1µl volume Max-light cartridge flow cell. All sample fractions were analyzed on Kinetx ® core-shell columns (Phenomenex, Torrance, CA, USA) fitted with Krudkatcher™ guard filters (Phenomenex, Torrance, CA, USA). Specifically, the monomeric phenolic and tannin fractions were analyzed on a 100 x 4.6mm C_{18} column packed with 2.6µm diameter particles with a 100Å pore size. The instrument was controlled using Agilent Chemstation software version B.04.03, service pack 2 with spectral software module. Samples were either filtered and then directly injected into the HPLC or separated into monomeric phenolic and tannin fractions using the method developed by Jeffery et al. (2008a) and modified by Manns and Mansfield (2012). Frozen samples were thawed and then centrifuged at 10,000x g for 5 min. Samples were then mixed with an equal amount of 0.01 N HCl in water and loaded onto a $3cm^3$, 60mg Oasis HLB solid phase extraction cartridge (Waters, Milford, MA, USA). Separated tannin fractions underwent phloroglucinolysis prior to HPLC analysis. Samples that were analyzed on the HPLC

by direct injection were centrifuged as previously described and filtered through a 0.22μm PES membrane immediately prior to sample analysis.

Sensory analysis. An informal sensory analysis of four monoclonal Riesling wines produced in 2014 was conducted in the summer of 2015 to determine if there were noticeable differences in the sensory qualities among the wines. Twenty-nine expert wine tasters (7 female; mean age 41 yrs.) from the New York Finger Lakes region participated in the test as part of an extension event. Panelists were presented with a flight of four monoclonal Riesling wine samples (clones 90, 110, 198, and an unknown Alsatian clone) and asked if they could detect differences among the samples. If differences were detected panelists were asked to identify which sample(s) varied from the others and what sensory characteristic(s) was different.

Statistical analysis. Statistical analysis of the Riesling clone sample data was performed using analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) test at a significance level of $p<0.05$. (JMP Pro 10 software, SAS Institute, Inc., Cary, NC, USA).

Results

Juice chemistry analysis.

Monoclonal Riesling grape samples. No significant differences in °Brix, pH, TA, or YAN content were seen among Riesling clones sampled within or across sites in 2011-2013 (Table 5.1).

Table 5.1. Juice chemistry data for Riesling clones sampled from two vineyard sites in 2011-2013 at or near commercial maturity[1].

Site	Year	Sample	Brix	pH	TA (g/L)	YAN[2] (mg N/L)
Vineyard 1	2011	21B	18.7a	2.95a	9.5a	
		49	17.5a	2.89a	9.0a	
		90	17.9a	2.95a	9.1a	
		110	17.9a	2.86a	9.4a	
		198	18.1a	2.87a	8.3a	
		239	18.7a	2.94a	9.2a	
	2012	21B	18.5a	2.97a	7.9a	31a
		49	17.8a	3.04a	8.2a	38a
		90	18.3a	3.025a	7.6a	24a
		110	17.7a	2.99a	8.2a	24a
		198	17.8a	2.97a	8.7a	45a
		239	16.3a	3.03a	7.9a	22a
	2013	21B	19.6a	3.04a	8.3a	56a
		49	17.2a	3.00a	8.6a	64a
		90	18.4a	3.025a	8.4a	75a
		110	17.4a	2.97a	9.1a	46a
		198	19.2a	3.03a	8.6a	57a
		239	18.7a	3.07a	8.4a	41a
Vineyard 2	2012	49	20.7a	3.18a	7.4a	136a
		198	20.2a	3.16a	6.1a	118a
		239	22.3a	3.25a	5.8a	115a
		HJW	21.6a	3.17a	6.7a	168a
	2013	49	16.4a	3.26a	7.4a	130a
		198	19.7a	3.24a	6.8a	114a
		239	17.4a	3.13a	7.7a	82a
		HJW	17.6a	3.28a	6.5a	115a

[1]Values connected by the same letter are not significantly different. ($p<0.05$). ANOVA with Tukey's HSD was performed across all Riesling clones, years, and vineyard sites.
[2]Vineyard 1 YAN recorded in 2012 and 2013 only.

Riesling juice chemistry analysis from 2008-2015. The analysis of 2008-2015 Riesling grape chemistry data reported in V2H newsletters published by the CEEL (http://grapesandwine.cals.cornell.edu/newsletters/veraison-harvest) is shown in Table 5.2. The CEEL data

did show some differences in berry weight, °Brix, pH, TA and YAN among regions within a sampling date. These differences could be contributed to site or terroir effects.

Table 5.2. 2008- 2015 Cornell Enology Extension Laboratory Veraison to Harvest Newsletter Riesling juice chemistry data[1,2].

Sampling Date	Site	Yearly Average				
		Berry Weight (g)	Brix	pH	TA (g/L)	YAN (g N/L)[3]
09/29/08	Finger Lakes	1.7 ±0.08bc	18.0 ±0.30abc	2.84 ±0.030	14 ±0.7bc	
	Lake Erie	1.8 ±0.1bc	16.4 ±0.14bc	3.00jk	12 ±0.4cd	
10/12/09	Finger Lakes	1.7 ±0.2bc	19.1 ±0.34ab	2.90 ±0.030kl	17 ±1a	
	Hudson Valley	2.1ab	17.7 ±1.5abc	3.30cdef	10 ±0.1def	
	Lake Erie	1.6 ±0.03c	17.9 ±0.38abc	3.20 ±0.010eg	15 ±0.6ab	
09/27/10	Finger Lakes	1.6±0.2c	19.6 ±1.4a	3.29 ±0.040bcdf	9.2 ±0.6defg	81.8 ±50ab
	Hudson Valley	1.5bc	17.1abc	3.54a	5.3h	134ab
	Lake Erie	1.5bc	17.8abc	3.27bcdef	6.9efgh	44.0ab
10/03/11	Finger Lakes	1.5 ±0.1c	18.3 ±1.0ab	3.04 ±0.030hijk	8.2 ±0.4fgh	37.8 ±26b
	Hudson Valley	1.7abc	14.2c	3.47abc	8.6defgh	196ab
	Lake Erie	2.6a	16.5abc	3.11efghijk	9.9defg	241a
09/24/12	Finger Lakes	1.6 ±0.09bc	19.6 ±0.44a	2.99 ±0.030jk	7.9 ±0.5fgh	56.0 ±27b
	Hudson Valley	1.8abc	18.7abc	3.33abcdef	6.0gh	119ab
	Lake Erie	1.8abc	19.9ab	3.24bcdefg	7.0efgh	133ab
10/09/13	Finger Lakes	1.6 ±0.1c	18.0 ±0.94ab	3.14 ±0.070e	8.4 ±0.7fgh	115 ±64ab
	Hudson Valley	1.7bc	16.6abc	3.48ab	7.4efgh	201ab
	Lake Erie	1.7abc	16.1abc	3.20defghi	7.6efgh	137ab
10/07/14	Finger Lakes	1.7 ±0.3bc	19.0 ±0.66ab	3.06 ±0.050ghij	10 ±1de	78.7 ±35ab
	Hudson Valley	2.0abc	19.0abc	3.40abcd	6.9efgh	139ab
	Lake Erie	2.0abc	17.3abc	3.12efghijk	8.9defgh	129ab
09/28/15	Finger Lakes	1.6 ±0.2c	19.2 ±1.3a	3.04 ±0.060hij	8.7 ±1fg	61.8 ±47b
	Hudson Valley	1.6bc	18.1abc	3.22cdefgh	7.0efgh	95.0ab

[1]Riesling juice data was obtained from Cornell Enology Extension Laboratory (CEEL) Veraison to Harvest Newsletters. (http://grapesandwine.cals.cornell.edu/newsletters/veraison-harvest)
[2]Values connected by the same letter are not significantly different. ANOVA with Tukey's HSD test was performed separetly for each sampling date across all sites ($p<0.05$).
[3]Juice YAN levels were not measured in 2008 and 2009.

Monoclonal Riesling wine sensory samples. Chemical parameters for 2014 monoclonal Riesling wine sensory samples is shown below in Table 5.3.

Table 5.3. Monoclonal Riesling juice and wine basic chemistry analysis.

Riesling Clone	Juice Analysis				Wine Analysis						
					After Cold Stabilization; Before TA Adjustments					Bottling	
	°Brix	pH	TA (g/L)	YAN (mg N/L)	pH	TA (g/L)	Tartaric Acid (g/L)	Malic Acid (g/L)	Citric Acid (g/L)	pH	TA (g/L)
90	22.0	3.13	7.2	133	2.96	9.2	4.1	2.4	0.14	3.09	8.2
110	21.0	3.09	8.3	118	2.95	9.4	4.3	2.5	0.15	3.08	8.4
198	21.3	3.09		118	2.97	9.6	4.0	2.5	0.15	3.15	8.0
Alsatian[c]	22.1	3.11	7.9	103	2.96	9.4	4.2	2.3	0.15	3.10	8.3

[c] Clone number unknown

Monomeric phenolic and tannin analysis of Riesling clone berry samples. No significant differences in individual or total monomeric phenolic compound concentrations were seen among Riesling clones harvested at a single site or across sites (Table 5.4). Further, few differences in tannin concentrations were seen for the factors clone, source, or year (2012-2013) with a few exceptions (Table 5.5). However, it should be noted that while these limited significant differences are present, it is likely that they are artifacts of the statistical analysis methods used, and will not be discussed here.

Table 5.4. Average Riesling clone monomeric phenolic data for each vineyard site from 2011-2013[1].

Location	Riesling Clone	[Compound][2] (ppm)												Total [Monomeric Phenolic] (ppm)
		Gallic Acid	Protocatechuic Acid	Catechin	Epicatechin[3]	GRP	t-Caftaric Acid	Caffeic Acid	c-Coutaric Acid	t-Coutaric Acid	t-Fertaric Acid	Quercetin-3-Galactoside	Quercetin-3-Glucoside	
Vineyard 1	21B	0.48a ±0.6	0.48a ±0.7	1.7a ±2	nd	6.9a ±6	31a ±13	0.53a ±0.5	0.60a ±0.5	0.80a ±0.5	3.3a ±2	nd	0.11a ±0.1	46a ±22
	49	0.090a ±0.2	0.38a ±0.5	1.0a ±0.6	0.070a ±0.09	4.0a ±4	30a ±14	0.37a ±0.3	0.34a ±0.5	0.86a ±0.4	3.1a ±2	nd	0.050a ±0.06	40a ±20
	90	0.55a ±0.5	0.30a ±0.1	1.9a ±1	0.41a ±0.1	5.4a ±4	29a ±15	0.80a ±0.1	0.61a ±0.5	1.0a ±0.6	3.5a ±2	nd	0.13a ±0.06	41a ±23
	110	0.49a ±0.6	nd	1.4a ±0.9	0.23a ±0.4	4.2a ±3	33a ±16	0.39a ±0.3	0.69a ±0.4	1.1a ±0.5	3.0a ±2	0.020a ±0.02	0.14a ±0.1	45a ±22
	198	0.49a ±0.7	0.21a ±0.2	1.4a ±0.8	0.23a ±0.3	6.1a ±6	38a ±14	0.58a ±0.5	0.79a ±0.5	1.3a ±0.6	3.6a ±2	nd	0.11a ±0.1	53a ±22
	239	0.070a ±0.1	0.42a ±0.6	1.5a ±2	0.14a ±0.2	5.5a ±5	27a ±17	0.64a ±0.6	0.35a ±0.5	0.95a ±0.7	2.9a ±2	nd	0.090a ±0.1	39a ±27
Vineyard 2	49	0.19a ±0.2	nd	1.8a ±0.2	nd	3.6a ±2	16a ±7	0.14a	0.86a ±0.6	0.42a ±0.4	2.7a ±1	nd	0.080a	26a ±15
	198	0.22a ±0.3	nd	1.9a	0.17a ±0.2	4.2a ±3	16a ±3	0.13a	0.98a ±0.7	0.76a	2.7a ±1	nd	0.10a	27a ±15
	239	0.20a ±0.3	nd	1.8a	nd	3.9a ±0.7	18a ±5	0.14a	1.0a ±0.7	0.77a	2.8a ±0.9	0.070a	1.0a ±1	30a ±17
	HJW	0.16a ±0.03	nd	2.2a	nd	6.3a ±2	17a ±5	0.20a	1.0a ±0.6	0.60a	3.2a ±0.5	0.060a ±0.08	0.73a ±0.4	31a ±17

[1]Values connected by the same letter are not significantly difference. ANOVA with Tukey's HSD was performed for each phenolic compound across all vineyard sites and Riesling clones ($p<0.05$)

[2]The compounds coumaric acid, ferulic acid, sinapic acid, caffeic acid ethyl ester, coumaric acid ethyl ester, rutin, quercetin-3-rhamnoside, myricetin, and quercetin were not detectable in any of the Riesling clone juice samples analyzed

[3]Epicatechin detected in 2013 only

Table 5.4. Riesling clone tannin data for each vineyard site from 2012-2013.

Source	Year	Riesling Clone	[Tannin] (ppm)[1]
Vineyard 1	2012	21B	3.80 ±3.5 bc
		49	7.05 ±5.3 abc
		90	3.30 ±1.1 c
		110	2.60 ±2.6 bc
		198	2.95 ±0.35 bc
		239	2.55 ±2.5 bc
	2013	21B	12.8 ±0.99 a
		49	11.0 ±0.21 abc
Vineyard 2	2012	49	11.0 ±2.0 ab
		198	4.90 ±2.4 abc
		239	7.30 ±1.7 abc
		HJW	7.30 ±0.57 abc
	2013	49	5.95 ±0.35 abc
		198	7.40 ±1.7 abc
		239	6.25 ±0.49 abc
		HJW	8.10 ±0.99 abc

[1]Values connected by the same letter are not significantly different. ANOVA with Tukey's HSD test was performed across all vineyard sites, years, and Riesling clones ($p<0.05$).

Sensory Data. In contrast to the monomeric phenolic and tannin profiles of the Riesling clones, differences in the sensory characteristics of monoclonal Riesling wines were found. In a preliminary sensory test with twenty-nine expert wine tasters, all of the participants reported differences among four monoclonal Riesling wines. In general, differences were attributed to variations in fruitiness, mouthfeel/palate weight, and acidity among the samples. However, there was not a consensus on which samples were the same, which were different, and what the specific differentiating characteristics between samples were.

Discussion

Riesling clones 21B, 49, 90, 110, 198, 239, and HJW grown in the New York Finger Lakes region and sampled in 2011-2013 could not be differentiated based on basic juice chemistry

analysis (°Brix, pH, TA, and YAN), monomeric phenolic compound profile, or sampling site. Further the factors clone, source, and year did not significantly affect the 2012 and 2013 Riesling clone juice tannin data. It should be stressed that the tannin concentrations of the samples are all relatively low compared to tannin concentrations found in red grapes (averaging 750ppm) or even other white grapes (averaging 20ppm) (Waterhouse 2002). Due to the low concentrations of juice tannins, it is unlikely that tannins have a substantial influence on the gustatory attributes of finished Riesling clone wines, as tannin addition rates as high as 600 to 830ppm catechin equivalents (CE) to red Cabernet Sauvignon wine with an innate tannin concentration of 554.0 ±11.5ppm catechin equivalents did not result in a difference in perceived astringency (Harbertson et al. 2012).

Vineyard site did not have appreciable effects on Riesling clone juice chemistry, monomeric phenolic concentration, or tannin concentration from grapes sampled at sites 1 and 2, located 25 miles apart. Due to the close proximity of the vineyards, it is possible that they experienced the same weather and climate conditions. Weather and climate are known to be critical wine quality determinates (Maltman 2008) and have greater influence on berry development than site-specific factors such as soil type and nutrient levels (Morlat and Jacquet 1993; Smart et al. 2006), or viticulture practices such as leaf thinning (Pereira et al. 2006). Interestingly, Nelson (2011) reported that no clear patterns were established when attempting to differentiate Finger Lakes Riesling vineyards by lake or clone during a single harvest year. However, it was proposed that winemaking and vineyard management factors may have the largest impact on differentiating Rieslings within the region (Nelson 2011). Additional studies comparing Riesling clones from a larger geographic region are needed to determine if growing site alone or terroir have an effect on the basic chemistry, monomeric phenolic, and tannin contents of Riesling clones.

V2H data obtained from grapes grown in the Finger Lakes, Hudson Valley, and Lake Erie regions showed some differences in berry weight, °Brix, pH, TA, and YAN when comparing clones within a single sampling date. A significant effect for sampling date was also seen in the ANOVA, likely due to yearly variations in weather and climate conditions. Further, sample collection dates varied by year due to V2H schedules, resulting in an additional source of variation. The data used for analysis represented the last sampling date prior to harvest for the majority of the Riesling sites. The larger distances between regions within the V2H sample set may have highlighted site differences that were not shown with the limited Riesling clone data collected in vineyards 1&2.

Despite a lack of chemical differences among Riesling clones, preliminary sensory test results suggest that wine experts can detect differences among monoclonal Riesling wines. However, this theory must be explored in more depth through formal sensory analysis. It is interesting to note that participants wrote the most extensive notes on the aromatic qualities of the wines, indicating the importance of aroma analysis during wine tasting compared to gustatory response. While the currently study only measured the phenolic compound content of Riesling clone grape juice, future studies analyzing the aromatic profiles of the clones and the resulting wines may provide more conclusive information. The effect of terroir on sensory perception of monoclonal or multiclonal Riesling wines was not assessed as part of this study, but is a possible topic for future work.

Conclusions

While Riesling clones could not be differentiated through the analysis of juice monomeric phenolic and total tannin contents, a preliminary sensory study with expert wine tasters suggested that monoclonal Riesling wines have perceivably different sensory qualities. Subsequent

descriptive analysis would allow for further identification of specific sensory characteristics, resulting from innate or extrinsic factors, that vary among Riesling clones. If present, sensory variations among monoclonal wines may provide an opportunity for the development of specialty wines that can be uniquely marketed, and potentially yield higher profit margins.

Finally, while total tannin concentrations in the Riesling clone juice samples were not different, the tannin contents of grape skins and seeds are likely more diverse and higher in concentration than juice tannins. As a result, the tannin profiles of the skins and seeds may serve as a better marker for differentiating among the grapes of Riesling clones and warrant additional study.

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CHAPTER 6

PROPOSED FUTURE WORK

The research presented in this dissertation supplements the greater wine community's understanding of key topics related to white wines, pre-fermentation cold soak (CS), and bitterness perception. Specifically, the research suggests that CS duration is not directly related to monomeric phenolic and monoterpene concentrations or the overall bitterness of white wines (See Chapter 2). However, as the influence of CS duration on the extraction of other gustatory and aroma compounds cannot be determined based on the data presented, additional work is required to fully understand the effect of CS on the composition and sensory qualities of wine. Meanwhile, based on the detection thresholds of select flavan-3-ols, it seems likely that any influences that these compounds have on wine sensory qualities occurs at subthreshold levels (See Chapter 3). Thus it is imperative to perform additional studies evaluating the effects of individual and mixtures of flavan-3-ol along with other compounds at subthreshold levels on sensory qualities. Furthermore, the influence of white wine color changes as a result of CS on bitterness perception was assessed but no association was observed (See Chapter 4). Even though a vision-taste crossmodal association was not observed here, there are many other factors that influence the wine tasting experience that have yet to be fully explored, such as the influence of wine tasting experience on taste and smell sensitivity. Finally, while Riesling clones could not be differentiated through monomeric phenolic or tannin profiling, preliminary sensory analysis suggests that monoclonal wines may be sensorially different (See Chapter 5). The work proposed below will build on the research presented in this dissertation in order to continue to expand the wine community's understanding of the influence of CS and specific wine components on the sensory qualities of white wines.

Proposed Future Work I. **Effect of pre-fermentation cold soak on the chemical components of aromatic white wines beyond monomeric phenolic compounds and monoterpenes as well as on the sensory qualities of finished wines.**

Previous studies have shown that CS of aromatic white grape cultivars leads to the extraction of volatile and non-volatile compounds, including monomeric phenolic and monoterpene concentrations, found in grape seeds, skin, and pulp, with the amount of compound extracted being related to the duration of skin contact. However, these results were not seen in the research presented in this dissertation (See Chapter 2). Specifically, CS of select aromatic white grape cultivars grown in the New York State Finger Lakes region for various lengths of time did not result in significant differences in monomeric phenolic compound profiles or bitterness intensity ratings among the wines. Monoterpene concentration was also not related to CS duration.

It is possible that differences in harvesting method, grape holding time and temperature prior to processing, CS duration, and temperature of CS result in variations in grape cell damage and sensory compound release, which in turn lead to the variations in CS study results. Alternatively, CS may have caused changes in other chemical components or sensory characteristics of wine that were not studied here. Such changes may include extraction of volatile compounds other than monoterpenes, as well as the extraction of non-volatile compounds like amino acids and anthocyanins. Sensory characteristics other than bitterness may have been modified by CS including astringency and other tactile sensations, aroma, sweetness, and sourness/ acidity. Additional studies looking at the effect of CS on these potential chemical and sensory changes in the Riesling, and Gewürztraminer, and Traminette wines studied, as well as other aromatic white grape cultivars, will provide a more global understanding of the influence of CS on sensory perception which in turn will aid winemakers when making processing choices.

Proposed Future Work II. **Effect of cultivar on individual compound detection thresholds in wine.**

The detection threshold of a compound varies depending on sample medium (water, model wine, wine), and is usually higher in more complex samples due to matrix effects. Matrix effects which can enhance or suppress the perception of a compound can be due to macro wine components such as sugar, acidity, and ethanol (Fontoin et al. 2008; Gawel et al. 2013; Wiley 1976), or microcomponents like phenolic and aroma compounds (Hufnagel and Hoffman 2008; Ferrer-Gallego et al. 2014). Monovarietal wines have unique sensory profiles that are based on the type and concentration of gustatory and aroma compounds present. As such, it is possible that the detection threshold of the same compound added to two different monovarietal wines will vary due to differences in enhancement, suppression, or other synergistic effects specific to each wine matrix.

The proposed work would determine whether grape cultivar has a significant effect on the detection threshold of compounds added to monovarietal wines. If differences in detection thresholds are found, non-volatile and volatile profiles of the wines will be assessed, and sensory profiles of the wines determined by descriptive analysis. With this data, researchers could begin to elucidate the specific wine components responsible for the differences in detection thresholds. Differences in sugar, ethanol, and acidity would be controlled for across wines. This research will provide insight on matrix effects such as the enhancement or suppression compound sensory qualities.

Proposed Future Work III. **Modeling changes in taste sensitivity based on wine tasting expertise.**

It is generally thought that detection thresholds decrease and gustatory and aroma sensitivity increase with increasing levels of wine tasting expertise. However, there is a lack of research quantifying detection thresholds and overall taste sensitivity differences among defined

groups with varying wine tasting expertise. Interestingly, it has been reported that the distributions of individual detection thresholds of several aromatic and taste compounds was greater than 3 $\log_{10}$ units in a sample of wine professionals comprised of enologists, wine growers, wine merchants, and wine brokers (Tempere et al. 2011). In the current work, a large discrepancy between panelist taste sensitivities to (+)-catechin and (-)-epicatechin was seen, likely due to varying levels of expertise among the panelists (See Chapter 3). A large distribution of gustatory and olfactory sensitivities among wine professionals may result from a lack of well-defined sub groups within the general category of "wine professionals" (i.e. not everyone who is a wine professional is also a Master Sommelier). There is, however, evidence to suggest that training level, as determined by academic degrees held, has an effect on the detection thresholds of ethylphenols and diacetyl (Tempere et al. 2011).

The proposed work will compare the detection thresholds of novice, moderate, and experienced wine consumers, trained panels, "expert wine tasters," and all levels of Sommeliers to select chemicals and overall taste sensitivities in order to determine if there are defined differences among the detection thresholds or taste sensitivities of each group. Factors that could be considered during the study include wine tasting expertise title (novice, expert, Master Sommelier, etc.), number of contact or wine training hours per year, age, health status, gender, and specific hyper- and hyposmias. Average detection thresholds and taste sensitivities of the groups will be compared to determine if differences show a linear relationship to wine tasting expertise, or if they are best represented using a different scale. In addition to analyzing overall wine taster groups, it would be optimal to measure the tasting abilities of individuals over time as they become more skilled at wine tasting, especially those who are working towards becoming wine professionals, or Advanced and Master Sommeliers. The results of the proposed study are

expected to lead to the construction of a model representing the impacts of training and wine professional status on wine tasting sensitivity.

***Proposed Future Work IV*. Effect of subthreshold compound concentrations on the perceived sensory qualities of white wines.**

A large number of phenolic compounds found in white wines are present at subthreshold levels. However, due to various mixture or synergistic effects, these compounds may still have an impact on the sensory qualities of wines (Ferrer-Gallego et al. 2014; Hufnagel and Hoffman 2008). The proposed study will attempt to elucidate the effect of subthreshold concentrations of phenolic compounds on the sensory qualities of aromatic white wines. This will be done through sensory analysis of water and model wine samples spiked with mixtures of phenolic compounds, and/or the analysis of commercial white wines with known phenolic compound profiles to which one or more phenolic compounds have been added. Alternatively, this study could be performed with suprathreshold compound additions to water, model wine, or wine samples.

***Proposed Future Work V*. Difference testing and descriptive analysis of monoclonal Riesling wines.**

In the current work, Riesling clones grown in the New York State Finger Lakes region could not be differentiated through monomeric phenolic or total tannin analysis. However, preliminary sensory results suggest that expert wine tasters can differentiate among monoclonal Riesling wines (See Chapter 5). If true, this ability will allow for the creation of more diverse wines. As a result, further difference testing and, if appropriate, descriptive analysis of the monoclonal Riesling wines is warranted to determine if monoclonal wines do indeed have differentiable sensory qualities. Finally, chemical analysis of monoclonal Riesling wines with distinct sensory characteristics should be performed in order to determine the specific source of the perceived differences.

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