AN ABSTRACT OF THE THESIS OF

Anthony Sereni for the degree of Master of Science in Food Science and Technology presented on July 18, 2016.

Title: Exploration into the Influence of Malolactic Fermentation Parameters and Pre-fermentation Juice Treatment on Chardonnay Mouthfeel

Abstract approved: _____

James P. Osborne

Mouthfeel is one of the most important quality parameters of Chardonnay wines. Malolactic fermentation (MLF) is an important process in wine production, and influential to wine mouthfeel, with the reduction in acidity being particularly important for cool climate wines that generally have higher acidity such as Chardonnay. MLF is typically induced by the addition of *Oenococcus oeni* after the completion of the alcoholic fermentation (AF) but can occur concurrent with AF by inoculating *O. oeni* simultaneously with the fermentative yeast *Saccharomyces cerevisiae*. We investigated the effect of MLF inoculation timing as well as the temperature of MLF and the presence of the non-*Saccharomyces* yeast *Torulaspora delbrueckii* on Chardonnay wine mouthfeel. Chardonnay wines were produced in 2014 with AF and MLF inoculated for simultaneous or sequential fermentations, and temperatures 15 and 21^{o}C, with or without the addition of *T. delbrueckii*. Mouthfeel attributes of the wines produced were assessed by a winemaker panel, using Napping® and Ultra-flash profiling. Significant differences

in mouthfeel perception were found based on timing and inoculation conditions, as well as between temperatures. Treatment type and temperature also effected the chemical composition of finished wines. Additionally, there are many interactions that occur between taste and aroma that may impact mouthfeel perception. This led us to investigate whether the aroma fraction of Chardonnay wine should be considered when investigating relationships between chemical composition and sensory perception of mouthfeel. Chardonnay wines were determined to have mouthfeel differences by altering the fermentation temperature of the alcoholic and malolactic fermentation as well as the timing of MLF and the presence of a non-*Saccharomyces* yeast during AF. Napping® and Ultra-flash-profiling were conducted using a panel of white winemakers. Each procedure was conducted twice: once with retro-nasal aroma and once without retronasal aroma. Napping® results showed that retronasal aroma impacted mouthfeel perception. Ultra-flash profiling displayed similar descriptive terms used with and without retronasal aroma, but terms were not consistently used for the same wine treatments with and without retronasal aroma. It is unclear if these differences are due to interactions or due to associated learning. These results suggest that for some mouthfeel terms the volatile fraction is playing a role and to establish relationships with chemical composition and mouthfeel perception it is important to consider both the volatile and nonvolatile wine fractions.

We then investigated the impact of pre-fermentation juice treatments on mouthfeel characteristics of Chardonnay wine. Chardonnay grapes were harvested from Oregon State University's vineyard in September, 2015. After destemming and pressing the juice was subjected to various treatments. These treatments included high, medium, and low

turbidity level, as well as hyper-oxidation, two-hour skin contact, and two-hour skin contact + hyper-oxidation. All treatments went through alcoholic and malolactic fermentations. Total phenolics and hydroxycinnamic acids differed between skin contact and hyper-oxidation treatments. Wines that underwent hyper-oxidation contained the lowest total phenolics. Hyper-oxidation following skin contact reduced total phenolics but retained more than the hyper-oxidation treatment. Sensory analysis using citation by frequency procedure showed that all treatments modified the mouthfeel of finished wines. However, chemical analysis did not fully elucidate the cause of these differences. Pre-fermentation juice treatments can be utilized to develop stylistic differences in finished Chardonnay wine.

The combined findings of this research demonstrate the usefulness of various enological practices to influence the sensory qualities of a Chardonnay wine, as well as emphasizing the importance of retro-nasal aroma's influence on the mouthfeel experience of Chardonnay wine.

EXPLORATION INTO THE INFLUENCE OF MALOLACTIC FERMENTATION PARAMETERS AND PRE-FERMENTATION JUICE TREATMENT ON CHARDONNAY MOUTHFEEL

by
Anthony Sereni

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented July 18, 2016
Commencement June 2017

Master of Science thesis of Anthony Sereni presented on July 18, 2016.

APPROVED:

Major Professor, representing Food Science & Technology

Head of the Department of Food Science & Technology

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Anthony Sereni, Author

ACKNOWLEDGEMENTS

I have to begin by thanking Dr. Elizabeth Tomasino and Dr. James Osborne for their patience, wisdom, and grace. Through their efforts and support I have learned about the many facets of enology, and gained valuable experience in wine production and assessment. Both of them have had a profound impact on my own thought process in reference to problem solving and intention. I'd also like to thank Stuart Cheshire for influencing me to join the wine lab at Oregon State University, and Dr. Thomas Shellhammer and Dr. Andrew Hunt for serving on my committee with Dr. Tomasino and Dr. Osborne.

This research would not be possible without the talents of Scott Robbins and Josh Price who grow disease free, high quality fruit at Woodhall vineyard. I also must thank Nadine Skillingstad and the all of the undergraduate research assistants working for Dr. Tomasino. Their efforts have been paramount in the sensory studies that accompanied this project. Thanks are also due to Daniel Kraft, Aubrey DuBois, Pallavi Mohekar, Mei Song, Jack Twilley, and Garrett Holzwarth for discussions on all things related to wine, as well as support and commiseration through graduate studies.

Thank you to the Food Science & Technology department for creating an amazing forum for learning. And thanks to all my family and friends through my crazy adventures in Corvallis and around the world; they have always helped bring context, appreciation, and fun to my experiences. Lastly I have to thank my loving wife Kassena for her support and patience along this windy road.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

Figure Page

LIST OF FIGURES (Continued)

Figure Page

LIST OF TABLES

CHAPTER ONE

LITERATURE REVIEW

Chardonnay

Chardonnay is a cultivar of the species *Vitis vinifera* that requires around 1300 (°C) growing degree days (GDD) to ripen. Because of the low GDD requirements Chardonnay is often grown in cooler grape growing regions. The grapes are generally thin skinned and at high risk for spring frost damage, powdery mildew, botrytis, and grapevine yellows. Vines are generally cane pruned because many of the buds close to the head of the vine are sterile and will not produce grapes (Robinson et al., 2012). It is one of the highest planted white wine grape cultivars in the world with roughly 400,000 acres planted across the globe as of 2008 (Brostrom & Brostrom, 2008, Cutler, 2012). Of all single cultivar wines Chardonnay is the most popular in US domestic sales (Stern, 2016).

The first mention of the Chardonnay grape is thought to be in an obscure text from 1583 under the name of "Beaunois". The name "Beaunois" was also used for the Aligote grape, and it remains controversial if "Beaunois" in fact refers to current day Chardonnay. There is no record of the name "Chardonnay" being used until between 1685 and 1690 when there was mention of a grape which produced the best wine: the "Chardonnet" grape, in the village of La Roche-Vineuse. But likely the modern name of the cultivar came from the village of Chardonnay close to La Roche-Vineuse and Uchizy in the Maconnais region of southern Burgundy. (Johnson et al., 2013) Through genetic testing we know that the Chardonnay cultivar was a crossing of Pinot Noir and Gouais Blanc, both of which were originally cultivated in France. The

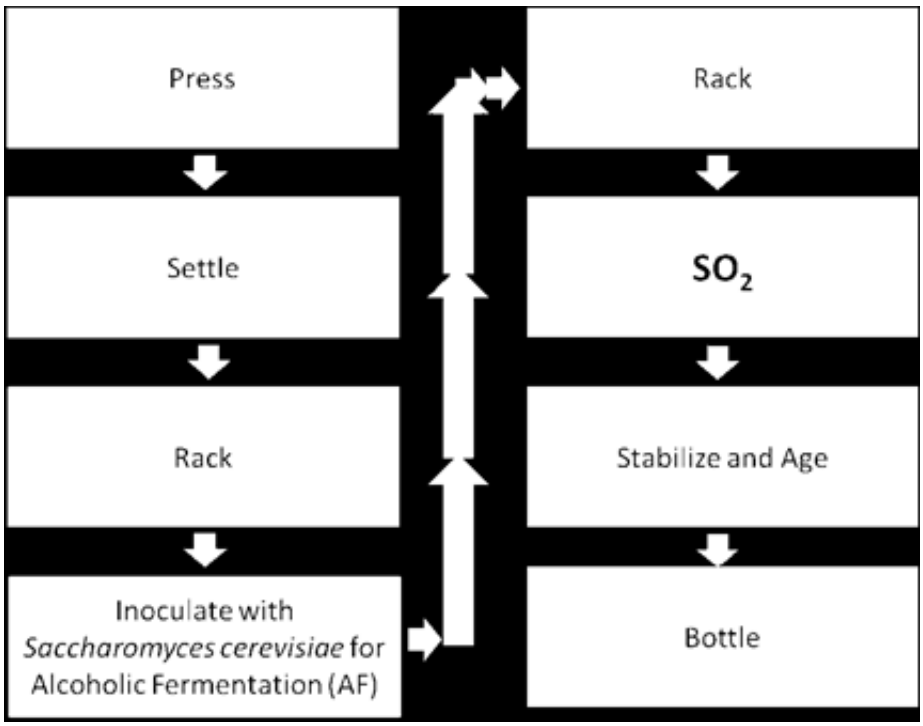
grapes birth location was traced to Saône-et-Loire, in eastern France; a region which runs from Burgundy to Champagne (Robinson et al., 2012). While Chardonnay wine is most famous from Burgundy, France, newer growing regions have gained global attention from this grape: such as California, Australia, Spain, Washington, and Oregon (Robinson et al., 2012; Johnson et al., 2013).

Chardonnay is referred to as a neutral aromatic cultivar; producing a wine which is not defined by a specific class of aroma compounds (Jackson, 2008). It has been crafted into many expressions of white wine. Chardonnay wine is possibly the most diverse white wine style, allowing for many variations in processing steps, including a variety of styles in sparkling wine and some dessert wines. However, it is most commonly used for the production of still white wine (Robinson et al., 2012).

White Wine Production

The process of white wines differs from the production of still red wines in that the grapes are pressed before fermentation, minimizing the extraction of compounds from grape skins and seeds. A basic white wine processing diagram is shown in Fig. 1.1. The grapes used for white wine are usually green or yellow skinned cultivars, though some popular white wine cultivars do contain higher amounts of coloration from anthocyanidins such as Pinot Gris and Gewürztraminer. (Jackson RS, 2008)

Figure 1.1 Process flow diagram for white wine.



Grapes are harvested when they have reached physiological maturity, generally decided upon by flavor, as well as the chemical measurement of sugar and acid. Sugar is measured as soluble solids, and is used to estimate the amount of alcohol that will result in the final wine. The strength of the acid in the must is measured by the pH of the solution, while the concentration is measured as titratable acidity (Deluc et al., 2007). These measurements have importance to the sensory properties and microbial stability of the final wine (Fernández-Novales et al., 2009).

The fermentative yeast *Saccharomyces cerevisiae* conducts the majority of the alcoholic fermentation (AF); converting sugars to ethanol, generating a number of secondary products which greatly impact the flavor, aroma, and mouthfeel of a finished wine. *S. cerevisae* will survive the acidic environment of wine (pH 3-4),

high alcohol content: 9-16% (v/v), and high levels of sulfur dioxide (sulfite): 30-80ppm typically used in winemaking. Sulfite is a by-products of yeast's metabolic pathway, and is also added by winemakers as an antioxidant, and antimicrobial addition to wine (Bakalinsky, 2000). The yeast is either inoculated or present in the winery environment. *S. cerevisiae* can sometimes be found on grapes, but generally other yeast species (non-*Saccharomyces*) which are less robust to grape juice conditions predominate the waxy grape surface environment in the vineyard (Rosini, 1984; Zahavi et al., 2002).

Chardonnay Wine Production Techniques

While in many ways the production of Chardonnay wine follows the same basic procedures as other white wines, there are a few production steps where winemakers utilize different techniques in order to produce varied styles of wine. For example, most white wines are fermented at lower temperatures than red wines in an effort to retain more volatile or aromatic compounds (Jackson, 2008). This usually occurs in temperature controlled tanks between 6-16^{o}C (Cottrell et al. 1986). While Chardonnay wine may also be produced at lower temperatures, it is one of the few white wines that is also commonly fermented at warmer temperatures ranging from 20-25^{o}C. Often the warmer fermentations are performed in barrel rather than stainless steel tanks.

Juice Turbidity

After pressing, Chardonnay juice contains a high amount of solids from the grape skins and pulp. In a review on grape solids by Casalta et al. (2016), they describe the

solid content of an average white grape must as containing 72% carbohydrate, 8% lipids, 5.5% minerals, 5.2% pectin and 2.6% nitrogen. These values can vary by cultivar and by the level of ripening. Depending on the starting concentration some of these compounds can negatively affect the sensory qualities of a finished wine leading some winemakers to utilize enzymes to degrade these compounds before fermentation as is the case with pectin, where winemakers will utilize pectinase to decrease the starting quantity (Casalta et al., 2016).

Must is generally settled prior to alcoholic fermentation (Fig. 1.1) for a period of time, or until a specific turbidity is reached. The specifics are variable by winemaking style. Higher must turbidity has been shown to correlate with an increase in the populations of two yeast species: *Candida zemplinina* and *Hanseniaspora* spp. (Albertin et al., 2014). High juice turbidity has been correlated with an increase in C_6 alcohols such as hexanol, and some C_6 aldehydes; all of which contribute to a "green" aroma character. Additionally, there are anecdotal claims by winemakers of higher levels of undesirable volatile thiols generated during ferments of white must with high turbidity. There is evidence to support an increase in fruity notes with increases in turbidity due to an increase in acetates and some higher alcohols. It is important to note that yeast strain selection has been demonstrated to be a greater influence than must turbidity on all of the above listed compounds (Nicolini et al., 2011).

While consistent difference in yeast assimilable nitrogen with higher must turbidity has not been demonstrated, there is evidence that yeast populations appear more robust with increases in must turbidity. Lower levels of residual sugar, shorter fermentation length, as well as lower levels of volatile acidity and acetaldehyde are

noted from higher rates of juice turbidity. High rates of glycerol production are also correlated with higher juice turbidity; although, they are generally not above sensory threshold limits. (Albertin et al., 2014)

If too much time is allowed for settling, or other pre-fermentation clarification treatments are used, such as fining, centrifugation, or pectinase, the must may be excessively clarified. Excessive clarification of must has been demonstrated to decrease long chain unsaturated fatty acids in yeast during ferment, which can cause an increase in acetic acid production (Nicolini et al., 2011). Boivin et al. (1998) found a decrease in mannoproteins in the cell wall of yeast by clarification of Chardonnay juice; with turbidity taken from 380 NTU to 34 NTU. The resulting cell walls were demonstrated to be more porous, and less robust to the fermentation environment, possibly leading to incomplete, or stuck, fermentations (Boivin et al., 1998).

Volatile and non-volatile fractions of Chardonnay wine fermented with varying levels of juice turbidity have been studied (Boivin et al., 1998, Nicolini et al., 2015). Research lacks sufficient sensory assessment to generate useful correlations with the overall wine experience. This is particularly important for the true assessment of the aroma experience, as the quantification of wine constituent compounds does little to aid in the understanding of the complex interactions that occur within the wine matrix. These studies also lack an assessment of the perceived texture, or mouthfeel, of the wine; instead relying on the non-volatile chemistry measurements of a wine alone. Much research has investigated nonvolatile composition to sensory perception with limited success (Rodriguez-Bencomo et al., 2011; Saenz-Navajas et al., 2012), and

therefore nonvolatile composition cannot be used to predict perceived sensory perception.

Skin Contact with Must

An additional technique that may be employed during Chardonnay wine production is an extended period of time that the juice and skin remains in contact before pressing. The goal of this process is to allow additional extraction of phenolic and flavor/aroma compounds from the skin before pressing. Some studies cite positive sensory ratings of wines after short periods of skin contact due to differences in aromatics, while other studies cite an increase in perceived viscosity as the main benefit of skin contact. Ferreira et al. (1995) found that skin contact caused an increase in C6 compounds, especially hexan-1-ol and hex-2-en-1-ol, in finished Chardonnay wines of Burgundy. They also found that excess settling time mitigated this increase; causing a neutralizing effect (Ferreira et al., 1995). The main downfall of skin contact is cited as the browning of finished wine with bottle aging (Cheynier et al., 1989; Gawel et al., 2014).

Browning has been demonstrated, by Fernandez-Zurbano et al., (1998) to be influenced by specific phenolic composition, and not total phenolic content. Flavanol content is cited as positively correlated with browning level due to oxidation, with no correlation due to hydroxycinnamic acids or esters. Flavanol compounds are derived from grape skins, and are found in much lower concentrations in white wines than in red wines. (Fernández-Zurbano et al., 1998)

Hyper-oxidation of Must

Hyper-oxidation is a technique during white winemaking where prior to fermentation the juice is oxidized by the addition of large amounts of air or oxygen. The goal is to oxidize the phenolic compounds that may be present in the juice so that these compounds will be removed during the alcoholic fermentation (by precipitation). This in turn will result in wine with lower phenolic compounds that could potentially be oxidized during the aging process leading to browning and flavor and aroma taints. The phenolic species are oxidized by polyphenol oxidase enzyme (PPO) in the presence of O_2 gas exposure, either by atmospheric gas, or pure O_2 gas pumped into the must. Post AF, wine made from hyper-oxidized Chardonnay juice have been demonstrated to hold stable color compared to control treatments (Schneider, 1998). There is no indication that hyper oxidation results in higher rates of acetic acid as previously thought (Cheynier et al., 1989). Wines fermented in this method have lower levels of all polyphenolic compounds compared to controls. These wines have also exhibited higher concentrations of volatile compounds with the exception of ethyl acetate, acetate, and ß-damascenone. Sensory analysis of these wines, compared to controls, have generally demonstrated a higher rate of fruity aromatics, and a lower rate of herbaceous, bitter, and flower characteristics (Schneider, 1998; María Jesús et al., 2011; Cejudo-Bastante et al., 2012). The disparity between the chemical findings on wine from hyper-oxidized must, and the subsequent sensory data emphasize the importance that future research on the volatile and non-volatile fraction influence of Chardonnay fermentation parameters be precisely correlated with sensory data.

Influence of Microbes on Wine Quality

Though grapes are pressed before fermentation in the production of white wines, bacteria and yeast present on grape skins and winery equipment can still play a role in the fermentation dynamics of the juice. The microbial counts on grapes at the time of harvest are highly variable with seasonal conditions. Most yeast species present on grapes cannot survive the high alcohol environment created by the fermentation by *S. cerevisiae* but high populations of bacteria and non-*Saccharomyces* yeast can sometimes interfere with the health of *S. cerevisiae* by limiting nutrient availability, or by generation of harmful compounds. These organisms can influence the sensory properties of a finished wine in both positive and negative ways. (Albertin et al., 2014)

Pre-fermentation must treatment has been shown to impact the kinetics of yeast species during fermentation, as well as the sensory properties of the finished wine. The addition of SO_2 generally decreases bacteria, as well as non-*Saccharomyces* yeast species, with less of an impact noted on total counts of *Candida zemplinina* than other non-*Saccharomyces* species. In addition, inoculation with a large population of a commercial *S. cerevisiae* culture can also ensure the initiation of the alcoholic fermentation and reduces the risk of growth of non-*Saccharomyces* yeast. For example, Albertin et al., (2014) noted that the inoculation of *S. cerevisiae* in Chardonnay allowed a competitive advantage against the native species *C. zemplinina* and *Hanseniaspora spp*. when fermentation was conducted at low temperatures (10-15^{o}C). Commercial culture inoculation of *S. cerevisiae* also appeared most effective

over a broad range of parameters at lowering populations of *Torulaspora delbrueckii*. (Albertin et al., 2014)

While the growth of non-*Saccharomyces* yeast is often associated with wine spoilage issues (Jolly et al., 2014), growth of certain species may have some beneficial impact on wine quality. For example, *Metschnikowia pulcherrima* has been shown to decrease final wine alcohol content from 0.9-1.6% (Contreras et al., 2014) that could be beneficial when producing wines from grapes with very high Brix. Positive sensory aspects were also noted for Shiraz wines, but negative aromatic influences were noted in Chardonnay wine due to increased levels of ethyl acetate (described as nail polish remover) (Contreras et al., 2014). In addition, *T. delbrueckii* when co-inoculated with *S. cerevisiae* has been shown to impact concentrations of 2-phenylethanol, isoamyl acetate, fatty acid esters, C_4-C_{10} fatty acids, lactones, and vinylphenols (Azzolini et al., 2014).

Malolactic Fermentation

Chardonnay is one of the few white wines that often undergo a malolactic fermentation (MLF). This process is generally conducted after AF and is induced by the addition of *Oenococcus oeni*. This bacteria converts the diprotic malic acid to lactic acid (single protic group) which results in a raise of pH in the wine and an increase in microbial stability due to the removal of malic acid (Silver et al., 1981). Because of the decrease in acidity this process is often utilized in the production of wines in cooler climates where grapes typically contain high concentrations of acids, especially malic acid. While MLF is frequently used in the production of red wines, it

is less common in white wines as reduction of white wine acidity may not improve quality. However, MLF is often used in the production of Chardonnay wine as it offers another tool that a winemaker can use to create a different style of Chardonnay (Gambetta et al., 2014).

Aside from impacting acidity, MLF may also impact other wine quality parameters. Avedovech et al. (1992) reported that tasters could discern differences in aroma between Chardonnay wines which have undergone MLF vs. non-MLF treatments. This is likely due to the changes in a number of volatile compounds that have been demonstrated to occur during MLF such as diacetyl, acetoin, volatile acids, diethyl succinate, volatile esters, ethyl acetate, n-propanol, 2-butanol, n-hexanol, ethyl lactate, and 2,3-butanediol. (Davis et al., 1985; Avedovech et al., 1992).

O. oeni is the predominant LAB utilized for MLF in wine. It is a fastidious organism with some important limitations. Clarification of must (by excess fining, filtration, or centrifugation) inhibits native growth of LAB. Sulfite inhibits most LAB, and is an important consideration for winemakers intending to put wines through MLF. Ethanol levels above 12% (v/v) generally inhibit *O. oeni*, but many commercial strains can tolerate ethanol levels above 14% (v/v). Ethanol indirectly impacts MLF by interfering with enzyme activity. CO_2 appears to stimulate *O. oeni* to convert malic acid into lactic acid in low pH, and high ethanol environments (Wibowo et al., 1985).

While MLF is typically conducted after the completion of the alcoholic fermentation, it may also occur at the same time as the alcoholic fermentation (AF). This is known

as either co-inoculation or simultaneous fermentation and can be induced by the inoculation of both the yeast and bacterial starter cultures at the same time. In red winemaking, co-inoculation has been studied as a possible means of reliably completing AF and MLF in a shorter period of time. AF and MLF were shown to more reliably complete fermentation during co-inoculation, than sequential AF and MLF (Guzzon et al., 2012). Co-inoculation for AF and MLF in white wines is not commonly conducted in most commercial settings due to anecdotal concerns of higher levels of volatile acidity, and stuck fermentations. This is due to the fact that *O. oeni* is a heterofermentative bacteria that can produce acetic acid via the metabolism of glucose. However, due to the bacteria's preference for malic acid metabolism at pH levels < 3.60 increased acetic acid has only been noted when co-inoculation occurred in high pH grapes (Mills et al., 2005).

Aside from shortening the time for the wine to complete MLF, co-inoculation has also been demonstrated to impact wine aroma and flavor. For example, Munoz et al. (2014) reported differences in quality parameters between yeast strains using the same bacteria strain (Lalvin VP41) in co-inoculated must. Wines produced with *S. cerevisae* strain ICV D80 had higher levels of residual fructose post fermentation, and higher levels of VA compared to sequential fermentations with the same strain. In contrast, wines fermented with *S. cerevisiae* Fermicru UY4 did not contain residual sugar and had no significant increase in VA levels compared to the sequential inoculation treatment (Muñoz et al., 2014).

In research on synthetic grape must Rossouw et al., (2012) found no difference in residual sugar levels between sequential and co-inoculated treatments (*S. cerevisae*

strain VIN13, *O. oeni* strain S6). There were differences in maximum yeast and bacteria populations, with lower total counts in co-inoculated treatments; however, there were no issues in the completion of AF and MLF. Co-inoculated synthetic must had higher levels of positive fruity aroma compounds ethyl lactate and octanoic acid. These treatments were also found to have lower levels of isobutanol, ethyl acetate, and isoamyl alcohol: three negative aroma compounds. (Rossouw et al., 2012)

Maarman et al. (2014) demonstrated that co-inoculation using two different yeast strains (Cross Evolution, and EC1118) and one strain of *O. oeni* (S5) consistently increased the concentration of volatile esters in the finished wine compared to sequential inoculations. While the majority of volatile esters are thought of as imparting positive sensory attributes, this study also found higher concentrations of ethyl acetate. At high concentrations ethyl acetate has a "solvent" aroma and is considered a defect (Medina et al., 2013). No significant difference was found between co-inoculated and sequential treatments in acetic acid production. Diacetyl was found to be significantly lower in co-inoculated treatments compared to sequential. This could be a positive, or a negative attribute depending on the style of Chardonnay desired. Sensory analysis of these wines was not conducted (Maarman et al., 2014). These studies demonstrate the variability in co-inoculation performance between yeast and bacteria strains.

Chardonnay Wine Mouthfeel

As can be clearly demonstrated, a large number of different winemaking techniques can be employed when producing a Chardonnay wine. While many of these

techniques are aimed at impacting wine flavor and aroma, many are targeted at improving the body or mouthfeel of the wine. However, compared to our understanding how winemaking techniques impact aroma and flavor compounds (Rapp et al., 1986, Noble et al., 1987, Allen et al., 1991, Guth, 1997, Parr et al., 2003), our understanding of how to impact mouthfeel through use of certain winemaking techniques is rather limited. The texture, or mouthfeel, of Chardonnay is one of the least understood areas in wine science, yet the importance of mouthfeel on wine assessment cannot be overstated.

Chardonnay is generally characterized as a full bodied white wine, meaning high perceived viscosity. Whether or not this is due to the fact that Chardonnay is the most frequent white wine to undergo MLF is unknown. Runnebaum et al. (2011) found that panelists' perception of higher viscosity in white wine was correlated with lactate (from MLF). They also noted that many white wines put through MLF are fermented, or aged, in oak barrels. This may impact the viscosity of the wine either directly through dissolved gas uptake and egallitannins, or indirectly through alterations in aroma compounds or through extended time on yeast lees.

Other factors that have been suggested to influence white wine mouthfeel include glycerol and phenolics. However, while glycerol has often been implicated in increasing white wine mouthfeel, Runnebaum et al., (2011) recently reported that this compound was not typically present in high enough concentrations to influence perceived viscosity. Phenolic compounds are generally lower in white wines than red, and have been shown to vary in level of astringency and mouthfeel impact with the wine chemistry measurements of acid and alcohol. The impact of phenolics on

texture is strongest in low alcohol wines (< 13% v/v). Astringency of wines at pH 3.3 has been shown to significantly increase with great concentration of phenolic compounds, however, no differences have been noted when the same phenolic addition was conducted in a wine of pH 3.0. In general, higher phenolic content increases bitterness, viscosity, and hotness. (Gawel et al., 2013).

Determining the factors influencing white wine mouthfeel is also complicated by the fact that many studies fail to account for the possible interactive effect between the volatile fraction and the nonvolatile of a wine. Modern research in food and sensory systems has demonstrated that volatile compounds can impact the sensory perception of touch and texture (Labbe et al., 2008; Kora et al., 2003; Chen et al., 2012; Koijck, et al., 2015). However, wine sensory research is yet to fully explore this phenomenon. Although the non-volatile fraction of wine has been demonstrated to strongly influence the intensity of the volatile or aromatic fraction of wines (Rodríguez-Bencomo et al., 2011), less is known about how the aroma of a wine impacts the perception of body or mouthfeel. For example, Pickering et al. (1998) found that in the absence of retro-nasal aroma, ethanol was positively associated with viscosity and density of white wines at 10 and 12 % (v/v) but was not significantly different between 7 and 14% (v/v) However these effects were not present when the aroma of the wines were expressed.

Other interactions between wine constituents have also been demonstrated to impact mouthfeel assessment. Vidal et al. (2004) reported that bitterness, a taste, was positively correlated with increases in ethanol concentration, while astringency was shown to decrease with increasing levels of ethanol (8 to 14%). The effects were

neutralized in wine containing high levels of glycoproteins (proteoglycans), such as barrel aged Chardonnay wines which are aged on lees. Glycoproteins have been demonstrated to decrease astringency, creating a "smoothing" effect on mouthfeel perception. Proteoglycans (which are from yeast cell wall components) and rhamnogalacturonan II (grape polysaccharides) have been positively associated with increased mouthfeel, or "fullness" in model wines which lack proanthocyanidins- corresponding to the assessment of white wines (Vidal et al., 2004). These interactions are particularly important in the understanding of Chardonnay mouthfeel, as the actual measurements of viscosity and identified non-volatile chemical parameters fall short of capturing the entire picture of human perception.

Due to the lack of understanding of how to manipulate white wine mouthfeel the objective of this study was to investigate the impact of a number of wine production methods on the sensory perception of Chardonnay wine mouthfeel. In particular, winemaking practices thought to influence mouthfeel such as malolactic fermentation, skin contact, hyper-oxidation, and increased juice solids content, were investigated. Further, the influence of retro-nasal aroma on the perception of Chardonnay wine mouthfeel was also determined.

LITERATURE CITED

Albertin, W., Miot-Sertier, C., Bely, M., Marullo, P., Coulon, J., Moine, V., Masneuf-Pomarede, I. (2014). Oenological prefermentation practices strongly impact yeast population dynamics and alcoholic fermentation kinetics in Chardonnay grape must. *International Journal of Food Microbiology*, *178*, 87–97. http://doi.org/10.1016/j.ijfoodmicro.2014.03.009

Allen, M. S., Lacey, M. J., Harris, R. L. N., & Brown, W. V. (1991). Contribution of Methoxypyrazines to Sauvignon blanc Wine Aroma. *American Journal of Enology and Viticulture*, *42*(2), 109–112.

Avedovech, R. M., Mcdaniel, M. R., Watson, B. T., & Sandine, W. E. (1992). An Evaluation of Combinations of Wine Yeast and Leuconostoc oenos Strains in Malolactic Fermentation of Chardonnay Wine. *American Journal of Enology and Viticulture*, *43*(3), 253–260.

Azzolini, M., Tosi, E., Lorenzini, M., Finato, F., & Zapparoli, G. (2014). Contribution to the aroma of white wines by controlled Torulaspora delbrueckii cultures in association with Saccharomyces cerevisiae. *World Journal of Microbiology and Biotechnology*, *31*(2), 277–293. http://doi.org/10.1007/s11274-014-1774-1

Bakalinsky, A., Why are Wine Yeasts Resistant to Sulfite? 2000. *In* The 3rd Burgundy-California-Oregon Wine Symposium, University of Dijon, France

Boivin, S., Feuillat, M., Alexandre, H., & Charpentier, C. (1998). Effect of must turbidity on cell wall porosity and macromolecule excretion of Saccharomyces cerevisiae cultivated on grape juice. *American Journal of Enology and Viticulture*, *49*(3), 325–332.

Brostrom, G. G., & Brostrom, J. (2008). *The Business of Wine: An Encyclopedia: An Encyclopedia*. ABC-CLIO.

Casalta, E., Vernhet, A., Sablayrolles, J., Tesniere, C., Salmon, J., (2016). Review: Characterization and Role of Grape Solids during Alcoholic Fermentation under Enological Conditions. *American Journal of Enology and Viticulture*, *67(2)*, 133-138.

Cejudo-Bastante, M. J., Hermosín-Gutiérrez, I., Castro-Vázquez, L. I., & Pérez-Coello, M. S. (2011). Hyperoxygenation and Bottle Storage of Chardonnay White Wines: Effects on Color-Related Phenolics, Volatile Composition, and Sensory Characteristics. *Journal of Agricultural and Food Chemistry*, *59*(8), 4171–4182. http://doi.org/10.1021/jf104744q

Cejudo-Bastante, M. J., Perez-Coello, M. S., Perez-Juan, P. M., & Hermosin-Gutierrez, I. (2012). Effects of hyper-oxygenation and storage of Macabeo and Airen white wines on their phenolic and volatile composition. *European Food Research and Technology*, *234*(1), 87–99. http://doi.org/10.1007/s00217-011-1619-1

Chen, J., & Eaton, L. (2012). Multimodal mechanisms of food creaminess sensation. *Food & Function*, *3*(12), 1265–1270. http://doi.org/10.1039/C2FO30116D

Cheynier, V., Rigaud, J., Souquet, J. M., BarillÈRE, J. M., & Moutounet, M. (1989). Effect of Pomace Contact and Hyperoxidation on the Phenolic Composition and Quality of Grenache and Chardonnay Wines. *American Journal of Enology and Viticulture*, *40*(1), 36–42.

Contreras, A., Hidalgo, C., Henschke, P. A., Chambers, P. J., Curtin, C., & Varela, C. (2014). Evaluation of Non-Saccharomyces Yeasts for the Reduction of Alcohol Content in Wine. *Applied and Environmental Microbiology*, *80*(5), 1670–1678. http://doi.org/10.1128/AEM.03780-13

Contreras, A., Hidalgo, C., Schmidt, S., Henschke, P. A., Curtin, C., & Varela, C. (2015). The application of non-Saccharomyces yeast in fermentations with limited aeration as a strategy for the production of wine with reduced alcohol content. *International Journal of Food Microbiology*, *205*, 7–15. http://doi.org/10.1016/j.ijfoodmicro.2015.03.027

Cottrell, T. H. E., & Lellan, M. R. M. (1986). The Effect of Fermentation Temperature on Chemical and Sensory Characteristics of Wines from Seven White Grape Cultivars Grown in New York State. *American Journal of Enology and Viticulture*, *37*(3), 190–194.

Cutler, L., Varietal Focus Chardonnay. Wine Business Monthly. January 2012. Wine Communications Group Inc

Davis, C. R., Wibowo, D., Eschenbruch, R., Lee, T. H., & Fleet, G. H. (1985). Practical Implications of Malolactic Fermentation: A Review. *American Journal of Enology and Viticulture*, *36*(4), 290–301.

Deluc, L. G., Grimplet, J., Wheatley, M. D., Tillett, R. L., Quilici, D. R., Osborne, C., Cramer, G. R. (2007). Transcriptomic and metabolite analyses of Cabernet Sauvignon grape berry development. *BMC Genomics*, *8*, 429. http://doi.org/10.1186/1471-2164-8-429

Domizio, P., Liu, Y., Bisson, L. F., & Barile, D. (2014). Use of non-Saccharomyces wine yeasts as novel sources of mannoproteins in wine. *Food Microbiology*, *43*, 5–15. http://doi.org/10.1016/j.fm.2014.04.005

Fernández-Novales, J., López, M.-I., Sánchez, M.-T., Morales, J., & González-Caballero, V. (2009). Shortwave-near infrared spectroscopy for determination of reducing sugar content during grape ripening, winemaking, and aging of white and red wines. *Food Research International*, *42*(2), 285–291. http://doi.org/10.1016/j.foodres.2008.11.008

Fernández-Zurbano, P., Ferreira, V., Escudero, A., & Cacho, J. (1998). Role of Hydroxycinnamic Acids and Flavanols in the Oxidation and Browning of White Wines. *Journal of Agricultural and Food Chemistry*, *46*(12), 4937–4944. http://doi.org/10.1021/jf980491v

Ferreira, B., Hory, C., Bard, M. H., Taisant, C., Olsson, A., & Le Fur, Y. (1995). Effects of skin contact and settling on the level of the C18:2, C18:3 fatty acids and C6 compounds in burgundy chardonnay musts and wines. *Food Quality and Preference*, *6*(1), 35–41. http://doi.org/10.1016/0950-3293(94)P4210-W

Gambetta, J. M., Bastian, S. E. P., Cozzolino, D., & Jeffery, D. W. (2014). Factors Influencing the Aroma Composition of Chardonnay Wines. *Journal of Agricultural and Food Chemistry*, *62*(28), 6512–6534. http://doi.org/10.1021/jf501945s

Gawel, R., Day, M., Van Sluyter, S. C., Holt, H., Waters, E. J., & Smith, P. A. (2014). White Wine Taste and Mouthfeel As Affected by Juice Extraction and Processing. *Journal of Agricultural and Food Chemistry*, *62*(41), 10008–10014. http://doi.org/10.1021/jf503082v

Gawel, R., Sluyter, S. C. V., Smith, P. A., & Waters, E. J. (2013). Effect of pH and Alcohol on Perception of Phenolic Character in White Wine. *American Journal of Enology and Viticulture*, *64*(4), 425–429. http://doi.org/10.5344/ajev.2013.13016

Gawel, R., Sluyter, S. V., & Waters, E. J. (2007). The effects of ethanol and glycerol on the body and other sensory characteristics of Riesling wines. *Australian Journal of Grape and Wine Research*, *13*(1), 38–45. http://doi.org/10.1111/j.1755-0238.2007.tb00070.x

Guth, H. (1997). Identification of Character Impact Odorants of Different White Wine Varieties. *Journal of Agricultural and Food Chemistry*, *45*(8), 3022–3026. http://doi.org/10.1021/jf9608433

Guzzon, R., Villega, T., Pedron, M., Malacarne, M., Nicolini, G., & Larcher, R. (2012). Simultaneous yeast–bacteria inoculum. A feasible solution for the management of oenological fermentation in red must with low nitrogen content - Springer. *Annals of Microbiology*, *63*(2), 805–808. http://doi.org/10.1007/s13213-012-0499-3

Jackson R., 2008. Wine Science Principles and Applications. Elsevier Inc., Amsterdam, NL.

Jaffe, J., Valentin, D., Meunier, J.-M., Siliani, A., Bertuccioli, M., & Le Fur, Y. (2011). The Chardonnay wine olfactory concept revisited: A stable core of volatile compounds, and fuzzy boundaries. *Food Research International*, *44*(1), 456–464. http://doi.org/10.1016/j.foodres.2010.09.022

Johnson, H., Robinson, J., 2013. The World Atlas of Wine. Mitchell Beazley, London, UK.

Koijck, L. A., Toet, A., & Van Erp, J. B. F. (2015). Tactile roughness perception in the presence of olfactory and trigeminal stimulants. *PeerJ*, *3*, e955. http://doi.org/10.7717/peerj.955

Kora, E. P., Latrille, E., Souchon, I., & Martin, N. (2003). Texture-Flavor Interactions in Low Fat Stirred Yogurt: How Mechanical Treatment, Thickener Concentration and Aroma Concentration Affect Perceived Texture and Flavor.

Journal of Sensory Studies, *18*(5), 367–390. http://doi.org/10.1111/j.1745-459X.2003.tb00395.x

Labbe, D., Gilbert, F., & Martin, N. (2008). Impact of Olfaction on Taste, Trigeminal, and Texture Perceptions. *Chemosensory Perception*, *1*(4), 217–226. http://doi.org/10.1007/s12078-008-9029-x

Maarman, B.C., 2014. Interaction Between Wine Yeast and Malolactic Bacteria and the Impact on Wine Aroma and Flavour. Thesis, Stellenbosch University, Stellenbosch, South Africa.

Medina, K., Boido, E., Fariña, L., Gioia, O., Gomez, M. E., Barquet, M., Carrau, F. (2013). Increased flavour diversity of Chardonnay wines by spontaneous fermentation and co-fermentation with Hanseniaspora vineae. *Food Chemistry*, *141*(3), 2513–2521. http://doi.org/10.1016/j.foodchem.2013.04.056

Mills, D. A., Rawsthorne, H., Parker, C., Tamir, D., & Makarova, K. (2005). Genomic analysis of Oenococcus oeni PSU-1 and its relevance to winemaking. *FEMS Microbiology Reviews*, *29*(3), 465–475. http://doi.org/10.1016/j.fmrre.2005.04.011

Muñoz, V., Beccaria, B., & Abreo, E. (2014). Simultaneous and successive inoculations of yeasts and lactic acid bacteria on the fermentation of an unsulfited Tannat grape must. *Brazilian Journal of Microbiology*, *45*(1), 59–66. http://doi.org/10.1590/S1517-83822014000100009

Nicolini, G., Moser, S., Roman, T., Mazzi, E., & Larcher, R. (2011). Effect of juice turbidity on fermentative volatile compounds in white wines. *Vitis*, *50*(3), 131–135.

Noble, A, Arnold, R, Buechsenstein, J, Leach, E, Schmidt, J, & Stern, M. (1987). Modification of a Standardized System of Wine Aroma Terminology. *American Journal of Enology and Viticulture*, 38(2), 143-146.

Parr, W. V., White, K. G., & Heatherbell, D. A. (2003). The nose knows: influence of colour on perception of wine aroma. *Journal of Wine Research*, *14*(2–3), 79–101. http://doi.org/10.1080/0957126041000167796 9

Peynaud, E., & Blouin, J. (1996). *The Taste of Wine: The Art Science of Wine Appreciation*. John Wiley & Sons.

Pickering, G. J., Heatherbell, D. A., Vanhanen, L. P., & Barnes, M. F. (1998). The Effect of Ethanol Concentration on the Temporal Perception of Viscosity and Density in White Wine. *American Journal of Enology and Viticulture*, *49*(3), 306–318.

Rapp, A, & Mandery H. (1986). Wine Aroma. *New Progress in Vine and Wine Research*, 42(8), 873-884

Reynolds, A. (2010) Managing Wine Quality. Woodhead Publishing Limited Cambridge, UK

Robinson, J., Harding, J., Vouillamoz, J., 2012. Wine Grapes. Ecco, New York.

Rodríguez-Bencomo, J. J., Muñoz-González, C., Andújar-Ortiz, I., Martín-Álvarez, P. J., Moreno-Arribas, M. V., & Pozo-Bayón, M. Á. (2011). Assessment of the effect of the non-volatile wine matrix on the volatility of typical wine aroma compounds by headspace solid phase microextraction/gas chromatography analysis. *Journal of the Science of Food and Agriculture, 91*(13), 2484–2494. http://doi.org/10.1002/jsfa.4494

Rosini, G. (1984). Assessment of dominance of added yeast in wine fermentation and origin of Saccharomyces cerevisiae in wine-making. *The Journal of General and Applied Microbiology, 30*(4), 249–256. http://doi.org/10.2323/jgam.30.249

Rossouw, D., Du Toit, M., & Bauer, F. F. (2012). The impact of co-inoculation with Oenococcus oeni on the trancriptome of Saccharomyces cerevisiae and on the flavour-active metabolite profiles during fermentation in synthetic must. *Food Microbiology, 29*(1), 121–131. http://doi.org/10.1016/j.fm.2011.09.006

Runnebaum, R. c., Boulton, R. b., Powell, R. l., & Heymann, H. (2011). Key Constituents Affecting Wine Body – an Exploratory Study. *Journal of Sensory Studies, 26*(1), 62–70. http://doi.org/10.1111/j.1745-459X.2010.00322.x

Sacchi, K. L., Bisson, L. F., & Adams, D. O. (2005). A Review of the Effect of Winemaking Techniques on Phenolic Extraction in Red Wines. *American Journal of Enology and Viticulture, 56*(3), 197–206.

Saenz-Navajas, Maria-Pilar. (2012) Contribution of Non-Volatile and Aroma fraction to In-Mouth Sensory Properties of Red Wines: Wine Reconstitution Strategies and Sensory Sorting Task. *Analytica Chimica Acta, 732*, 64-72

Schneider, V. (1998). Must Hyperoxidation: A Review. *American Journal of Enology and Viticulture, 49*(1), 65–73.

Silver, J., & Leighton, T. (1981). Control of Malolactic Fermentation in Wine. 2. Isolation and Characterization of a New Malolactic Organism. *American Journal of Enology and Viticulture, 32*(1), 64–72.

Singleton, V. L., & Trousdale, E. K. (1992). Anthocyanin-Tannin Interactions Explaining Differences in Polymeric Phenols Between White and Red Wines. *American Journal of Enology and Viticulture, 43*(1), 63–70.

Stern, E., Wine Business Monthly. May 2016. Volume 23:5. Wine Communications Group Inc.

Vidal, S., Courcoux, P., Francis, L., Kwiatkowski, M., Gawel, R., Williams, P., Cheynier, V. (2004). Use of an experimental design approach for evaluation of key wine components on mouth-feel perception. *Food Quality and Preference, 15*(3), 209–217. http://doi.org/10.1016/S0950-3293(03)00059-4

Wibowo, D., Eschenbruch, R., Davis, C. R., Fleet, G. H., & Lee, T. H. (1985). Occurrence and Growth of Lactic Acid Bacteria in Wine: A Review. *American Journal of Enology and Viticulture, 36*(4), 302–313.

Zahavi, T., Droby, S., Cohen, L., Weiss, B., Ben-Arie, R., (2002). Characterization of the Yeast Flora on the Surface of Grape Berries in Israel. *Vitis, 41*(4), 203-208.

CHAPTER TWO

IMPACT OF THE TIMING AND TEMPERATURE OF MALOLACTIC FERMENTATION ON THE MOUTHFEEL PROPERTIES OF CHARDONNAY WINE

Anthony Sereni [+], James Osborne[+] and Elizabeth Tomasino [*+]

Department of Food Science & Technology, Oregon State University, Corvallis, OR 97331, USA

*Correspondence: Tel.: +1-541-737-4866

[+]These authors contributed equally to the work

ABSTRACT

Malolactic fermentation (MLF) is an important process in wine production with the reduction in acidity being particularly important for cool climate wines that generally have higher acidity. MLF is typically induced by the addition of *Oenococcus oeni* after the completion of the alcoholic fermentation (AF) but can occur concurrent with AF by inoculating *O. oeni* simultaneously with the fermentative yeast *Saccharomyces cerevisiae*. This study investigated the effect of MLF inoculation timing as well as the temperature of MLF and the presence of the non-*Saccharomyces* yeast *Torulaspora delbrueckii* on Chardonnay wine mouthfeel. Chardonnay wines were produced in 2014 with AF and MLF inoculated for simultaneous or sequential fermentations, and temperatures 15 and 21°C, with or without the addition of *T. delbrueckii*. Mouthfeel attributes of the wines produced were assessed by a winemaker panel, using Napping® and Ultra-flash profiling. Significant differences in mouthfeel perception were found based on timing and inoculation conditions, as well as between temperatures. At 21°C sequential MLF wine was described as "round, smooth, and acidic", and co-inoculate MLF wine was described as "prickly, salty, balanced, and chewy". Differences between sequential, and simultaneous MLF inoculation were also noted with *T. delbrueckii* inoculation. At 15 °C wines produced from sequential MLF were discussed as "thin, dry, and astringent" while "smooth, round, and acidic" terms were used for wines produced by co-inoculated MLF. Treatment type and temperature also effected the chemical composition of finished wines. These findings demonstrate the usefulness of various fermentation practices to influence the sensory qualities of a Chardonnay wine.

INTRODUCTION

Chardonnay is referred to as a neutral aromatic cultivar; producing a wine which is not defined by a specific set of aroma compounds (Jackson, 2008; Jaffe et al., 2011). Chardonnay wine is possibly the most diverse white wine style, allowing for many variations in processing steps, including a variety of styles in sparkling wine and some dessert wines. However, it is most commonly used for the production of still white wine (Robinson et al., 2012). Chardonnay grapes are typically pressed before alcoholic fermentation (AF) to minimize contact with the skin and seeds (Jackson, 2008). In the absence of the aromatic constituents of grape skins, Chardonnay is most often fermented at one of two temperature ranges to retain aroma compounds; 6-16^{o}C or 20-25^{o}C (Cottrell et al., 1986). However, the impact of fermentation temperature on mouthfeel has been little studied. Of almost all white wines, texture and mouthfeel are considered of extreme importance for Chardonnay wine style (Cutler, 2012).

Another important winemaking process for many styles of Chardonnay wine is malolactic fermentation (MLF). This process results in a raise of pH in the wine and an increase in microbial stability (Silver et al., 1981) due to the removal of malic acid (a potential nutrient source for spoilage lactic acid bacteria). Because of the decrease in acidity this process is conducted in cooler climate cultivars such as Chardonnay which generally have higher acidity than warm climate grapes (Gambetta et al., 2014). MLF is generally conducted after AF, with the addition of *Oenococcus oeni*, for the conversion of the diprotic malic acid to lactic acid (single protic group). This process is also known to alter sensory characteristics of wines. Avedovech et al., (1992) found that tasters, and subsequent volatile composition analysis by GCMS,

could discern differences in aroma between Chardonnay wines which have undergone MLF vs. non-MLF treatments. MLF produced a variety of desired aroma compounds, including diacetyl, acetoin, volatile acids, diethyl succinate, volatile esters, ethyl acetate, n-propanol, 2-butanol, n-hexanol, ethyl lactate, and 2,3-butanediol.

While MLF is typically conducted after the completion of the alcoholic fermentation, it may also occur at the same time as the alcoholic fermentation (AF). This is known as either co-inoculation or simultaneous fermentation and can be induced by the inoculation of both the yeast and bacterial starter cultures at the same time. In red winemaking, co-inoculation has been studied as a possible means of reliably completing AF and MLF in a shorter period of time. For example, AF and MLF were shown to more reliably complete fermentation during co-inoculation, than sequential AF and MLF (Guzzon et al., 2012). Co-inoculation for AF and MLF in white wines is not commonly conducted in most commercial settings due to anecdotal concerns of higher levels of volatile acidity, and stuck fermentations. This is due to the fact that *O. oeni* is a heterofermentative bacteria that can produce acetic acid via the metabolism of glucose (Mills et al., 2005). However, due to the bacteria's preference for malic acid metabolism at pH levels < 3.60 increased acetic acid has only been noted when co-inoculation occurred in high pH grapes (Wibowo et al., 1985).

A number of studies have reported on the impact of co-inoculation on wine aroma (Abrahamse and Bartowsky, 2012; Rossouw et al., 2012; Munoz et al. 2014; Maarman et al., 2015). Unfortunately, few have reported on the influence of MLF timing on wine mouthfeel. For example, Maarman et al., (2015) found increases in volatile esters including ethyl acetate when using co-inoculation versus sequential

inoculations. However, sensory analysis was not conducted on these wines so no comparison of wine mouthfeel could be made. In a study conducted in Shiraz, Abrahamse and Bartowsky (2012) reported significant differences in volatile compounds as well as anthocyanin and pigmented polymer composition. Again however, no sensory analysis of the wines was conducted so the influence of MLF timing on wine mouthfeel could not be determined.

If co-inoculated fermentations are to be performed the presence of other micro-organisms at the beginning of fermentation must be considered. After grapes are harvested and processed a large number of yeast and bacteria species may still be present on the grapes. How the presence of these microbes influences the ability of *O. oeni* to conduct a co-inoculated MLF is relatively unknown. A number of studies have investigated how the presence of microorganism naturally present on the grapes at harvest impact *Saccharomyces cerevisiae*. Many of these yeast species present on grapes cannot survive the high alcohol environment created by the fermentation by *Saccharomyces cerevisiae* but can still interfere with the health of *Saccharomyces cerevisiae* by limiting nutrient availability, or by generation of harmful compounds (Zahavi et al., 2002; Albertin et al., 2014). Arnink et al., (2005) demonstrated the negative impact of nutrient stress on *S. cerevisiae* and *O. oeni* during AF and MLF, with particular importance on nitrogen availability. High microbial loads can also influence the sensory properties of a finished wine in both positive and negative ways. (Albertin et al., 2014)

Some non-*Saccharomyces* yeast species have also been found to positively impact wine flavor and aroma when present before or during fermentation with *S. cerevisiae*.

In fact, a number of non-*Saccharomyces* yeast are now also available as commercial cultures. *Metschnikowia pulcherrima* has been shown to decrease final wine alcohol content from 0.9-1.6%, with positive sensory aspects noted for Shiraz wines, but a negative aromatic influence imparted into Chardonnay wine due to increased levels of ethyl acetate (described as nail polish remover) (Contreras et al., 2014). *Hanseniaspora vineae,* an apiculate yeast species, has been cited to remain active in ferment until up to 9% alcohol (v/v), and in Chardonnay wines can be associated with an increase in "fruit intensity, described as banana, pear, apple, citric fruits and guava." Trained panelists noted Chardonnay fermented with *H. vineae* as being more full bodied, and longer lasting on the pallet. (Medina et al., 2013). *T. delbrueckii* when co-inoculated with *S. cerevisiae* has been shown to impact concentrations of 2-phenylethanol, isoamyl acetate, fatty acid esters, C_4-C_{10} fatty acids, lactones, and vinylphenols (Azzolini et al., 2014). It produces lower levels of acetic acid and ethanol, as well as higher concentrations of mannoproteins (Domizio et al., 2014, Contreras et al., 2015). Despite the importance of non-*Saccharomyces* yeast to the winemaking practice little is known about how their presence may impact the MLF. This is of particular importance when considering co-inoculated fermentations as it is unknown how *O. oeni* will react when inoculated into a grape juice/must where a high population of non-*Saccharomyces* may be present. In the present study this will be explored as *O. oeni* will be inoculated into Chardonnay juice containing a high population of a commercially available culture of *T. delbrueckii.*

The objective of this study therefore was to investigate how the timing of MLF impacted the mouthfeel of Chardonnay wine. Two different temperatures of

fermentation were explored as Chardonnay is commonly fermented at either a cool (13-15°C) or warm (18-21°C) temperature to produce different styles of wine. The impact of a high population of a non-*Saccharomyces* yeast on co-inoculated fermentations was also investigated as the use of these yeast in winemaking is increasing but little is known about how their use may impact the MLF.

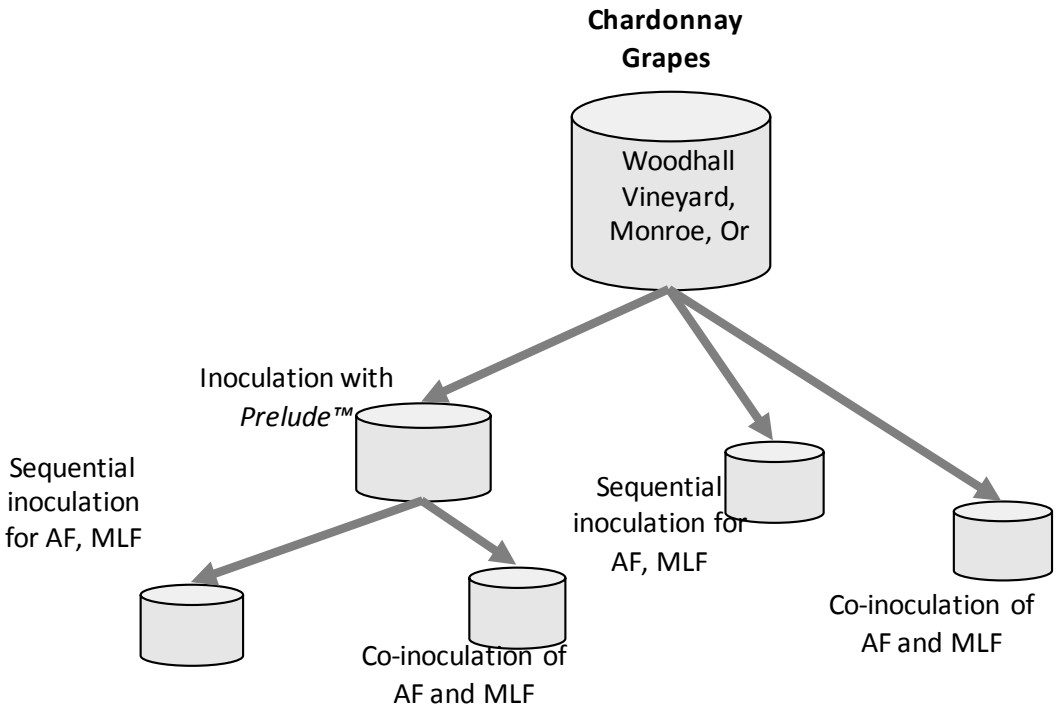
MATERIALS AND METHODS

Winemaking

Chardonnay grapes were harvested in September of 2014 from Oregon State University's Woodhall vineyard (Monroe, Oregon). A destemmer (VLS technologies, Treviso, Italy) was used to destem the grapes which were then pressed using a membrane press (Velo technologies, Treviso, Italy). The resulting juice was settled for 12 hours at 8^0C. After racking, juice was divided into 24 one-gallon glass carboys, 3 liters per carboy, and secured with airlocks. To one set of carboys a commercial culture of *Torulaspora delbrueckii* (Vinoflora Prelude™) (Chr. Hansen, Hørsholm, Denmark) was added at a rate of 0.25 g/L of must after hydration according to manufacturer's specification, and juice held at either 15 or 21^0C for 48 hrs. After 48 hours carboys were inoculated with *Saccharomyces cerevisiae* D47 (Lallamend, Montreal, Canada) at a rate of 0.25 g/L of must after hydration according to manufacturer's specification. Carboys of juice to which *T. delbrueckii* was not added were also inoculated with *S. cerevisiae* D47. At the time of *S. cerevisiae* inoculation half of the carboys were also inoculated with *Oenococcus oeni* Beta

(Lallamend) to induce MLF. *O. oeni* was inoculated at approximately 1 x 10^6 cfu/mL following manufacturer's instructions. For the remaining carboys, Beta was inoculated at the completion of alcoholic fermentation. Triplicate fermentations of all treatments were performed at either 15 or 21^{0}C. Figure 2.1 displays a flow chart of the treatment details.

Figure 2.1 Chardonnay winemaking treatments (AF=alcoholic fermentation, MLF=malolactic fermentation)



At the completion of AF and MLF (glucose/fructose < 4g/L, malic acd < 50mg/L) an addition of 50 mg/L SO_2 was made to the wines before they were placed at 4^{0}C to settle. After 14 days settling the wines were racked, sterile filtered (0.45µm PES cartridge filter), and bottled in 375 mL green glass bottles and sealed with aluminum screw cap closures (Stelvin TM, Amcor, Australia) previously sparged with nitrogen. Prior to bottling, samples were taken and frozen at -20 ^{0}C until required for analysis.

Chemical Analysis

Basic juice analysis included ^{o}Brix, pH and a titratable acidity. Brix was monitored throughout AF using a digital densitometer (Anton Paar, Santner Foundation, Graz, Austria). pH was determined by ion-selective electrode (ThermoFisher Scientific, MA, USA), and titratable acidity determined following standard methods by titration with 0.1M NaOH. Glucose/fructose, malic acid, and acetic acid were measured by enzymatic test kits (r-Biopharm, Darmstadt, Germany) while ethanol was determined using an Alcolyzer (Anton Paar, Santner Foundation, Graz, Austria).

Sensory Analysis

After five months of bottle aging, sensory analysis was conducted on the 27th and 28th of May, 2015. The panel was composed of 17 white winemakers from the Willamette Valley, Oregon. Age range of panelists were 25 to 66, and each winemaker had a minimum of 5 years' experience producing white wine. Panelists were screened for oral lesions, specific anosmia, and cigarette use. A positive response for any of the questions resulted in exclusion. The tastings were held at the Oregon State University Yamhill County Extension Office (McMinnville, Oregon) from 2:30 to 4:30pm. Each panelist tasted 10 wines presented in random order using an incomplete block design which included the 8 treatments listed and two randomly designated replicate samples. Wine glasses were labeled with randomly generated three digit identifiers. Any background odors were eliminated with air purifiers and temperature of the room was kept at 20 ±2^{o}C.

This experiment utilized Napping® followed by Ultra-flash-profiling (UFP) (Pagès, 2005; Reinbach et al., 2014). In brief, sketch paper (50lb., 45.7 x 61cm) and pens were placed in front of the panelist. Panelists were asked to refrain from smelling the wine samples as mouth feel analysis was the main objective of the sensory tests. They were instructed to immediately take the sample into their mouth. Tasters grouped the wines based on similarity of mouthfeel, with wines placed closer on the paper to wines of similar mouthfeel, and wines which were very different in mouthfeel being placed further apart. Once the wines were placed on the paper each panelist was asked to enrich the wine(s) with descriptors related to mouth feel which would characterize the differences between wines written near the wine/group (UFP). UFP terms were combined when obvious synonyms were utilized by panelists. This study utilized an incomplete block design for replication, where each panelist received two replicate samples per tasting which resulted in a complete replication of each treatment across all panelists.

Data Analysis

Analysis of variance was used to interpret the chemical parameters with treatment types using R studio version 3.2.1 (R consortium, Boston, MA). Tukey's HSD test and 95% confidence intervals were utilized to assess the impact of winemaking treatment on alcohol concentration, acetic acid concentration, malic acid degradation, as well as time to complete MLF. Sensory data analysis was conducted using XLSTAT (Addingsoft co., New York, NY), and the FactoMineR package from R version 3.2.1 (Lê et al. 2008). Napping® data was obtained using a tape measure (millimeters) from the left (X) and bottom edges (Y) relative to the original

orientation of the paper to the panelists. These measurements were utilized to generate a dissimilarity matrix using Euclidean distance. Multiple factor analysis (MFA) was run on the X and Y co-ordinates for each wine to look at effect of treatments. Correspondence analysis was used to evaluate the UFP terms.

RESULTS

All treatments completed alcoholic fermentation within 35 days although treatments where Prelude™ was added were initially slower to degrade glucose/fructose (Figure 2.2). Malolactic fermentation completed in 8 days in all co-inoculated treatments (malic acid < 0.5 mg/L), while taking between four and five weeks to complete in sequential inoculations (Figure 2.3). When combining the length of time for the completion of both the alcoholic and malolactic fermentation there were significant differences between the treatments. Chardonnay wines produced with a co-inoculation strategy completed the fermentations in 26 days while those produced using a sequential fermentation strategy took between 62 and 82 days to complete (Table 2.1). While there was no significant difference in acetic acid concentration between fermentation treatments conducted at the same temperature (Table 2.1), there was a significant difference in acetic acid between wines fermented at different temperatures. Ferments conducted at 15°C contained significantly higher concentrations of acetic acid compared to ferments conducted at 21°C (Table 2.1).

Figure 2.2 Changes in glucose and fructose during fermentation of Chardonnay juice at either 15 or 21°C with the following inoculation treatments: (▲) Sequential inoculation with Prelude™. (♦) Co-inoculation with Prelude™. (■) Co-inoculation. (●) Sequential inoculation.

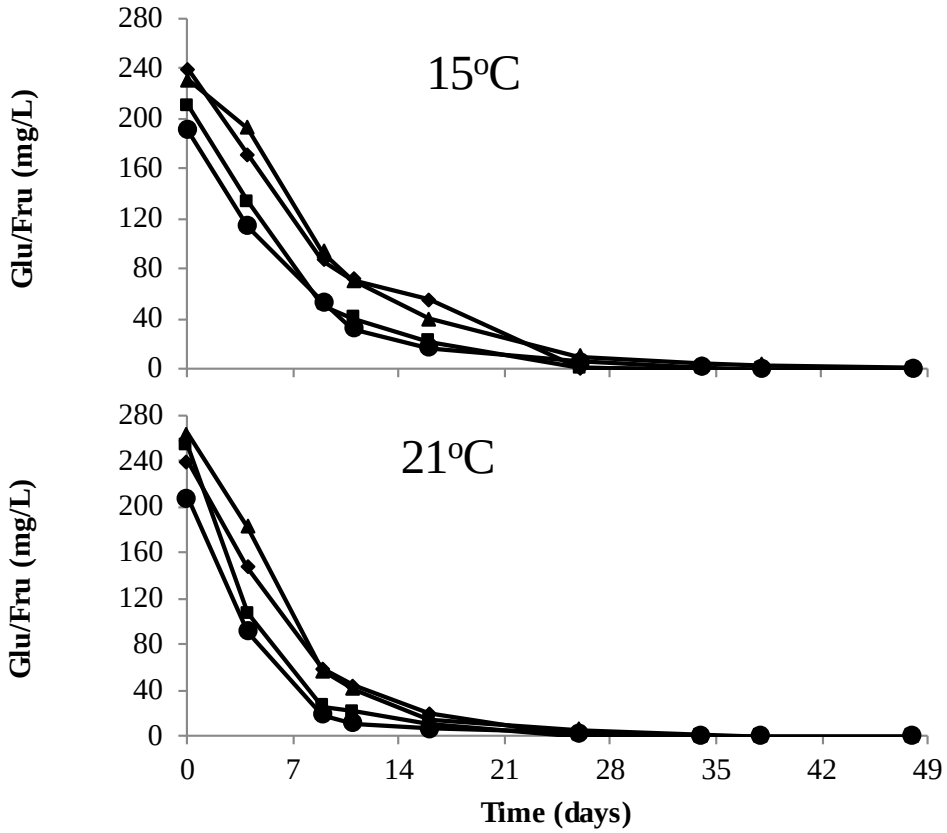


Figure 2.3 Malic acid concentration during malolactic fermentation conducted by the following inoculation treatments: (▲) Co-inoculation with Prelude™. (♦) Sequential inoculation with Prelude™. (■) Sequential inoculation. (●) Co-inoculation.

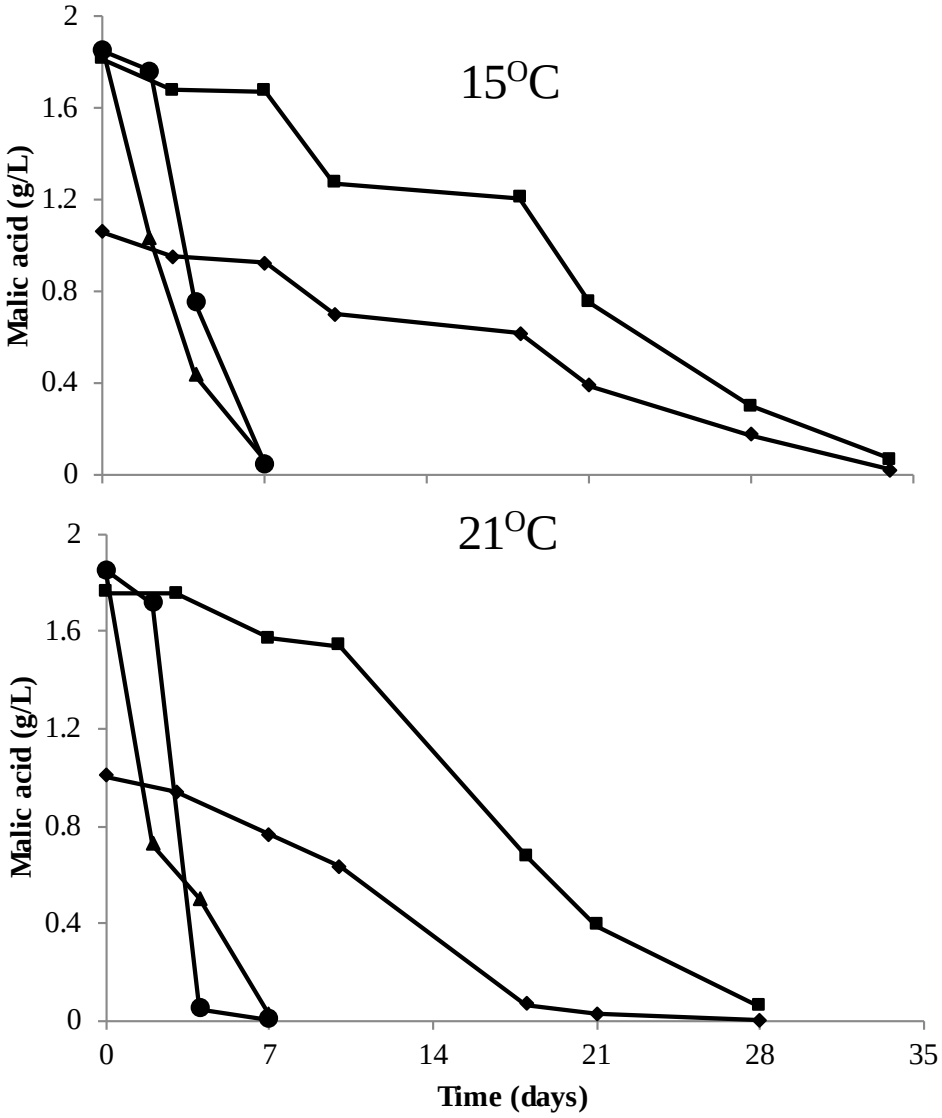


Table 2.1 Fermentation time and basic chemistry of Chardonnay wines produced with either co-inoculation or sequential inoculation at two temperatures with or without the addition of *Torulaspora delbrueckii* (Prelude) pre-fermentation.

	Days to complete AF & MLF	Alcohol % (v/v)	Acetic acid (g/L)	Wine glucose/fructose (g/L)	wine pH
Co-inoculation at 15°C	26[a]	14.14[b]	0.72[a]	2.1	3.37
Co-inoculation + Prelude at 15°C	26[a]	13.87[a]	0.72[a]	1.0	3.36
Sequential at 15°C	68[b]	14.64[d]	0.70[a]	1.4	3.44
Sequential + Prelude at 15°C	82[c]	14.54[c]	0.71[a]	1.1	3.38
Co-inoculation at 21°C	26[a]	14.18[b]	0.58[b]	2.0	3.42
Co-inoculation + Prelude at 21°C	26[a]	13.82[a]	0.59[b]	2.5	3.43
Sequential at 21°C	62[b]	14.55[c]	0.56[b]	0.4	3.44
Sequential + Prelude at 21°C	62[b]	14.43[c]	0.58[b]	0.7	3.44

[a,b,c,d] indicates average results reported which were statistically significant by Tukey's HSD. Each result is indicated with a corresponding superscript by column denoting results which ranked as not significantly different (by the letter) and significantly different by p-value <0.05 (different letter).

There were also significant differences in the final ethanol concentrations of the wines. For ferments conducted at 15°C there were significant differences between all treatments for ethanol concentration (Table 2.1). Wines produced by co-inoculation plus Prelude™ addition pre-fermentation had the lowest ethanol (13.87% v/v) while ferments conducted by sequential fermentation contained the highest ethanol content (14.64% v/v). At 21°C both the co-inoculated wines contained lower alcohol than wines produced by sequential inoculation (Table 2.1). Overall, the highest ethanol concentration was measured in wines fermented at 15^0C where MLF occurred after alcoholic fermentation (14.64% v/v) while the lowest was in wines fermented at 21^0C where Prelude™ had been inoculated and MLF occurred simultaneously (13.82 % v/v).

Napping® yielded broadly defined groupings without obvious consistency between temperature or treatment type. While three of the four co-inoculated treatment wines were found on one side of the dendogram (Figure 2.4), three of the four treatments inoculated with Prelude™ were also found on one side. While it did appear that wine treatment correlated with differences in mouthfeel, the differences did not appear consistent between temperatures.

MFA incorporated the Napping® data with the UFP data (Figure 2.5). In total F1 and F2 explains 46% of the total variance (F1-26%, F2-20%). As seen in Figure 3, three of the four co-inoculated treatments are differentiated by groupings on the negative F2 axis; three of the four Prelude™ treatments also lie on the negative F2 axis. The wines appear to vary in their degree of difference in Napping® location, and UFP data.

Figure 2.4 Dendogram by wine location groupings. Arm 1: co-inoculation and Prelude at 15°C (cp15), sequential inoculation and Prelude at 21°C (sp21). Arm 2: co-inoculation at 15°C (c15), co-inoculation and Prelude at 21°C (cp21). Arm 3: sequential inoculation at 15°C (s15), sequential inoculation at 21°C (s21). Arm 4: sequential inoculation and Prelude at 15°C (sp15), co-inoculation at 21°C (c21).

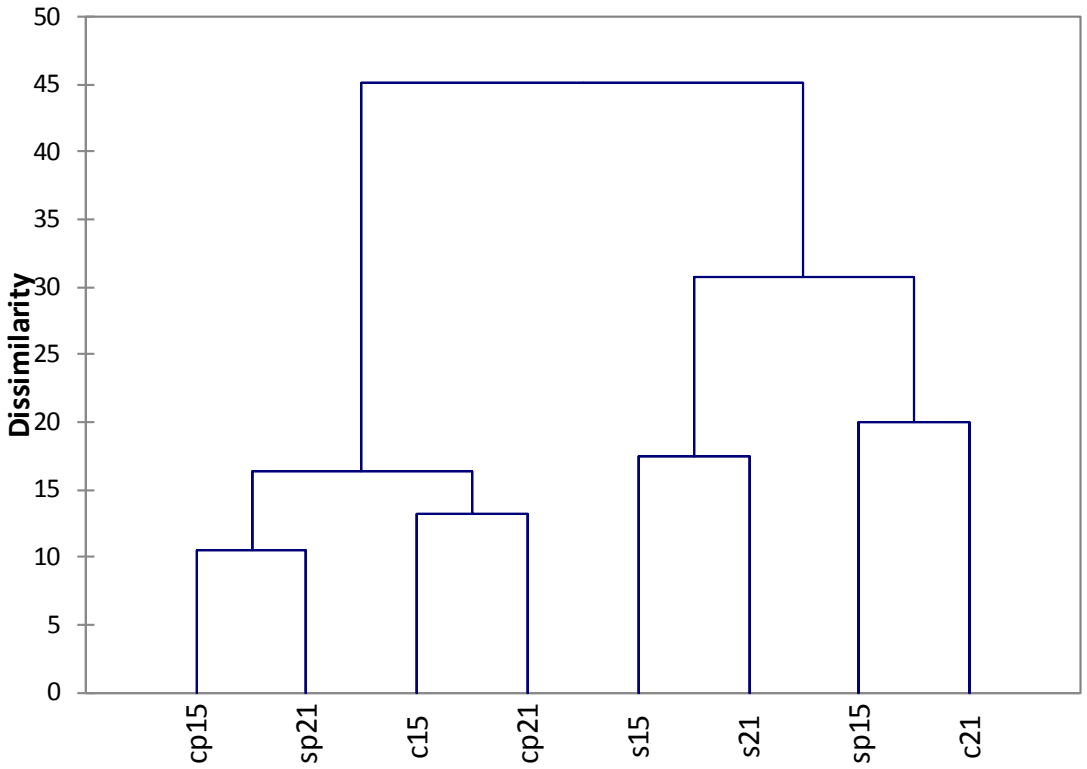
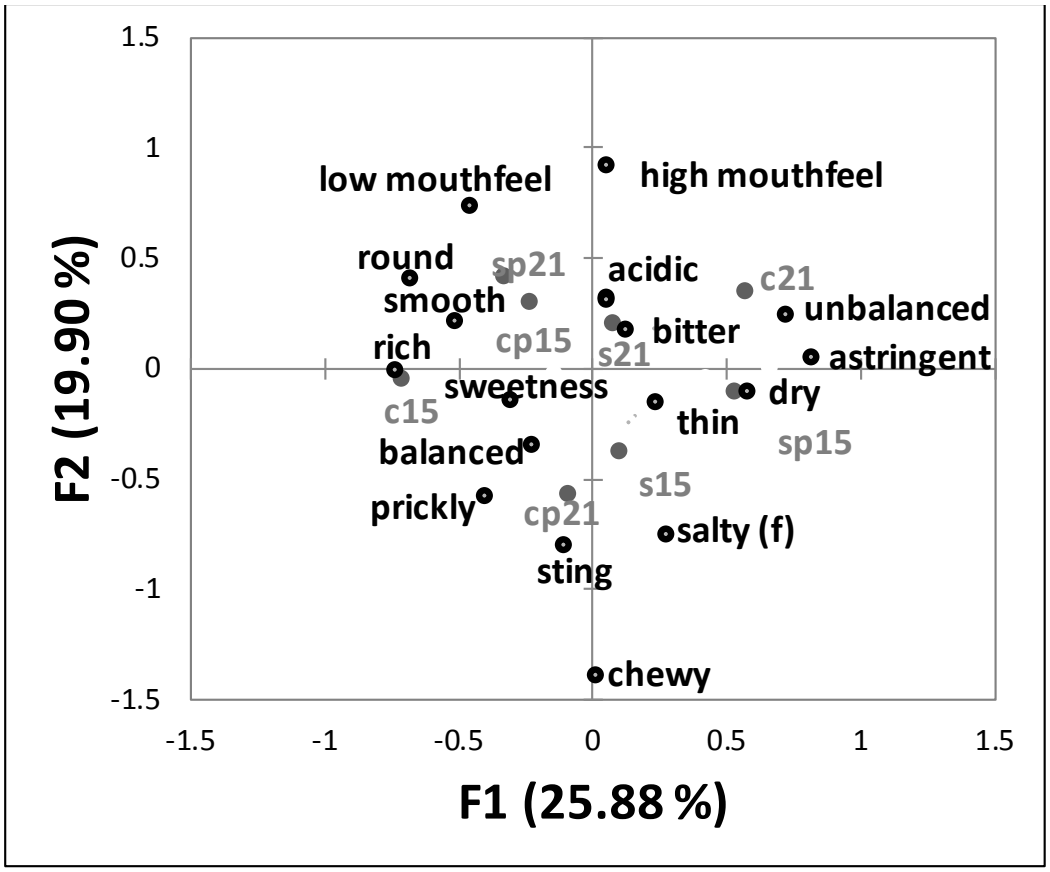


Figure 2.5 Correspondence analysis of Napping data with ultra-flash profiling (UFP) descriptors; co-inoculation at 15°C (c15), co-inoculation at 21°C (c21), co-inoculation and Prelude at 15°C (cp15), co-inoculation and Prelude at 21°C (cp21), sequential inoculation at 15°C (s15), sequential inoculation at 21°C (s21), sequential inoculation and Prelude at 15°C (sp15), sequential inoculation and Prelude at 21°C (sp21). UFP descriptors are in black.



DISCUSSION

Co-inoculation of alcoholic and malolactic fermentation during Chardonnay wine production was explored in the present study. While there have been contradictory reports in literature regarding the benefits of this technique regarding fermentation kinetics and sensory impact (Davis et al., 1985; Edwards et al 1999; Jussier et al., 2006) the results from the present study support the use of co-inoculation as a method to significantly reduce the length of alcoholic and malolactic fermentations. This likely is due to the choice of yeast and malolactic bacteria used in the present study as others have noted that the specific yeast and bacteria combination can have a significant influence on the success of the fermentations (Beelman et al., 1982; Henick-Kling and Park 1994; Osborne and Edwards 2006; Munoz et al., 2014). In the present study the difference between when fermentations were completed in the co-inoculated ferments vs. sequential ferments was as large as 56 days. Co-inoculated ferments allow for earlier SO_2 additions to minimize oxidation and microbial spoilage as well as earlier release of product to market (Maarman, et al., 2014). The addition of a high population of the non-*Saccharomyces* yeast *Torulaspora delbrueckii* did not impact the co-inoculated alcoholic and malolactic fermentations. While interactions between non-*Saccharomyces* yeast and *Saccharomyces cerevisiae* have been reported previously (Jolly et al., 2003; Fleet 2008) little is known regarding how these yeast will impact *O. oeni*. It is important to understand what the impact of the common non-*Saccharomyces* yeast, such as *H. uvarrum*, will have on *O. oeni* to ensure that these yeast will not cause issues for co-inoculated fermentations. However, results from this study suggest that high populations of *T.delbruekii* will not hinder *O. oeni* conducting the MLF, the fermentation kinetics of Chardonnay must, nor the final sensory

assessment of the wines. This indicates that co-inoculation for AF and MLF may still be a viable option in years of high microbial load on grape skins.

Varying the timing of the fermentations (AF and MLF) impacted the mouthfeel attributes of the Chardonnay wines in this study, however, the impact was not consistent across the two temperatures of fermentation utilized in this study. Instead it appears that the fermentation timing and temperature combination can alter the mouthfeel of finished wine in a unique manner.

Jussier et al., (2006) found no difference between sequential and co-inoculated MLF treatments of Chardonnay wine. They also did not see any significant differences in wine ethanol level, contrary to the findings of this study. The differences found in the present study could be due to differences in wine and yeast strain combinations utilized for co-inoculation. A number of studies utilizing different yeast and bacteria strains have reported aromatic differences when conducting co-inoculated fermentations (Abrahamse and Bartowsky 2012; Maarman et al., 2014). Further, the wines of the study by Jussier et al., (2006) were fined with bentonite and filtered through a plate and frame filter before sterile filtration. These treatments have been demonstrated to decrease compounds associated with wine aroma and mouthfeel, possibly reducing the distinction between wine treatments (Puig-Deu et al., 1996, Rodrigues et al., 2012). Further the present study expands these results to fermentation temperature influence as well as the presence of *T. delbruekii*.

The present study is one of the few to report mouthfeel differences. The differences noted from the sensory analysis did not necessarily align with differences in pH, acetic acid, and residual sugar content. Wines also did not group based on temperature

of fermentation or timing of the MLF. These findings suggest that the range of winemaking procedures investigated in this study all have the potential to impact the mouthfeel of Chardonnay wine rather than there being a dominant factor driving differences in mouthfeel.

Assessing the Dendogram grouping by chemical parameters; while co-inoculated and sequential inoculated treatments had significant differences in final wine alcohol concentration, there did not appear to be consistent grouping by panelists. Treatment C21, and SP21 are the treatments that do not align with the co-inoculated vs. sequential inoculated split. Treatment C21, has the highest average alcohol concentration of the co-inoculated treatments (14.18% v/v), while treatment SP21 has the lowest alcohol concentration for a sequential treatment (14.43% v/v). Differences in pH, acetic acid, and residual sugar content do not appear correlated to wine grouping. Treatments C15, and CP21 do group together, and they are the two treatments with the highest residual sugar measurement, however this chemical parameter does not appear to consistently impact the groupings between other treatments, as C21, and SP21 group together (R.S. of 2 g/L and 0.7 g/L respectively).

Although the addition of *T. delbruekii* did not impact fermentation kinetics it did impact other wine parameters. For example, a drop in malic acid due to the addition of *T. delbrueckii* was noted. Other non-*Saccharomyces* yeast have been reported to have the ability to partially degrade malic acid (Ciani and Comintini 2010; Benito et al., 2015) and based on our results *T. delbruekii* also has this trait. *T. delbrueckii* could be a viable alternative to *O. oeni* inoculation for partial degradation of malic acid in cool climates. This is an important consideration for wines with particularly high

levels of malic acid, such as sparkling wine production, when MLF can be challenging for *O. oeni* to complete. A combination of a non-*Saccharomyces* yeast that can partially degrade the malic acid and *O. oeni* may be helpful in these situations. It could also be a tool for winemakers who do not wish for a complete MLF, as *T. delbrueckii* cannot completely utilize the malic acid in must, but can partially degrade it (Belda et al., 2014).

The role of *T. delbrueckii* on the mouthfeel perception differences between treatments is not completely understood. Three of the four treatments inoculated with *T. delbrueckii* were consistently grouped together, and were all influenced by the descriptive terms balanced, rich and sweetness. Sequential inoculation and Prelude at 15°C treatment was grouped separately and was characterized by the terms astringent, unbalanced, thin and dry. Unlike the co-inoculation grouping this treatment did not have significantly different residual sugar or ethanol which might explain this discrepancy. *T. delbrueckii* has been demonstrated to positively impact Chardonnay wine aromatics when utilized in conjunction with *S. cerevisiae* (Azzolini et al., 2014). Domizio et al., (2014) has previously demonstrated the increase in mannoprotein content of finished wines when *T. delbrueckii* is inoculated. However, mannoprotein differences alone do not appear to account for the differences between treatments of this study. Understanding the metabolism of *T. delbruckii* and the secondary metabolites of malic acid degradation could lead to new understanding of this yeast's impact on wine sensory evaluation.

CONCLUSIONS

Co-inoculation for AF and MLF did not result in problems regarding fermentation kinetics. AF and MLF were completed, on average, in 26 days for co-inoculated treatments at 15 and 21°C. Sequentially inoculated treatments took, on average, 62 days at 21°C and between 68 and 82 days at 15°C. *T. delbrueckii* did not negatively impact fermentation kinetics and also resulted in significant differences in mouthfeel perception compared to controls.

LITERATURE CITED

Abrahamse, C., & Bartowsky, E. (2012). Timing of Malolactic Fermentation Inoculation in Shiraz Grape Must and Wine: Inluence on Chemical Composition. *World Journal of Microbiology and Biotechnology. 28(1),* 255-265

Albertin, W., Miot-Sertier, C., Bely, M., Marullo, P., Coulon, J., Moine, V., Masneuf-Pomarede, I. (2014). Oenological prefermentation practices strongly impact yeast population dynamics and alcoholic fermentation kinetics in Chardonnay grape must. *International Journal of Food Microbiology*, *178*, 87–97. http://doi.org/10.1016/j.ijfoodmicro.2014.03.009

Arnink, K., & Henick-Kling, T. (2005). Influence of Saccharomyces cerevisiae and Oenococcus oeni Strains on Successful Malolactic Conversion in Wine. *American Journal of Enology and Viticulture*, *56*(3), 228–237.

Avedovech, R. M., Mcdaniel, M. R., Watson, B. T., & Sandine, W. E. (1992). An Evaluation of Combinations of Wine Yeast and Leuconostoc oenos Strains in Malolactic Fermentation of Chardonnay Wine. *American Journal of Enology and Viticulture*, *43*(3), 253–260.

Azzolini, M., Tosi, E., Lorenzini, M., Finato, F., & Zapparoli, G. (2014). Contribution to the aroma of white wines by controlled Torulaspora delbrueckii cultures in association with Saccharomyces cerevisiae. *World Journal of Microbiology and Biotechnology*, *31*(2), 277–293. http://doi.org/10.1007/s11274-014-1774-1

Beelman, R., Keen, R., Banner, M., King, S., (1982). Interactions Between Wine Yeast and Malolactic Bacteria Under Wine Conditions. *Developments in Industrial Microbiology*, *23*, 107-121

Belda, I., Navascués, E., Marquina, D., Santos, A., Calderon, F., & Benito, S. (2014). Dynamic analysis of physiological properties of Torulaspora delbrueckii in wine fermentations and its incidence on wine quality. *Applied Microbiology and Biotechnology*, *99*(4), 1911–1922. http://doi.org/10.1007/s00253-014-6197-2

Benito, Á., Calderón, F., Palomero, F., & Benito, S. (2015). Combine Use of Selected Schizosaccharomyces pombe andLachancea thermotolerans Yeast Strains as an Alternative to theTraditional Malolactic Fermentation in Red Wine Production. *Molecules*, *20*(6), 9510–9523. http://doi.org/10.3390/molecules20069510

Ciani, M., & Comitini, F. (2010). Non-Saccharomyces wine yeasts have a promising role in biotechnological approaches to winemaking. *Annals of Microbiology*, *61*(1), 25–32. http://doi.org/10.1007/s13213-010-0069-5

Contreras, A., Hidalgo, C., Schmidt, S., Henschke, P. A., Curtin, C., & Varela, C. (2015). The application of non-Saccharomyces yeast in fermentations with limited aeration as a strategy for the production of wine with reduced alcohol

content. *International Journal of Food Microbiology*, *205*, 7–15. http://doi.org/10.1016/j.ijfoodmicro.2015.03.027

Cottrell, T. H. E., & Lellan, M. R. M. (1986). The Effect of Fermentation Temperature on Chemical and Sensory Characteristics of Wines from Seven White Grape Cultivars Grown in New York State. *American Journal of Enology and Viticulture*, *37*(3), 190–194

Cutler, L., Varietal Focus Chardonnay. Wine Business Monthly. January 2012. Wine Communications Group Inc

Davis, C. R., Wibowo, D., Eschenbruch, R., Lee, T. H., & Fleet, G. H. (1985). Practical Implications of Malolactic Fermentation: A Review. *American Journal of Enology and Viticulture*, *36*(4), 290–301.

Domizio, P., Liu, Y., Bisson, L. F., & Barile, D. (2014). Use of non-Saccharomyces wine yeasts as novel sources of mannoproteins in wine. *Food Microbiology*, *43*, 5–15. http://doi.org/10.1016/j.fm.2014.04.005

Edwards, C. G., Reynolds, A. G., Rodriguez, A. V., Semon, M. J., & Mills, J. M. (1999). Implication of Acetic Acid in the Induction of Slow/Stuck Grape Juice Fermentations and Inhibition of Yeast by Lactobacillus sp. *American Journal of Enology and Viticulture*, *50*(2), 204–210.

Fleet, G. H. (2008). Wine yeasts for the future. *FEMS Yeast Research*, *8*(7), 979–995. http://doi.org/10.1111/j.1567-1364.2008.00427.x

Gambetta, J. M., Bastian, S. E. P., Cozzolino, D., & Jeffery, D. W. (2014). Factors Influencing the Aroma Composition of Chardonnay Wines. *Journal of Agricultural and Food Chemistry*, *62*(28), 6512–6534. http://doi.org/10.1021/jf501945s

Guzzon, R., Villega, T., Pedron, M., Malacarne, M., Nicolini, G., & Larcher, R. (2012). Simultaneous yeast–bacteria inoculum. A feasible solution for the management of oenological fermentation in red must with low nitrogen content - Springer. *Annals of Microbiology*, *63*(2), 805–808. http://doi.org/10.1007/s13213-012-0499-3

Henick-Kling, T., & Park, Y. H. (1994). Considerations for the Use of Yeast and Bacterial Starter Cultures: SO2 and Timing of Inoculation. *American Journal of Enology and Viticulture*, *45*(4), 464–469.

Jackson R., 2008. Wine Science Principles and Applications. Elsevier Inc., Amsterdam, NL.

Jaffe, J., Valentin, D., Meunier, J.-M., Siliani, A., Bertuccioli, M., & Le Fur, Y. (2011). The Chardonnay wine olfactory concept revisited: A stable core of volatile compounds, and fuzzy boundaries. *Food Research International*, *44*(1), 456–464. http://doi.org/10.1016/j.foodres.2010.09.022

Jussier, D., Morneau, A. D., & Orduña, R. M. de. (2006). Effect of Simultaneous Inoculation with Yeast and Bacteria on Fermentation Kinetics and Key Wine Parameters of Cool-Climate Chardonnay. *Applied and Environmental Microbiology*, *72*(1), 221–227. http://doi.org/10.1128/AEM.72.1.221-227.2006

Lê, S., Josse, J. & Husson, F. (2008). FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software*. **25(1)**. pp. 1-18.

Maarman, B.C., 2014. Interaction Between Wine Yeast and Malolactic Bacteria and the Impact on Wine Aroma and Flavour. Thesis, Stellenbosch University, Stellenbosch, South Africa.

Medina, K., Boido, E., Fariña, L., Gioia, O., Gomez, M. E., Barquet, M., … Carrau, F. (2013). Increased flavour diversity of Chardonnay wines by spontaneous fermentation and co-fermentation with Hanseniaspora vineae. *Food Chemistry*, *141*(3), 2513–2521. http://doi.org/10.1016/j.foodchem.2013.04.056

Muñoz, V., Beccaria, B., & Abreo, E. (2014). Simultaneous and successive inoculations of yeasts and lactic acid bacteria on the fermentation of an unsulfited Tannat grape must. *Brazilian Journal of Microbiology*, *45*(1), 59–66. http://doi.org/10.1590/S1517-83822014000100009

Osborne, J. P., & Edwards, C. G. (2007). Inhibition of malolactic fermentation by a peptide produced by Saccharomyces cerevisiae during alcoholic fermentation. *International Journal of Food Microbiology*, *118*(1), 27–34. http://doi.org/10.1016/j.ijfoodmicro.2007.05.007

Pagès, J. (2005). Collection and analysis of perceived product inter-distances using multiple factor analysis: Application to the study of 10 white wines from the Loire Valley. *Food Quality and Preference*, *16*(7), 642–649. http://doi.org/10.1016/j.foodqual.2005.01.006

Puig-Deu, M., Lopez-Tamames, E., Buxaderas, S., & Torre-Boronat, M.(1996). Influence of Must Racking and Fining Procedures on the Composition of White Wine. *Vitis. 35(3)*, 141-145

Reinbach, H. C., Giacalone, D., Ribeiro, L. M., Bredie, W. L. P., & Frøst, M. B. (2014). Comparison of three sensory profiling methods based on consumer perception: CATA, CATA with intensity and Napping®. *Food Quality and Preference*, *32*, *Part B*, 160–166. http://doi.org/10.1016/j.foodqual.2013.02.004

Robinson, J., Harding, J., Vouillamoz, J., 2012. Wine Grapes. Ecco, New York.

Rodrigues, A., Ricardo-da-Silva, J. M., Lucas, C., & Laureano, O. (2012). Influence of fining and tartaric stabilisation procedures on white wine mannoprotein content. *South African Journal of Enology and Viticulture*, *33*(1), 88–94.

Rossouw, D., Du Toit, M., & Bauer, F. F. (2012). The impact of co-inoculation with Oenococcus oeni on the trancriptome of Saccharomyces cerevisiae and on the flavour-active metabolite profiles during fermentation in synthetic must. *Food Microbiology*, *29*(1), 121–131. http://doi.org/10.1016/j.fm.2011.09.006

Silver, J., & Leighton, T. (1981). Control of Malolactic Fermentation in Wine. 2. Isolation and Characterization of a New Malolactic Organism. *American Journal of Enology and Viticulture*, *32*(1), 64–72.

Zahavi, T., Droby, S., Cohen, L., Weiss, B., Ben-Arie, R., (2002). Characterization of the Yeast Flora on the Surface of Grape Berries in Israel. *Vitis, 41*(4), 203-208.

CHAPTER THREE

EXPLORING RETRO-NASAL AROMA'S INFLUENCE ON MOUTHFEEL PERCEPTION OF CHARDONNAY WINES

Anthony Sereni [+], James Osborne and Elizabeth Tomasino [*+]

Department of Food Science & Technology, Oregon State University, Corvallis, OR 97331, USA

*Correspondence: Tel.: +1-541-737-4866

[+]These authors contributed equally to the work

As seen in Beverages (2016): Volume 2, Issue 1 (http://www.mdpi.com/2306-5710/2/1/7)

ABSTRACT

There are many interactions that occur between taste and aroma that may impact perception. The main objective of this study was to ascertain whether the aroma fraction of wine should be considered when investigating relationships between chemical composition and sensory perception of mouthfeel. Chardonnay wines with different mouthfeels were produced by altering the fermentation temperature (15°C, 21°C) of the alcoholic and malolactic fermentation (MLF) as well as the timing of MLF and the presence of a non-*Saccharomyces* yeast during alcoholic fermentation. Napping® and Ultra-flash-profiling were conducted using a panel of white winemakers. Each procedure was conducted twice: once with retro-nasal aroma (+R) and once without retronasal aroma (-R). Napping® results showed that retronasal aroma impacted mouth feel perception. Ultra-flash profiling of +R and -R displayed similar descriptive terms used. Several terms appear to be related to retronasal aroma as they were used in +R and not in –R. It is unclear if these terms are due to interactions or due to associated learning. These results suggest that for some mouthfeel terms the volatile fraction is playing a role and to establish relationships with chemical composition and mouthfeel perception it is important to consider both the volatile and nonvolatile wine fractions.

INTRODUCTION

The assessment of white wine involves the appreciation of the appearance, ortho-nasal and retro-nasal aroma, flavor, and texture or mouthfeel of that wine [1]. Mouthfeel is one of the least understood areas in the assessment of wine due to the number of factors involved, direct and indirect, as well as the difficulty in agreed upon descriptions or analogous terms [2]. Traditionally mouth feel is thought to be caused by nonvolatile compounds, including polysaccharides, mannoproteins, sugar, ethanol, glycerol, pH, tartaric acid, and phenolics [3]. To change the mouthfeel of wines, winemakers attempt to alter these compositional elements. However when tasting a glass of wine there are more than just mouthfeel components present. Both volatile and non-volatile contents are perceived together. Therefore, mouthfeel perception may be the result of interactions between nonvolatile and volatile compounds perceived through retronasal olfaction. This study proposes evidence that the aromatic fraction of the wine plays a role in the final perception of the mouthfeel of Chardonnay wines.

Mouthfeel of White Wine

The components thought to influence mouthfeel are difficult to quantify and qualify due to the number of components involved, poor understanding of the interactions between them, and the use of descriptors based on tactile sensation [2,4]. Researchers interested in the mouthfeel of white wines generally use the in-mouth sensations of acidity, astringency, prickling, temperature, body, and burning to qualify the differences in mouthfeel between wines [5]. Terms used to describe wines are generally fabrics, such as velvet, suede, or silk, and not terms directly based on the sensory experience [2].

Another complication to mouthfeel perception is that many individuals have difficulty in distinguishing between flavor sensations and chemesthesis sensations. Specifically, it is common for wine consumers to mistake the bitterness on the tongue (taste) for astringency (touch, or chemesthesis) [6,7]. Astringency is caused by the polymerization of phenolics with a salivary protein and not by interactions with taste buds located on the tongue [7]. Further complicating the perception of astringency is the reported differences between individuals in saliva quantity per volume of wine, varying protein concentration of individual's saliva, as well as variation in the sip-size each person imbibes [6]. All these factors have been found to impact the perception of astringency.

Napping®

Napping® is a recently developed sensory procedure where untrained panelists are asked to separate samples spatially, on a table cloth or Nappe, based on how similar or different the samples are [8,9]. It is a sub-form of projective mapping which dates back to the mid 1990s [10]. This procedure has proven comparable to analysis by trained panelists using descriptive analysis [11]. Pairing the Napping® procedure with Ultra-flash-profiling (UFP) has been utilized to interpret wine groupings by the Napping® procedure [9,11,12].

The Napping® procedure has been successful in the grouping of food products by specific attributes [13], as well as to separate in mouth textural responses in food science studies [14], but as of yet, no research has utilized this procedure for wine mouthfeel assessment. It has been postulated that assessment of food products is a cultural phenomenon with regional variations, and as such, the Napping® procedure has been demonstrated to be precise only when utilizing panelists of similar regional

and cultural locals [15]. Therefore, in this study, the panelists were recruited and selected from a selection of winemakers in Oregon's Willamette Valley wine region.

Mouthfeel Linked to Winemaking Processes

The concentration of non-volatile chemical constituents of wine such as phenolics can be influenced by a number of factors including timing of picking [16], grape pressing and maceration, and time on skins. Juice settling time and filtration can also influence the grape solids and polysaccharide content of must and wine [17]. Fermentation parameters such as non-*Saccharomyces* yeast species can be used to increase mannoprotein content, which has been correlated with a general increase in mouthfeel [18]. Aging conditions can drastically effect a finished wine due to oxidative processes [19]. A producer's desired style influences the decision of harvest dates between cultivar, and region; time of harvest correlates with differences in acid degradation, sugar accumulation, and phenolic advancement [16] The effects of these chemicals are discussed further in the following section.

Chemicals Involved in Mouthfeel Perception

Studies have shown that altering the composition of a wine can influence mouthfeel sensations [17,20]. Wines with a lower pH are generally perceived as more astringent, irrespective of their phenolic content. pH has also been demonstrated to effect the perception of viscosity, however a clear relationship has not been displayed [17]. Viscosity of wine, a major determinant of wine body, is influenced by sugar, polysaccharide composition, and, to a lesser extent, ethanol and glycerol [21,22]. Studies are conflicting regarding the impact on mouthfeel perception from glycerol and

polysaccharides, with a purport that phenolic compounds may have a greater influence on the perception of viscosity [17]. This has led some researchers to conclude that the concentration of phenolic compounds are the key to understanding mouthfeel in white wine. However, research in this area has failed to demonstrate that quantity and concentration of phenolic compounds have a direct relationship with perceived viscosity [23].

Phenolic compounds are known to elicit two separate sensory experiences: bitterness and astringency. Bitterness is a taste, perceived by taste buds in the tongue. In wine it is generally caused by flavan-3-ols, some flavonols, hydroxycinnamates, and benzoic acid derivatives [6]. Astringency is a more complicated sensation perceived via chemesthesis. It is described as a prickling sensations on the mouth and tongue, and a drying of the mouth [6,24]. Low molecular weight polyphenols and polymeric tannins bind with salivary proteins to elicit a drying, and puckering response. However astringency in wine is described by specific textures, often related to tactile memory of fabric [2]. Schobel and others suggest that there may be specialized neuronal connections in the mouth which bind with low molecular weight polyphenols and polymeric tannins, and aid in the creation of distinct astringent sensation: which they describe as either “puckering” astringency, or “velvety” [7]. There are astringent phenolic compounds which are also perceived as bitter via taste buds, causing some individuals to assimilate the two sensations into one experience; such misinformation causes confused assessments of food products since many compounds elicit a bitter taste without astringency, or an astringent sensation without bitterness [24]. The sensations involved in the mouthfeel of a wine generally involve multiple chemical groups, some with known influence, and others with a more indirect influence. Many

of these compounds may interact with each other, or, potentially, with the volatile fraction of the wine matrix to elicit the full experience of white wine mouthfeel.

Indirect Sensory Attributes, and Interactions

While phenolic compounds have shown a direct impact to mouth feel perception they are also involved in some indirect effects on sensory perception. Ethanol and phenolics have been found to have a synergistic effect- causing an increase in the perception of astringency, and burning [25]. Another indirect effect of compounds important to mouthfeel perception is the interaction of sugar and acid, which alter sweetness and sourness perception [26], and ethanol and glycerol which effect the perception of sweetness. An increase in either compound results in a reported increase of sweetness [27].

Interactions between ortho-nasal, and retro-nasal aroma on the perception of the texture in food systems have been investigated [28]. Olfactory influence on the perception of texture has been observed in other complex food systems, such as yogurt and milk products [29,30]. An investigation into the effect of the nonvolatile fraction of wine on the intensity of the volatile fraction has shown a positive influence [31], and some work has been done linking wine volatiles to the perception of mouthfeel sensations, however this study was limited to the specific parameter of “astringency” [32]. In this study we begin to investigate how the volatile fraction of white wine can influence the perception of all mouthfeel parameters.

MATERIALS AND METHODS

Wine Production

Chardonnay grapes from Oregon State University's Woodhall vineyard (Monroe, Oregon) were de-stemmed, pressed and the juice was settled for 12 hours at 8°C. After racking, the juice (pH 3.31, TA 7.2 g/L, Brix 24.4) was divided into one-gallon glass carboys, three liters per carboy, and secured with airlocks. Fermentation treatments varied based on timing of yeast and bacteria inoculations as well as fermentation temperatures. Yeast strains used include Prelude ™ (*Torulaspora delbrueckii*) (Chr. Hansen, Hørsholm, Denmark) and *Saccharomyces cerevisiae* strain D47 (Lallemand). Malolactic bacteria used was *Oenococcus oeni* strain Beta (Lallemand, Montreal, Canada). All treatments were performed in triplicate. Each treatment was performed at two different temperatures, 15 and 21^{0}C, by placing carboys in temperature controlled rooms. *S. cerevisiae* strain D47 and *O. oeni* strain Beta were inoculated at approx. 1 x 10^6 cfu/mL. Prelude™ was inoculated at approx. 1 x 10^5 cfu/mL. At completion of alcoholic (reducing sugars < 0.5 g/L) and malolactic fermentation (malic acid < 50 mg/L) an addition of 50 mg/L SO_2 was made and wines were placed at 4^{0}C to settle. After settling, wines were racked and sterile filtered with a 0.45µm PES cartridge filter (Millipore, MA, USA) , and bottled in 375 mL aluminum screw cap (Stelvin, Amcor, Australia) closed bottles. Wines were stored at 13 °C until required for analysis. Glucose/fructose, malic acid, glycerol and acetic acid of each sample were measured using enzymatic assays (r-Biopharm, Darmstadt, Germany). Ethanol concentration was determined using an Anton Paar Alcolyzer (Santner Foundation, Graz, Austria)

Sensory Analysis using Napping and Ultra-flash-profiling

Sensory analysis of the wines was conducted five months after bottling. After screening for specific anosmia, oral legions, and cigarette use 17 white winemakers from the Willamette Valley wine region were selected for a sensory study on Chardonnay mouthfeel using Napping® followed by Ultra-flash-profiling (UFP). The age range of the panelists was 25-66 and each winemaker had a minimum of 5 years' experience with Chardonnay wines. Panelists were split between tasting A, held on the 27th of May, 2015, and tasting B, on the 28th of May, 2015. The tastings were held from 2:30 to 4:30pm, at the Oregon State University Yamhill County Extension Office (McMinnville, Oregon, USA). The room was lit with fluorescent lighting and kept at approximately 68°F for the duration of both sessions. Two air purifiers were used to eliminate any background aroma. Each panelist participated in 2 tasting sessions separated by a 15 minute break. During each session panelists were presented with 10 wines; the 8 wines previously described and 2 replicate samples. Replicates were included randomly and all wines were presented in random order determined using an incomplete block design. All wines were assigned randomly generated three digit identifiers. Panelists were randomly selected to begin either with, or without, nose clips (A-M systems, Sequim, WA). When using nose clips panelists were instructed that nose clips were to remain on for the entirety of the flight.

Napping® procedure was used according to Pages et al [9, 11]. Sketch paper (50lb., 45.7 x 61cm) was placed in front of the panelist in addition to a ball point pen (for UFP). Panelists were asked to refrain from smelling the wine samples as mouth feel analysis was the main objective of the sensory tests. A practice wine was given to each

taster to acclimate themselves to assessment of the wines while wearing the nose clips. Tasters grouped the wines based on similarity of mouthfeel. Once the 10 wines were placed on the paper panelists were asked to enrich each wine/group with descriptors, related to mouth feel, written near the wine/group using ultra flash profiling as described by Perrin et al [11]. Tasters were instructed only to include flavor descriptors if they were important to mouthfeel. UFP descriptors were assessed for synonyms and erroneous descriptors to simplify the data analysis.

Data Analyses

Data analysis conducted using XLSTAT (Addingsoft co.), and R version 3.2.1. Hierarchical clustering analysis (HCA) was used to determine consistency of replicates. A dissimilarity matrix using Euclidean distance and Napping® data was assessed using multiple factor analysis (MFA) of the X and Y co-ordinates (unstandardized variables) of each wine on the paper placemat. These coordinates were obtained using a tape measure in millimeters from the left edge (X), and bottom edge (Y), relative to the original paper orientation for each panelist. The data set for Napping contained 18 variables corresponding to the 18 winemakers participating in the study. UFP resulted in two contingency tables, one for +R and 1 for –R. The variable names in the contingency table correspond to the sample attribute and the value of that attribute corresponds to the frequency of the attribute for all samples and all assessors. UFP contingency tables were analyzed using correspondence analysis.

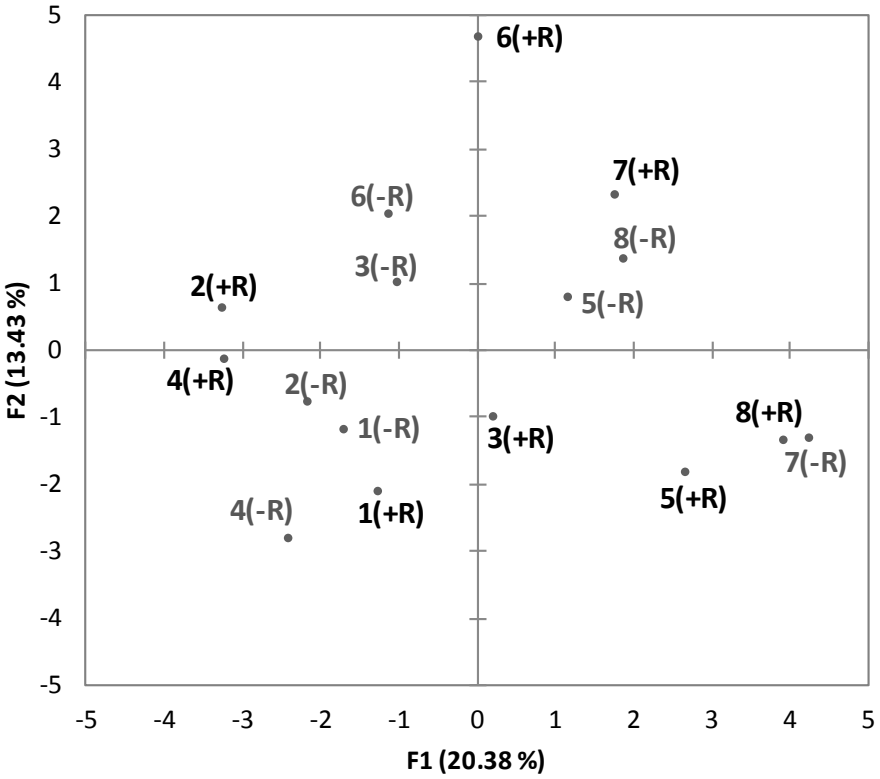
RESULTS

Napping®

Agglomerative Hierarchical clustering was used to show consistency of panelists with the replicate wines. All wines but one were consistently grouped using napping® for both +R and –R. However the wines that were not consistently grouped were different between the two analyses. For +R analysis treatment 8 was grouped with both treatment 2 wines and with treatment 4 wines. For –R analysis treatment 1 wine was grouped with both treatment 3 and 4 wines. This inconsistency was due to results from one panelists that did not group replicates together. This individual's data was excluded from further analysis.

Multiple factor analysis from Napping® showed that retronasal aroma impacted sorting of wines based on mouth feel (Figure 3.1). Some wines were found to have a large influence of retronasal aroma as they are located quite far apart while other wines had less impact of retronasal aroma, as they are located closer together. Treatment 1, 2, and 4 wines, both +R and –R, are located near each other although there are some small differences. All other wines were not located spatially close together. For example, 5 (+R) is found in the positive F1 direction and negative F2 direction and 5 (-R) is found in the positive F1 and F2 direction. 6(+R) is the only wine that is not spatially located near another wine, found in the positive F1 direction.

Figure 3.1 Multiple factor analysis of Napping® results of Chardonnay wines analyzed +R (**black**) and –R (**grey**).



Ultra-flash Profiling

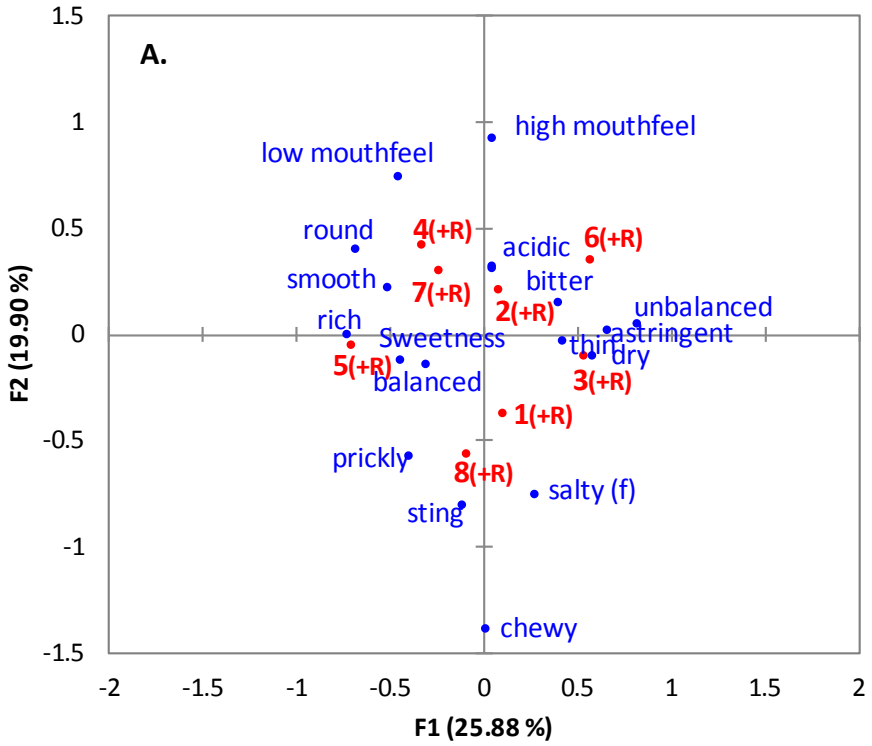
Descriptors included in Correspondence Analysis (CA) had a quotient factor greater than 3 and a total of 27 descriptors were used (Table 3.1). The first two factors are responsible for 46% of the total inertia, 26% and 20% respectively; with clear separation both in the F1 and F2 directions. Of the 27 descriptors used only 17 contributed highly to the formation of axes, based on their squared correlations provided in analysis output (data not shown). The F1 axis is mainly comprised of sweetness, dry, round, astringent and unbalanced. The F2 axis is primarily composed of chewy, high mouthfeel and acidic. The placement of wine group centroids with the attribute points display those mouthfeel descriptors that are associated with each wine (Figure 3.2).

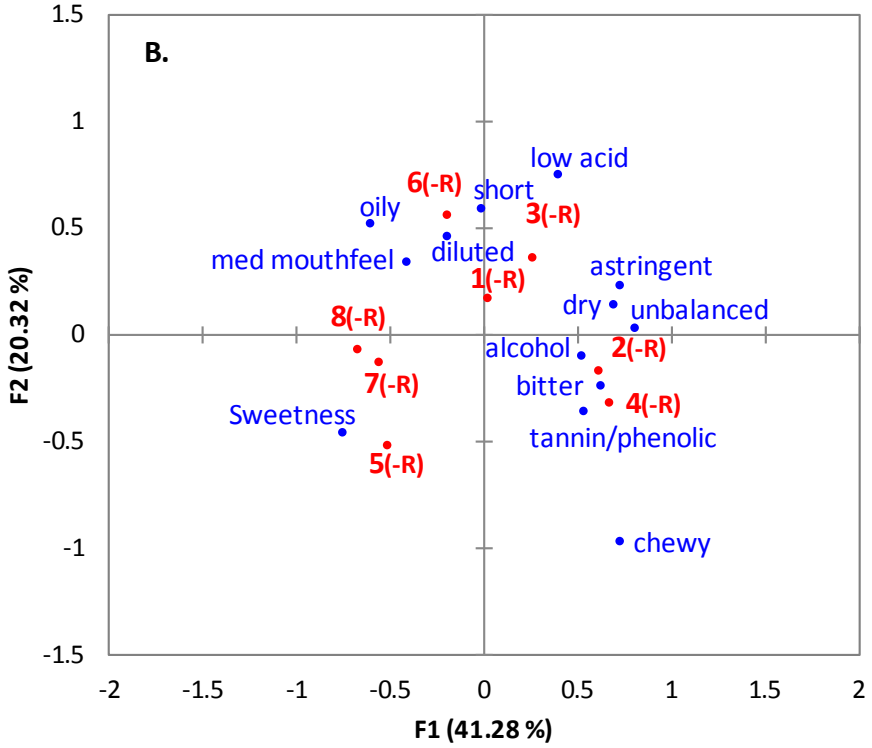
As with +R analysis those descriptors that were used more than 3 times were included in the Correspondence analysis. In total 22 terms were incorporated in the analysis, although only 12 highly contributed to the formation of the axis based on the squared correlations. The first 2 factors account for 61% of the variance (F1 41% and F2 20%). As with +R results, sweetness was the term that contributed the most both for the F1 and F2 axis. Other terms important for F1 axis include bitter, alcohol and astringent. Terms that are important to the F2 axis include chewy, low acid, and short. Terms that appear to be related to retronasal aroma, terms used in +R but not –R, include flabby, fresh, smooth, soft, prickly, high mouthfeel, sting and salty.

Table 3.1 Frequency of mouthfeel descriptors used for UFP with retronasal aroma (+R) and without retronasal aroma (-R).

Term	+R	-R
Sweetness	31	31
Acidic	28	33
Alcohol	16	17
Balanced	15	13
Dry	12	6
Bitter	12	13
Length/Persistence	11	6
Round	11	8
Short	11	5
Tannin/phenolic	10	9
Thin	10	13
Astringent	8	8
Flabby	8	
Medium mouthfeel	8	10
Diluted	7	7
Rich	7	8
Fresh	6	
Smooth	5	
Soft	5	
Bright	4	4
Chewy	4	4
Prickly	4	
Low mouthfeel	4	11
High mouthfeel	4	
Unbalanced	4	4
Sting	4	
Salty	4	

Figure 3.2. Correspondence analysis of terms used for UFP analysis (A = +R, B = -R.)





DISCUSSION

Napping®

MFA results (Figure 3.1) show that retronasal aroma is playing a role with mouthfeel perception, especially since the majority of reapplications were sorted together. The wines that were sequentially inoculated were found spatially closer together than those that were co-inoculated, specifically 1, 2 and 4 can all be seen in the negative F1 and F2 directions. This would suggest that the aromas produced during co-inoculation influence mouthfeel, while the aromatics of the sequentially inoculated wines were more similar, although it should be noted the aromas of the wines were not analyzed in this study. But since the only difference in the analysis of wines was the presence of retronasal aroma, the results show that there is some influence of retronasal aroma to mouthfeel perception. This is supported by previous research where aroma was found to influence wine texture [29]. The application of this research in other food systems has demonstrated similar results [30, 33], however these interactions have yet to be demonstrated in a wine matrix

Ultra-flash-profiling (UFP)

Many terms used were spatially located in a similar area for both the +R and –R analysis. This suggests that while slightly different terms may be used, the panelists were describing a similar mouthfeel parameter. For instance, in –R, both bitter and tannin/phenolic were spatially located together and these two terms are known to be associated with phenolic compounds in wine [34]. Other terms were not as consistent, for example, low and high

mouthfeel, in the +R group (Figure 3.2). In the –R group: astringent, dry and unbalanced were located quite close together (Figure 3.2). This may be due to the individual's difference in mouth feel intensity, but it also suggests that panelists are using different language for a similar sensation. Interestingly the term medium mouthfeel was found to be important for –R and yet only low and high mouthfeel were found in the +R analysis (Figure 3.2). This again supports the possibility that perception of mouthfeel is linked to retronasal qualities and it is perhaps the aromatic extremes or unbalances more than compositional differences that are important within a similar wine style. Clearly there is much work needed to develop consistent terms for specific mouth feel parameters.

The separation of specific taste and mouthfeel descriptors known to be related, and those known to be antonyms, are important to note. Specifically, sweetness and dry are located in opposite directions along the F1 axis in +R (Figure 3.2). These two attributes are related to residual sugar content with sweet wines having more residual sugar and dry wines less residual sugar. Sweetness and rich are also both found on the positive direction of F1. These two descriptors have been found to be related although sweetness is not the only factor attributed to richness, as fats, proteins and polysaccharides have been found to play a role in "rich mouthfeel" of other foods [35]. We can see that terms that have a known relationship to chemical composition are being perceived in an expected manner. Although the small difference between low and high mouthfeel for +R suggests that there is either difficulty in determining what low and high mouthfeel is or that several interactions occur for lower mouthfeel wines that are not directly related to the same compositional elements.

Differences in the usage of terms between +R and –R analysis are most likely due to the influence of retronasal aroma to mouth feel. Specifically, it would appear that retronasal aroma is in fact very important for several mouthfeel associated descriptors. Terms found in +R and not –R include flabby, fresh, smooth, soft, prickly, high mouthfeel, sting and salty. Several of these terms are known to be in some way related to aroma or taste. For instance, salty is considered to be a taste caused by ions in the wine that are then perceived in the taste bud [36]. However salty is also thought to be a component of the aroma term minerality [37,38] and the perception of saltiness has been found to be linked to aromas in other foods and model solutions [39,40]. It appears that while salty is considered a taste the panelists were relating it to a retronasal aroma. Fresh is another term that appears to be related to retronasal aroma, as it was incorporated in the +R analysis but not in the –R. The term "fresh: is typically related to fresh fruits or clean aromas and while aromatic information was not collected it would seem that the usage of this term may be due directly to aroma since it was not used in –R analysis.

A number of terms used in the +R analysis are not known to be linked to retronasal aroma. These include flabby, smooth, soft, prickly, high mouthfeel and sting (Table 3.1). These terms are clearly related to tactile sensations. One possible explanation for their usage in R and not in –R is that there is little consensus on the use of these terms. However flabby, flesh, smooth and soft were used between 5-8 times, while the others were used less. The usage of terms with retronasal aroma suggests that an aroma is eliciting a response to these tactile sensations and that while there is no actual perception of smooth, the aroma is reminding the taster of something that can be described as "smooth". These types of

interpretations around associative learning are well known in sensory science [41, 42]. A further investigation into these terms and the role of retronasal aroma would be of great interest as these results clearly show the impact that retronasal aroma is having to mouthfeel. Determining which mouthfeel terms are due to a combination of retronasal aroma and mouthfeel would be extremely valuable when trying to establish chemical relationships to mouthfeel terms.

Another interesting point in the UFP analysis is that the use of terms associated with taste descriptors are used most frequently. This may be due to the fact that it is possible to train individuals to recognize sweetness, acidic, alcohol and bitter by the use of chemical standards [43], making it easier for the taster to use and perceive these tastes. Familiarity with taste perception may also explain the usage of these terms as taste is a part of all food and beverages while mouthfeel will vary depending on the product. Additionally, panelists used two terms that are not actual sensory perceptions but chemicals, tannin/phenolic and alcohol. The tannin/phenolic terms would appear to be related to the tannin and/or phenolic content of the wine, as these compounds are known to impact mouthfeel perception [3, 44]. However, it is interesting to discuss the "alcohol" term. This could be referring to a sensory perception of heat, as higher alcohol content is known to producer warmer or hot wines [45]. However, the term alcohol could also relate to the smell of alcohol which is many times referred to as solvent or lifted. There are many alcohols present in wine beyond ethanol and the usage of this term and not the term "heat" warrants further investigation. It is most likely that these terms were used as they are common in most winemakers'

professional vocabulary and would not necessarily be used if panelists were wine consumers.

CONCLUSIONS

The differences in sorting, and in the use of descriptive terms to describe the mouthfeel of each wine, between +R and –R emphasizes the importance of the volatile fraction of a wine in the appreciation of mouthfeel. It is unclear if the influence of the volatile fraction is due to interactions between chemical groups, by indirect effects of the volatile fraction, or by associative learning. The contradictions between UFP groupings in the –R procedure imply that something pivotal is missing from each wine which would allow more consistent descriptive terms to be assigned by wine professionals. Since the volatile fraction remains in the wine the difference appears to be inimitable to the panelists, and as such could infer an interaction between processing modality of olfactory and chemesthesis, or possibly, in the absence of retronasal aroma, the associations made by wine professionals could be rendered inaccessible. More research is needed to investigate these findings, but this work provides a first step in examining the intricacies and interactions at work for mouthfeel perception.

LITERATURE CITED

1. Peynaud, E.; Blouin, J. The Taste of Wine: The Art Science of Wine Appreciation; JohnWiley & Sons: Hoboken, NJ, USA, 1996.
2. Pickering, G.J.; Demiglio, P. The White Wine Mouthfeel Wheel: A Lexicon for Describing the Oral Sensations Elicited by White Wine. J. Wine Res. **2008**, 19, 51–67.
3. Vidal, S.; Courcoux, P.; Francis, L.; Kwaitkowski, M.; Gawel, R.; Williams, P.; Waters, E.; Cheynier, V. Use of an experimental design approach for evaluation of key wine components on mouth-feel perception. Food Qual. Preference **2004**, 15, 209–217.
4. Gawel, R.; Oberholster, I.; Leigh Francis, I. A "Mouth-feel Wheel": Terminology for communicating the mouth-feel characteristics of red wine. Aust. J. Grape Wine Res. **2000**, 6, 203–207.
5. Jackson, R.S. Wine Science: Principles and Applications; Academic Press: Cambridge, MA, USA, 2008.
6. Ma,W.; Guo, A.; Zhang, Y.;Wang, H.; Liu, Y.; Li, H. A review on astringency and bitterness perception of tannins in wine. Trends Food Sci. Technol. **2014**, 40, 6–19.
7. Schöbel, N.; Radtke, D.; Kyereme, J.;Wollmann, N.; Cichy, A.; Obst, K.; Kallweit, K.; Kletke, O.; Minovi, A.; Dazert, S.; et al. Astringency Is a Trigeminal Sensation That Involves the Activation of G Protein–Coupled Signaling by Phenolic Compounds. Chem. Senses **2014**, 39, 471–487.
8. Pagès, J. Recueil direct de distances sensorielles: Application à l'évaluation de dix vins blancs du Val-de-Loire. Sci. Aliment. **2003**, 23, 679–688.
9. Pagès, J. Collection and analysis of perceived product inter-distances using multiple factor analysis: Application to the study of 10 white wines from the Loire Valley. Food Qual. Preference **2005**, 16, 642–649.
10. Risvik, E.; McEwan, J.A.; Colwill, J.S.; Rodgers, R.; Lyon, D.H. Projective mapping: A tool for sensory analysis and consumer research. Food Qual. Preference **1994**, 5, 263–269.
11. Perrin, L.; Symoneaux, R.; Maître, I.; Asselin, C.; Jourjon, F.; Pagès, J. Comparison of three sensory methods for use with the Napping® procedure: Case of ten wines from Loire valley. Food Qual. Preference **2008**, 19, 1–11.
12. Perrin, L.; Pages, J. Construction of a product Space from the Ultra-Flash Profiling method: Application to 10 Red Wines from the Loire Valley. J. Sens. Stud. **2009**, 24, 372–395.
13. Dehlholm, C.; Brockhoff, P.B.; Meinert, L.; Aaslyng, M.D.; Bredie, W.L.P. Rapid descriptive sensory methods—Comparison of Free Multiple Sorting, Partial Napping, Napping, Flash Profiling and conventional profiling. Food Qual. Preference **2012**, 26, 267–277.

14. Grygorczyk, A.; Lesschaeve, I.; Corredig, M.; Duizer, L. Impact of Structure Modification on Texture of a Soymilk and Cow's Milk Gel Assessed Using the Napping Procedure. J. Texture Stud. **2013**, 44, 238–246.
15. Kim, Y.-K.; Jombart, L.; Valentin, D.; Kim, K.-O. A cross-cultural study using Napping®: Do Korean and French consumers perceive various green tea products differently? Food Res. Int. **2013**, 53, 534–542.
16. Pirie, A.J.G.; Mullins, M.G. Concentration of Phenolics in the Skin of Grape Berries during Fruit Development and Ripening. Am. J. Enol. Vitic. **1980**, 31, 34–36.
17. Gawel, R.; Day, M.; Van Sluyter, S.C.; Holt, H.;Waters, E.J.; Smith, P.A. White Wine Taste and Mouthfeel as Affected by Juice Extraction and Processing. J. Agric. Food Chem. **2014**, 62, 10008–10014.
18. Domizio, P.; Liu, Y.; Bisson, L.F.; Barile, D. Use of non-Saccharomyces wine yeasts as novel sources of mannoproteins in wine. Food Microbiol. **2014**, 43, 5–15.
19. Kennedy, J.A. Grape and wine phenolics: Observations and recent findings. Cienc. E Investig. Agrar. **2008**, 35, 107–120.
20. Runnebaum, R.C.; Boulton, R.B.; Powell, R.L.; Heymann, H. Key Constituents AffectingWine Body—An Exploratory Study. J. Sens. Stud. **2011**, 26, 62–70.
21. Walker, S.; Prescott, J. The Influence of Solution Viscosity and Different Viscosifying Agents on Apple Juice Flavor. J. Sens. Stud. **2000**, 15, 285–307.
22. Vidal, S.; Francis, L.; Williams, P.; Kwiatkowski, M.; Gawel, R.; Cheynier, V.; Waters, E. The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. Food Chem. **2004**, 85, 519–525.
23. Oberholster, A.; Francis, I.L.; Iland, P.G.; Waters, E.J. Mouthfeel of white wines made with and without pomace contact and added anthocyanins. Aust. J. Grape Wine Res. **2009**, 15, 59–69.
24. Breslin, P.A.S.; Gilmore, M.M.; Beauchamp, G.K.; Green, B.G. Psychophysical evidence that oral astringency is a tactile sensation. Chem. Senses **1993**, 18, 405–417.
25. Pickering, G.J.; Heatherbell, D.A.; Vanhanen, L.P.; Barnes, M.F. The Effect of Ethanol Concentration on the Temporal Perception of Viscosity and Density in White Wine. Am. J. Enol. Vitic. **1998**, 49, 306–318.
26. Nurgel, C.; Pickering, G. Contribution of Glycerol, Ethanol and Sugar to the Perception of Viscosity and Density Elicited by Model White Wines. J. Texture Stud. **2005**, 36, 303–323.
27. Gawel, R.; Sluyter, S.V.; Waters, E.J. The effects of ethanol and glycerol on the body and other sensory characteristics of Riesling wines. Aust. J. Grape Wine Res. **2007**, 13, 38–45.
28. Byrnes, N.K.; Nestrud, M.A.; Hayes, J.E. Perceptual Mapping of Chemesthetic Stimuli in Naive Assessors. Chemosens. Percept. **2015**, 8, 19–32.

29. Demattè, M.L.; Sanabria, D.; Sugarman, R.; Spence, C. Cross-Modal Interactions between Olfaction and Touch. Chem. Senses **2006**, 31, 291–300.
30. Kora, E.P.; Latrille, E.; Souchon, I.; Martin, N. Texture-Flavor Interactions in Low Fat Stirred Yogurt: How Mechanical Treatment, Thickener Concentration and Aroma Concentration Affect Perceived Texture and Flavor. J. Sens. Stud. **2003**, 18, 367–390.
31. Rodríguez-Bencomo, J.J.; Muñoz-González, C.; Andújar-Ortiz, I.; Martín-Álvarez, P.J.; Moreno-Arribas, M.V.; Pozo-Bayón, M.Á. Assessment of the effect of the non-volatile wine matrix on the volatility of typical wine aroma compounds by headspace solid phase microextraction/gas chromatography analysis. J. Sci. Food Agric. **2011**, 91, 2484–2494.
32. Saenz-Navajas, M.-P.; Campo, E.; Fernandez-Zurbano, P.; Valentin, D.; Ferreira, V. An assessment of the effects of wine volatiles on the perception of taste and astringency in wine. Food Chem. **2010**, 121, 1139–1149.
33. Labbe, D.; Gilbert, F.; Martin, N. Impact of Olfaction on Taste, Trigeminal, and Texture Perceptions. Chemosens. Percept. **2008**, 1, 217–226.
34. Ferrer-Gallego, R.; Hernández-Hierro, J.M.; Rivas-Gonzalo, J.C.; Escribano-Bailón, M.T. Sensory evaluation of bitterness and astringency sub-qualities of wine phenolic compounds: Synergistic effect and modulation by aromas. Food Res. Int. **2014**, 62, 1100–1107.
35. Dwyer, E.; Gallagher, E. Functional Ingredients as Fat Replacers in Cakes and Pastries; Teagasc: Dublin, Ireland, 2001.
36. Busch, J.L.H.C.; Tournier, C.; Knoop, J.E.; Kooyman, G.; Smit, G. Temporal Contrast of Salt Delivery in Mouth Increases Salt Perception. Chem. Senses **2009**, 34, 341–348.
37. Vignon, X.; Pillet, O.; Kessler, G.; Lhotellier, N.; Le Bras, G. Salinité et Minéralité. Le chaînon manquant. Rev. Oenol. **2012**, 143, 49–53.
38. Ballester, J.; Mihnea, M.; Peyron, D.; Valentin, D. Exploring minerality of Burgundy Chardonnay wines: A sensory approach with wine experts and trained panellists: Exploring minerality in wine. Aust. J. Grape Wine Res. **2013**, 19, 140–152.
39. Nasri, N.; Septier, C.; Beno, N.; Salles, C.; Thomas-Danguin, T. Enhancing salty taste through odour–taste–taste interactions: Influence of odour intensity and salty tastants' nature. Food Qual. Preference **2013**, 28, 134–140.
40. Nasri, N.; Beno, N.; Septier, C.; Salles, C.; Thomas-Danguin, T. Cross-modal interactions between taste and smell: Odour-induced saltiness enhancement depends on salt level. Food Qual. Preference **2011**, 22, 678–682.
41. Baeyens, F.; Eelen, P.; Van den Bergh, O.; Crombez, G. Flavor-Flavor and Color-Flavor Conditioning in Humans. Learn. Motiv. **1990**, 21, 434–455.

42. Barkat, S.; Poncelet, J.; Landis, B.N.; Rouby, C.; Bensafi, M. Improved smell pleasantness after odor–taste associative learning in humans. Neurosci. Lett. **2008**, 434, 108–112.
43. Ross, C.F. Physiology of Sensory Perception. In The Sensory Evaluation of Dairy Products; Springer US: New York, NY, USA, 2009; pp. 17–42.
44. Stéphane Vidal, L.F. Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. Anal Chim Acta. Anal. Chim. Acta **2004**, 513, 57–65.
45. Jones, P.R.; Gawel, R.; Francis, I.L.; Waters, E.J. The influence of interactions between major white wine components on the aroma, flavour and texture of model white wine. Food Qual. Preference **2008**, 19, 596–607.
46. R Core Team. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria, 2015.
47. Lê, S.; Josse, J.; Husson, F. About the FactoMineR package. J. Stat. Softw. **2008**, 25, 1–18.

CHAPTER FOUR

INFLUENCE OF JUICE TURBIDITY, HYPER-OXIDATION, AND SKIN-CONTACT ON CHARDONNAY WINE MOUTHFEEL

Anthony Sereni [+], Elizabeth Tomasino [+], and James Osborne [*,+]

Department of Food Science & Technology, Oregon State University, Corvallis, OR 97331, USA

*Correspondence: Tel.: +1-541-737-6494

[+]These authors contributed equally to the work

ABSTRACT

The impact of pre-fermentation treatments on mouthfeel characteristics of Chardonnay wine was investigated. Chardonnay grapes were harvested from Oregon State University's vineyard in September, 2015. After destemming and pressing the juice was subjected to various treatments. These treatments included high, medium, and low turbidity level, as well as hyper-oxidation, two-hour skin contact, and two-hour skin contact + hyper-oxidation. Three liter fermentations of each treatment were performed in triplicate. All treatments went through both alcoholic and malolactic fermentations. Total phenolics and hydroxycinnamic acids differed between the treatments. Wines that underwent hyper-oxidation contained the lowest total phenolics. Hyper-oxidation following skin contact reduced total phenolics but retained more than the hyper-oxidation treatment. Sensory analysis using citation by frequency procedure showed the treatments did modify the mouthfeel of finished wines. High turbidity treatment wines were characterized as spritz, dusty, medium acid, while medium turbidity is characterized by adhesive, tingle and complex, and low turbidity treatment wines were described as chalky and sour. Skin contact treatment was characterized by spritz, dusty, and medium acid while hyper-oxidation was characterized by chalky and sour. The combination of the two treatments: Skin contact + hyper oxidation, was characterized as fleshy. Pre-fermentation juice treatments can be utilized to develop stylistic differences in finished Chardonnay wine.

INTRODUCTION

Chardonnay wine is produced in a wide variety of styles that utilize a number of different winemaking procedures (Robinson et al., 2012). For example, Chardonnay wines often undergo malolactic fermentation (MLF) with the addition of *Oenococcus oeni* and barrel aging. Additional techniques that may be employed include altering the amount of juice solids, keeping the juice in contact with the skins for extended periods before pressing, and oxidizing the juice before alcoholic fermentation (Gawel et al., 2014). These winemaking techniques employed during Chardonnay wine production are typically aimed at improving the mouthfeel or body of the final wine, as mouthfeel is one of the most important aspects of Chardonnay quality (Cutler, 2012). However, while much work has been conducted to elucidate the influence of these various winemaking procedures on the aroma of finished Chardonnay wine, the impact of these treatments on the mouthfeel of Chardonnay is relatively unknown.

Winemakers generally alter the amount of juice solids present after pressing in order to modify a white wines aroma and mouthfeel characteristics. Typically, after the grapes are pressed the juice may be settled for an extended period of time or until a specific turbidity level is reached. However, how wine sensory changes are related to juice solids or turbidity is not clear. It has been shown that settling time can impact the population of wine microorganisms present with higher juice turbidity levels correlating with an increase in non-*Saccharomyces* yeast (Albertin et al., 2014). In addition, turbidity level

has also been demonstrated to influence the aroma of finished wine with high juice turbidity being correlated with an increase in C_6 alcohols such as hexanol, and some C_6 aldehydes; which can contribute to a "green" aroma character (Gambetta et al., 2014). There is also some evidence to support an increase in fruity notes with increases in turbidity due to an increase in acetates and some higher alcohols (Nicolini et al., 2011).

High rates of glycerol production are also correlated with higher juice turbidity. However, levels are generally not above known sensory threshold limits (Albertin et al., 2014). In contrast, excessive clarification of must has been demonstrated to decrease long chain unsaturated fatty acids in yeast during ferment, which can cause an increase in acetic acid production (Nicolini et al., 2011). Boivin et al., (1998) also found a decrease in mannoproteins, compounds important to Chardonnay wine mouthfeel (Boivin et al., 1998), in the cell walls of yeast that fermented clarified Chardonnay juice. While many of these studies have investigated individual compounds found in wine, few studies have investigated the full impact of the composite parts on finished wine.

An additional winemaking treatment that may impact Chardonnay mouthfeel is skin contact. Pomace or skin contact of Chardonnay grapes is sometimes used to extract phenolic compounds from grape skins to influence mouthfeel and aromatics. The effect to perception based on skin contact is unclear, some studies show a positive influence to wine aroma (Ferreira et al., 1992, Gawel et al., 2014), while others an increase in perceived viscosity (Cheynier et al., 1989). Ferreira et al., (1995) found that skin contact caused an increase in C6 compounds, especially hexan-1-ol and hex-2-en-1-ol, in

finished Chardonnay wines of Burgundy. They also found that excess settling time mitigated this increase; causing a neutralizing effect (Ferreira et al., 1995). The main downfall of skin contact is cited as the browning of finished wine with bottle aging (Cheynier et al., 1989; Gawel et al., 2014).

Another technique that can be used to impact the phenolic content of a white wine is a process known as hyper-oxidation. This involves "browning" or oxidation of the juice in the press pan or by vigorous pumping into the settling tank. The goal is to oxidize the phenolic compounds that may be present in the juice so that these compounds will be removed during the alcoholic fermentation (by precipitation). This in turn results in wine with lower amounts of phenolic compounds that could potentially be oxidized during the aging process leading to browning and flavor and aroma taints. The phenolic species are oxidized by polyphenol oxidase enzyme (PPO) in the presence of O_2 gas exposure, either by atmospheric gas, or pure O_2 gas pumped into the must post alcoholic fermentation. Final wines produced from hyper-oxidized Chardonnay juice appear clarified. These wines hold stable color compared to control treatments over longer durations of aging. In addition to impacting polyphenol content, hyper-oxidation also impacts the concentration of volatile compounds in the wines, exhibiting higher concentrations of volatile compounds with the exception of ethyl acetate, acetate, and ß-damascenone (REF). Sensory analysis have generally demonstrated a higher rate of fruity aromatics, and a lower rate of herbaceous, bitter, and floral characteristics (Cheynier et al., 1989; Schneider, 1998; María Jesús et al., 2011; Cejudo-Bastante et al., 2012). As with the

previously discussed winemaking techniques, the impact of hyper-oxidation on the mouthfeel of Chardonnay wine is little understood, with limited mention in literature.

Because of the importance of mouthfeel to Chardonnay wine quality, this study investigated a number of winemaking procedures thought to influence Chardonnay wine mouthfeel. Specifically, we examined the pre-fermentation treatments of hyper oxidation, skin contact, and must turbidity and how these techniques impacted the characterization of Chardonnay mouthfeel. In addition, we investigated whether any differences in the perception of Chardonnay wine mouthfeel were related to differences in basic wine chemical measurements of phenolics, acidity, and ethanol content. These measurements are part of routine analysis in most wineries and the correlation of wine chemistry with wine style is of importance to industry.

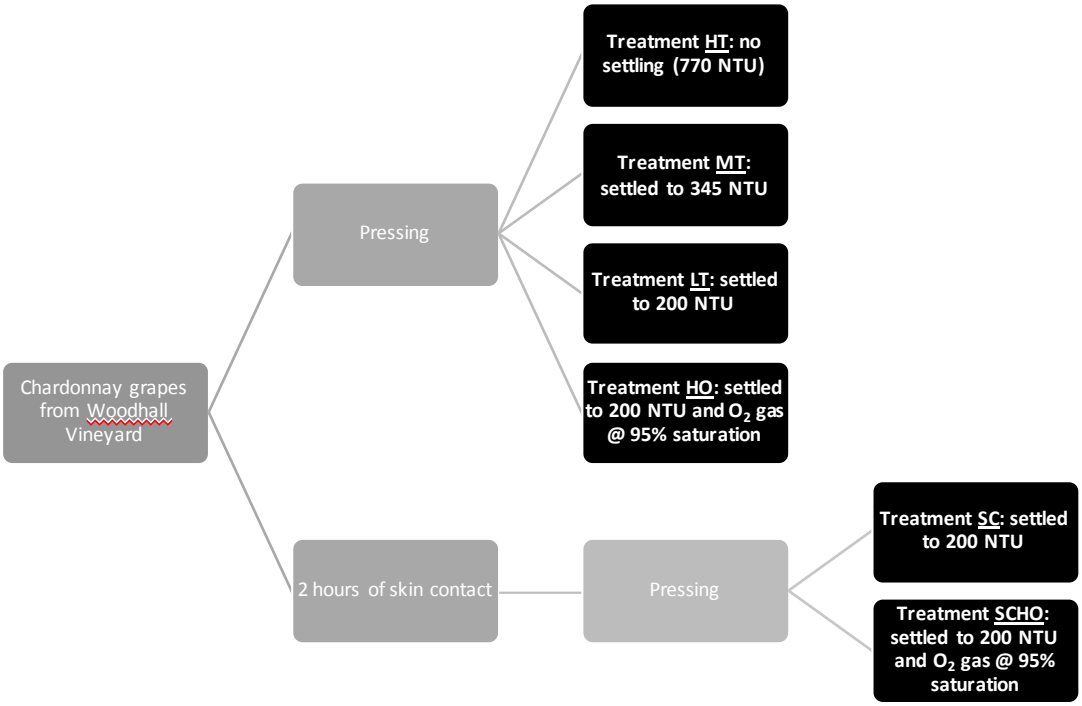
MATERIALS AND METHODS

Winemaking

Chardonnay grapes were harvested from Oregon State University's Woodhall vineyard, located in Monroe, OR. The grapes were destemmed with a Velo destemmer (VLS technologies, Treviso, Italy) and utilized for a number of experimental treatments as outlined in Figure 1. A portion of the grapes were placed in a 100L stainless steel tank and kept cool at 4°C for two hours of extended skin contact. The remainder of the grapes were pressed for 10 minutes at 0.5 bar (Velo membrane press, VLS technologies, Treviso, Italy). After pressing the juice was placed in a 500 L stainless steel tank before

being split into various treatments. One portion of the juice was immediately removed and designated the high turbidity (HT) treatment. This juice had a turbidity reading of 775 NTU as measured by a nephelometer (Hach industries, Loveland, CO). The remaining juice was allowed to settle. Periodically a sample was taken and assessed for turbidity. When the juice reached a turbidity of approximately 350 NTU a portion was removed from the juice lees and designated as the medium turbidity treatment (MT). The remaining juice was settled overnight (total settling time 12 hours) before being removed from the settled juice lees. This juice was designated the low turbidity treatment (LT) and had an NTU of 200. A portion of the LT juice was then oxidized by rapid bubbling of oxygen gas into it until it reached 95% saturation (>15 mg/L) as measured by a dissolved oxygen meter (Hanna edge® DO meter, Hanna Instruments, Limena, Italy). This was designated as the hyper-oxidized (HO) treatment. Grapes that had undergone 2 hours skin contact after destemming were pressed for 10 minutes at 0.5 bar and settled to an NTU of 200. The juice was then split into two portions. One portion was designated the skin contact treatment (SC) while the second portion was subjected to oxidation as described above. This treatment was designated skin contact + hyper-oxidation (SC + HO). A flow chart summarizing the treatments is displayed in Figure 4.1.

Figure 4.1 Flow chart of pre-fermentation juice treatment for the *Influence of Juice Turbidity, Hyper-oxidation, and Skin-contact on Chardonnay Wine Mouthfeel.*



Three liter fermentations of each juice treatment were conducted in triplicate in one-gallon glass carboys (three liters of must) at 15°C. All treatments were inoculated with *S. cerevisiae* strain D47 (Lallemand, Montreal, Canada) after rehydration according to the manufacturer. Alcoholic fermentation was monitored by measuring °Brix daily with an Anton-Paar DMA 35N Density Meter (Graz, Austria). Completion of alcoholic fermentation (reducing sugar concentration below 0.5g/L) was confirmed by testing with Bayer Clinitest tablets (Morristown, New Jersey, USA). At this point wines were

inoculated for MLF by addition of *Oenococcus oeni* strain Beta (Lallemand, Montreal, Canada) at approximately 1 x 10^6 cfu/mL. After completion of MLF (< 30 mg/L malic acid as measured by enzymatic test kit (R-Biopharm, Darmstadt, Germany)) an addition of 50 mg/L SO_2 was made and wines were placed in 4°C to settle for two weeks. Wines were then racked, sterile filtered (0.45µm PES cartridge filter), and bottled in 375 mL green glass bottles and sealed with aluminum screw cap closures (Stelvin ™, Amcor, Australia) previously sparged with nitrogen. Prior to bottling samples were taken and frozen at -20^{0}C until required for analysis.

Chemical Analysis

pH was determined using an ion-selective electrode (ThermoFisher Scientific, MA, USA), and titratable acidity was determined by titration with 0.1M NaOH following standard procedures. Ethanol content was determined with Anton Paar Alcolyzer (Santner Foundation, Graz, Austria). Total phenolic and hydroxycinnamic acids were determined by UV3101 pc spectrophotometer at 280nm and 320nm respectively (Shimadzu, Kyoto, Japan).

Sensory Analysis

Sensory analysis was conducted on March 24th and 25th, 2016, at the Oregon State University Yamhill County Extension Office (McMinnville, Oregon) after 3 months of bottle aging. Background odors were eliminated with a WINIX air cleaner, model 5300 (WINIX, Dundee, IL), and temperature of the room was kept at 20 ± 2°C. 15

winemakers from the Willamette Valley participated, 9 males and 6 females (age range from 30-65), all with at least 5 years of experience making white wine. Panelists were screened before the sessions for oral lesions, specific anosmia, and cigarette use. Wines were tasted by panelists with a randomized design where each panelist received two randomized replicate samples in their tasting, allowing for a complete replication of each treatment across the panel. They were instructed to refrain from smelling the wines before tasting as this study focused on the in-mouth experience. Each wine glass was labeled with randomly generated three digit numbers. Panelists evaluated wines using a citation-by-frequency method (Campo et al., 2010, Schuttler et al., 2015). Each taster was presented with lists of flavor and mouthfeel descriptors associated with Chardonnay wines. Descriptors were taken from previous research on Chardonnay mouthfeel (Gawel et al., 2015, Le Fur et al., 2003, Sereni et al., 2016). Nose clips were not utilized in this study as previous research has suggested that retro-nasal aroma plays an important but indeterminate role in the human perception of mouthfeel (Labbe et al., 2008, Saenz-Navajas et al., 2010, Sereni et al., 2016). Individuals were asked to choose five flavor attributes and five mouthfeel attributes from the list of descriptors which they felt best described each wine.

Data Analysis

Phenolic absorbance data was analyzed by ANOVA using R studio version 3.1.2 (R consortium, Boston, MA). Data analysis of sensory results were conducted using XLSTAT (Addingsoft co., New York, NY), and the FactoMineR package from R version

3.2.1 (Lê et al. 2008). Descriptive terms that were chosen less than 15% across panelists were removed from the data prior to analysis. Correspondence analysis was used to determine which of the chosen attributes best described each wine. Mouthfeel descriptors that had contributions greater than 1/n (n=3of mouthfeel descriptors), were considered to be pertinent for the specific factors.

RESULTS

Wine Analysis

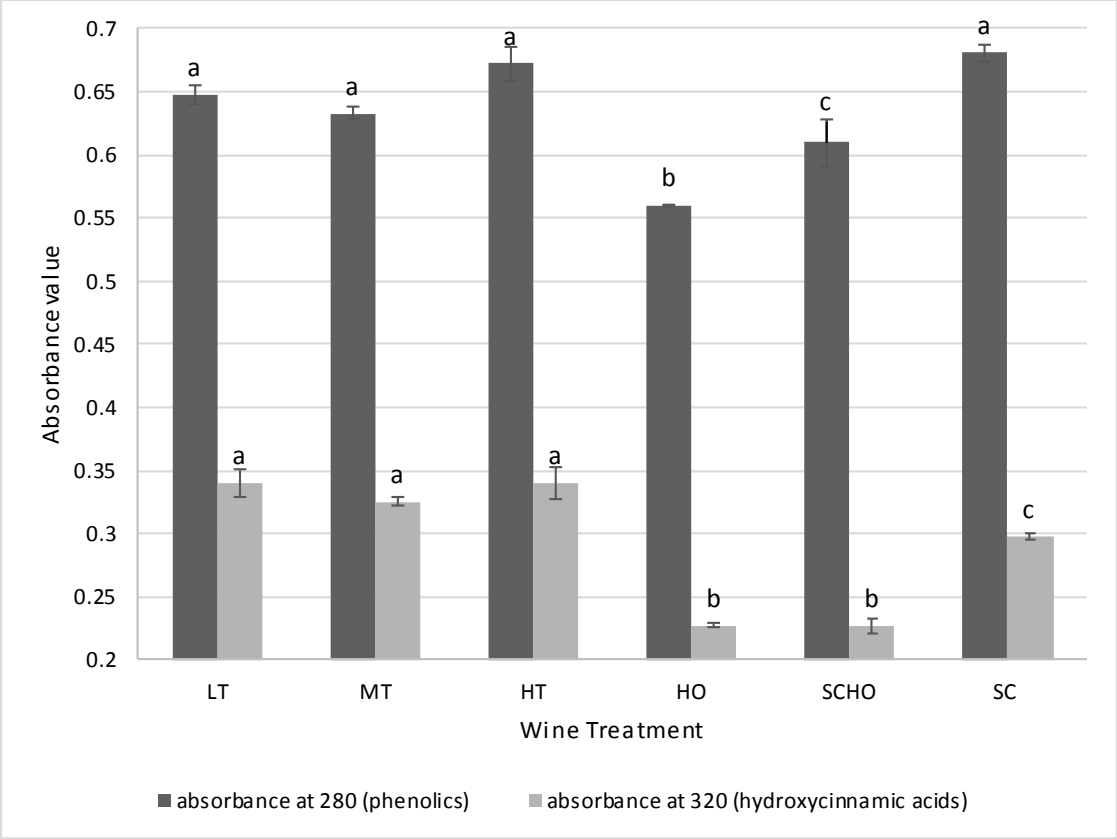
Pre-fermentation must treatments did not impact the completion of alcoholic fermentation with all fermentations being completed six weeks after inoculation. No differences were noted in residual sugar levels or pH (Table 4.1). Ethanol concentrations also did not differ between wines produced from the various pre-fermentation treatments (Table 4.1) with a high of 14.34 (v/v) in the hyper oxidation treatment, and a low of 13.93 (v/v) in the skin contact treatment. No differences in the time taken to complete MLF were noted with all wines completing MLF in five weeks.

Table 4.1 Final wine Ethanol, pH, °Brix, and T.A. Ethanol, pH, and °Brix data are averages from replicate treatments, standard deviations for pH and °Brix were marginal. T.A. data is of the final homogenized wines.

Wine Treatment	Ethanol (v/v)	pH	°Brix	T.A.
SCHO	14.12 ± 0.08	3.37	< 0.0	5.80
HO	14.34 ± 0.05	3.38	< 0.0	5.79
HT	14.04 ± 0.16	3.38	< 0.0	5.75
MT	14.25 ± 0.02	3.38	< 0.0	5.80
LT	13.97 ± 0.21	3.38	< 0.0	5.85
SC	13.93 ± 0.03	3.37	< 0.0	5.91

Total phenolic and the hydroxycinnamic acid values, as assessed by absorbance at 280 or 320 nm, are shown in Fig. 4.2. The highest value for total phenolics in wine were obtained by the high turbidity and skin contact treatments. Hyper-oxidation resulted in the lowest concentration of total phenolics, as well as hydroxycinnamic acids. Skin contact followed by hyper-oxidation showed significantly decreased total phenolics and hydroxycinnamic acids. No significant differences in hydroxycinnamic acids were found between treatments that underwent hyper-oxidation. Turbidity level did not significantly impact the hydroxycinnamic acid fraction. However significant differences were noted in total phenolic content.

Figure 4.2 Absorbance at 280nm (total phenolics) and 320nm (hydroxycinnamic acids) of Chardonnay wines produced from juice that had undergone the following treatments: Low turbidity juice (LT), medium turbidity juice (MT), high turbidity juice (HT), hyper-oxidized juice (HO), hyper-oxidized juice following skin contact (SCHO), and Skin contact juice (SC). N=3 with S.D. denoted by error bars.



[a,b,c] indicates average results reported which were statistically significant by Tukey's HSD. Each result is indicated with a corresponding superscript by absorbance wavelength denoting results which ranked as not significantly different (same letter) and significantly different (different letter) by p-value <0.05.

Sensory Analysis

Pre-fermentation must treatments altered the characterization of Chardonnay wine mouthfeel based on sensory analysis. Wine groupings were established by in mouth flavor experience (retro-nasal aroma, taste and mouthfeel). Twenty two of the 44 descriptors were found to contribute significantly to the first two dimension, comprising 57.2 of the total variance. These attributes include seven retro-nasal aroma, two taste and 13 mouthfeel descriptors. Both the figure and table with descriptor contributions can be found in the Appendix. Figure 4.3 shows the treatments in conjunction with only the mouthfeel descriptors. Table 4.2 shows the contributions of each mouthfeel descriptor for each factor.

Clear separation of wines based on treatment can be seen both in the F1 and F2 axes (Figure 4.3). Four groupings can be seen; group 1 - MT by itself, group 2 - SCHO by itself, group 3 - LT and HO, and group 4 - HT and SC. Mouthfeel descriptors were found to be important for separation, as even when retro-nasal aroma was included in the analysis (Appendix 1), more mouthfeel descriptors contributed to the formation of the factors, and separation of treatments are very similar between the two analysis. MT is characterized by complex, adhesive, sappy and tingle, SCHO by fleshy, LT and HO by sour, and HT and SC by medium acid, thin, spritz and dusty.

Figure 4.3. Correspondence Analysis mouthfeel attributes used to characterize different treatments (HT= high turbidity, HO=hyperoxidation, LT=low turbidity, MT=medium turbidity, SC=skin contact, SCHO=skin contact+hyperoxidation).

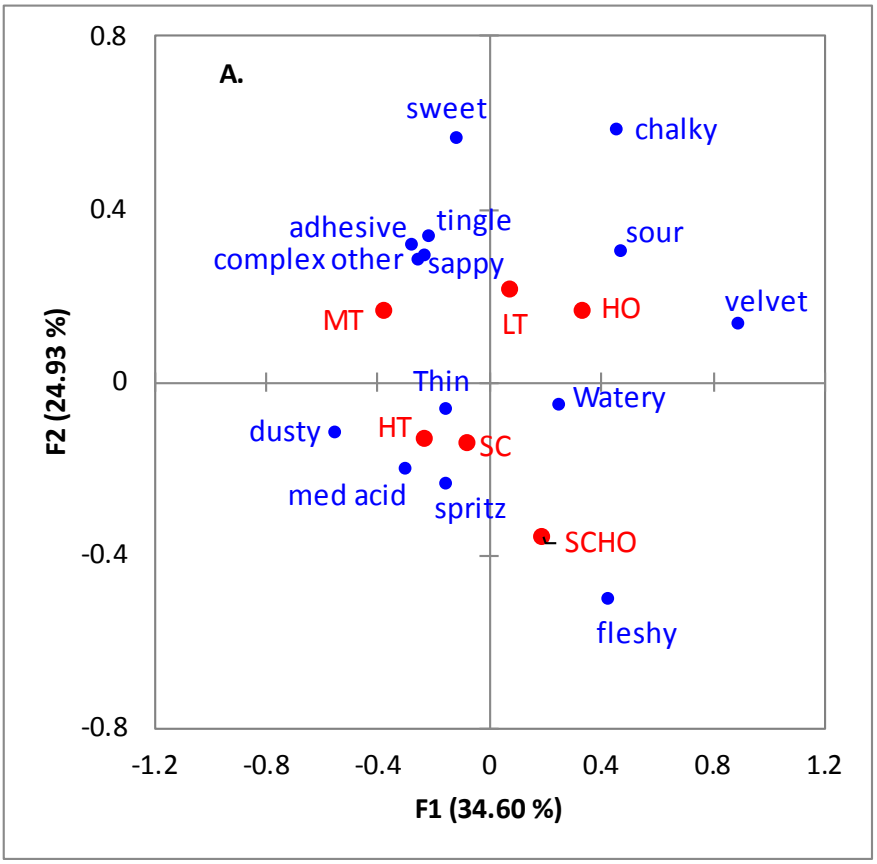


Table 4.2 – Contributions of mouthfeel descriptors to each factor of correspondence analysis. Contributions above 0.04 are considered significant.

	F1	F2	F3	F4	F5
high acid	0.00	0.03	0.00	0.01	0.00
low acid	0.00	0.01	0.06	0.01	0.07
Thin	0.02	0.01	0.00	0.01	0.12
med acid	0.08	0.05	0.01	0.00	0.01
warm	0.00	0.00	0.03	0.04	0.01
spritz	0.02	0.06	0.01	0.05	0.02
syrup	0.01	0.00	0.03	0.00	0.00
Watery	0.05	0.00	0.06	0.02	0.03
Full	0.01	0.01	0.01	0.01	0.26
dry	0.00	0.01	0.01	0.01	0.05
sappy	0.03	0.07	0.02	0.00	0.00
adhesive	0.04	0.07	0.00	0.00	0.02
velvet	0.35	0.01	0.00	0.11	0.08
complex other	0.03	0.05	0.10	0.03	0.03
unripe-other	0.00	0.03	0.06	0.03	0.00
tingle	0.02	0.07	0.00	0.00	0.07
rich	0.01	0.00	0.35	0.01	0.00
sour	0.08	0.04	0.00	0.12	0.01
fleshy	0.07	0.12	0.01	0.00	0.00
dry other	0.03	0.03	0.24	0.04	0.01
sweet	0.00	0.13	0.00	0.05	0.00
dusty	0.08	0.01	0.00	0.10	0.01
grainy	0.01	0.03	0.00	0.04	0.02
chalky	0.06	0.13	0.00	0.16	0.02
supple	0.00	0.02	0.02	0.00	0.00
mouthcoat	0.00	0.02	0.00	0.14	0.14

DISCUSSION

During the production of Chardonnay wine a number of pre-fermentation juice treatments are often employed by winemakers to alter the aroma and mouthfeel of the final wine. A number of these treatments were investigated in the present study with a focus on whether the mouthfeel of the wine was modified. Skin-contact and hyper-oxidation treatments were studied due to their potential impact to the phenolic content of wine. As expected, hyper-oxidation reduced total phenolics and hydroxycinnamic acids compared to all other treatments, in agreement with previous work (Schneider, 1998).

While the influence of phenolics on mouthfeel is well documented in red wines, their impact on white wine mouthfeel is less well understood. In addition, the impact of hydroxycinnamic acids on wine mouthfeel is also relatively unknown (Runnebaum et al., 2011). Researchers have noted that hydroxycinnamic acids may have an influence on wine mouthfeel, however whether this is a direct impact, or an interactive effect based on reactions with other phenolic compounds is still unknown (Fernández-Zurbano et al., 1998; Hufnagel & Hofmann, 2008). Furthermore, the few studies investigating hyper-oxidation of Chardonnay have focused on wine aroma rather than mouthfeel (Cejudo-Bastante et al., 2011). Interestingly though, one study noted no difference on the aromatic qualities of hyper-oxidation treatment Chardonnay wine, and their sensory results noted a preference by panelists for hyper-oxidation Chardonnay treatments suggesting that changes other than aroma, such as mouthfeel, occurred (Cheynier et al., 1989).

The process of leaving the juice in contact with the skins for two hours did not increase the amount of total phenolics and hydroxycinnamic acids compared to the control, (low

turbidity treatment) in opposition to previously reported studies (Ferreira et al., 1992). It is possible that the length of skin contact in the present study may not have been sufficient for large changes in phenolic content to occur. As evidence, previous studies of skin contact with Chardonnay grapes have noted a decrease in acidity [with at least 2 hours of skin contact] due to the increased potassium ions extracted from the skins (Gambetta et al., 2014). However, in the present study no changes in pH or titratable acidity were noted. It is also possible that the differences in the phenolic data of the present study and the phenolic data of previous research could be influenced by differences in phenolic and precursor compounds associated with berry ripening level (Adams, 2006). Finally, the measurement of absorbance at 280nm is a simple method used to determine total phenolic content but does not reveal any information about the composition of the phenolic compounds present. It is possible that compositional changes may have been present between the treatments but that the method utilized was not able to detect them. Further, Gawel et al., (2014) has demonstrated that skin contact of Chardonnay juice can increase polysaccharides in finished wine, which could impact mouthfeel. HPLC analysis should be conducted in future work investigating the phenolic content and polysaccharides of Chardonnay as this will provide compositional data that may help explain differences in mouthfeel.

While the phenolic data did not explain mouthfeel differences between treatments, neither did the final basic chemistries of the wines. Although alcohol and pH have been demonstrated to influence the perception of phenolics in white wine, in the present study alcohol and pH do not differ significantly enough to be of influence (Gawel et al., 2013). A possible explanation for the mouthfeel characterizations may be differences in the

aromatic qualities of the wines. Recent studies have demonstrated how retro-nasal aroma can impact the perception of mouthfeel by sensory panelists (Labbe et al., 2008, Saenz-Navajas et al., 2010, Sereni et al., 2016). Given that skin contact and hyper-oxidation have both been demonstrated to change the aroma composition of Chardonnay wine (Gawel et al., 2014) it is possible that differences in wine aroma may have influenced the perception of mouthfeel in the present study. Previous research does not support any differences in yeast cell wall components, or fermentation dynamics due to hyper-oxidation when it is applied before the exponential growth phase (Schneider, 1998). Future work in this area should include volatile analysis of wines to investigate possible correlation of aroma on mouthfeel perception.

Aside from pre-fermentation treatments that impact phenolics, the amount of juice turbidity was also investigated for its' impact on mouthfeel. Turbidity has been reported to impact wine aroma in particular and is often attributed to differences in microbial populations associated with the juice lees. For example, Albertin et al., (2014) saw an increase in *Candida zemplinina* and *Hanseniaspora spp*. with increased levels of must turbidity. Both of these yeasts have been extensively researched for their aromatic contribution to white wine, as well as their influence on final wine mannoprotein size and quantity (Domizio et al., 2014; Jolly et al., 2014). Must turbidity levels have also been linked to cell wall porosity and mannoproteins content of *S. cerevisiae* in wine, with decreased quantity of mannoproteins associated with lower must turbidity (Boivin et al., 1998). Mannoproteins are glycoproteins which are a component of yeast cell walls. They have been demonstrated to influence the aromatic qualities of white wine, as well as produce wines with increased tartrate stability, and a "fuller" mouthfeel (Goncalves et al.,

2002; Vidal et al., 2004). However, because the present study did not include any time where the wine was in extended contact with the yeast lees it is unlikely that mannoprotein differences were a significant factor in the mouthfeel changes noted between the turbidity treatments.

An additional consequence of altering juice turbidity is encouraging a successful malolactic fermentation. Boivin et al., (1998) saw decreases in yeast secreted macromolecules with higher levels of must turbidity. Decreased macromolecules from yeast growth can impact the ability of *O. oeni* to conduct MLF (Alexandre et al., 2004) and so high turbidity levels may cause issues. *O. oeni* growth and the MLF have been demonstrated to be influential to the perception of mouthfeel in Chardonnay wine (Runnebaum et al., 2011) and so the consequences of pre-fermentation juice treatments to this process must be considered.

CONCLUSION

The pre-fermentation must treatments of hyper-oxidation, skin contact, skin contact + hyper-oxidation, and turbidity levels significantly altered the mouthfeel characterization of the resulting wines. Basic wine chemistry such as pH, TA, and ethanol were not impacted by pre-fermentation treatments while hyper-oxidation reduced total phenolics and hydroxycinnamic acid content. These changes did not directly correlate to the observed differences in mouthfeel. This suggest that other factors known to impact mouthfeel but not measured in the present study, such as polysaccharides, mannoproteins, and volatile compounds may have played a role. Additionally, differences in phenolic composition rather than total phenolic content may have driven the mouthfeel differences

and requires additional investigation. While this study does demonstrate that these techniques can be utilized to influence Chardonnay mouthfeel, there are broader implications to production and research. This study emphasizes the importance to improve predictive measurements for research and industry as the chemical parameters thought to be most influential to white wine mouthfeel did not correlate with significant differences.

LITERATURE CITED

Albertin, W., Miot-Sertier, C., Bely, M., Marullo, P., Coulon, J., Moine, V., Masneuf-Pomarede, I. (2014). Oenological prefermentation practices strongly impact yeast population dynamics and alcoholic fermentation kinetics in Chardonnay grape must. *International Journal of Food Microbiology*, *178*, 87–97. http://doi.org/10.1016/j.ijfoodmicro.2014.03.009

Boivin, S., Feuillat, M., Alexandre, H., & Charpentier, C. (1998). Effect of must turbidity on cell wall porosity and macromolecule excretion of Saccharomyces cerevisiae cultivated on grape juice. *American Journal of Enology and Viticulture*, *49*(3), 325–332.

Campo, E., Ballester, J., Langlois, J., Dacremont, C., & Valentin, D. (2010). Comparison of conventional descriptive analysis and a citation frequency-based descriptive method for odor profiling: An application to Burgundy Pinot noir wines. *Food Quality and Preference*, *21*(1), 44–55. http://doi.org/10.1016/j.foodqual.2009.08.001

Cejudo-Bastante, M. J., Perez-Coello, M. S., Perez-Juan, P. M., & Hermosin-Gutierrez, I. (2012). Effects of hyper-oxygenation and storage of Macabeo and Airen white wines on their phenolic and volatile composition. *European Food Research and Technology*, *234*(1), 87–99. http://doi.org/10.1007/s00217-011-1619-1

Cheynier, V., Rigaud, J., Souquet, J. M., BarillÈRE, J. M., & Moutounet, M. (1989). Effect of Pomace Contact and Hyperoxidation on the Phenolic Composition and Quality of Grenache and Chardonnay Wines. *American Journal of Enology and Viticulture*, *40*(1), 36–42

Cutler, L., Varietal Focus Chardonnay. Wine Business Monthly. January 2012. Wine Communications Group Inc

Fernández-Zurbano, P., Ferreira, V., Escudero, A., & Cacho, J. (1998). Role of Hydroxycinnamic Acids and Flavanols in the Oxidation and Browning of White Wines. *Journal of Agricultural and Food Chemistry*, *46*(12), 4937–4944. http://doi.org/10.1021/jf980491v

Ferreira, B., Hory, C., Bard, M. H., Taisant, C., Olsson, A., & Le Fur, Y. (1995). Effects of skin contact and settling on the level of the C18:2, C18:3 fatty acids and C6 compounds in burgundy chardonnay musts and wines. *Food Quality and Preference*, *6*(1), 35–41. http://doi.org/10.1016/0950-3293(94)P4210-W

Gawel, R., Day, M., Van Sluyter, S. C., Holt, H., Waters, E. J., & Smith, P. A. (2014). White Wine Taste and Mouthfeel As Affected by Juice Extraction and Processing. *Journal of Agricultural and Food Chemistry*, *62*(41), 10008–10014. http://doi.org/10.1021/jf503082v

Gawel, R., Sluyter, S. C. V., Smith, P. A., & Waters, E. J. (2013). Effect of pH and Alcohol on Perception of Phenolic Character in White Wine. *American Journal of Enology and Viticulture*, *64*(4), 425–429. http://doi.org/10.5344/ajev.2013.13016

Gawel, R., Sluyter, S. V., & Waters, E. J. (2007). The effects of ethanol and glycerol on the body and other sensory characteristics of Riesling wines. *Australian Journal of Grape and Wine Research*, *13*(1), 38–45. http://doi.org/10.1111/j.1755-0238.2007.tb00070.x

González-Royo, E., Urtasun, A., Gil, M., Kontoudakis, N., Esteruelas, M., Fort, F., Zamora, F. (2013). Effect of Yeast Strain and Supplementation with Inactive Yeast during Alcoholic Fermentation on Wine Polysaccharides. *American Journal of Enology and Viticulture*, *64*(2), 268–273. http://doi.org/10.5344/ajev.2012.12071

Ibarburu, I., Soria-Díaz, M. e., Rodríguez-Carvajal, M. a., Velasco, S. e., Tejero-Mateo, P., Gil-Serrano, A. Dueñas, M. t. (2007). Growth and exopolysaccharide (EPS) production by Oenococcus oeni I4 and structural characterization of their EPSs. *Journal of Applied Microbiology*, *103*(2), 477–486. http://doi.org/10.1111/j.1365-2672.2006.03266.x

Jaffe, J., Valentin, D., Meunier, J.-M., Siliani, A., Bertuccioli, M., & Le Fur, Y. (2011). The Chardonnay wine olfactory concept revisited: A stable core of volatile compounds, and fuzzy boundaries. *Food Research International*, *44*(1), 456–464. http://doi.org/10.1016/j.foodres.2010.09.022

Jolly, N., Augustyn, O., & Pretorius, I. (2003). The Effect of Non-Saccharomyces Yeasts on Fermentation and Wine Quality. *South African Journal for Enology and Viticulture 23(2)*. 55-62

Labbe, D., Gilbert, F., & Martin, N. (2008). Impact of Olfaction on Taste, Trigeminal, and Texture Perceptions. *Chemosensory Perception*, *1*(4), 217–226. http://doi.org/10.1007/s12078-008-9029-x

Lasik, M. (2013). The application of malolactic fermentation process to create good-quality grape wine produced in cool-climate countries: a review. *European Food Research and Technology*, *237*(6), 843–850. http://doi.org/10.1007/s00217-013-2083-x

Lê, S., Josse, J. & Husson, F. (2008). FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software*. **25(1)**. pp. 1-18.

Le Fur, Y., Mercurio, V., Moio, L., Blanquet, J., & Meunier, J. M. (2003). A New Approach To Examine the Relationships between Sensory and Gas Chromatography−Olfactometry Data Using Generalized Procrustes Analysis Applied to Six French Chardonnay Wines. *Journal of Agricultural and Food Chemistry*, *51*(2), 443–452. http://doi.org/10.1021/jf0205458

Nicolini, G., Moser, S., Roman, T., Mazzi, E., & Larcher, R. (2011). Effect of juice turbidity on fermentative volatile compounds in white wines. *Vitis*, *50*(3), 131–135.

Pickering, G. J., Heatherbell, D. A., Vanhanen, L. P., & Barnes, M. F. (1998). The Effect of Ethanol Concentration on the Temporal Perception of Viscosity and Density in White Wine. *American Journal of Enology and Viticulture*, *49*(3), 306–318

Robinson, J., Harding, J., Vouillamoz, J., 2012. Wine Grapes. Ecco, New York.

Runnebaum, R. c., Boulton, R. b., Powell, R. l., & Heymann, H. (2011). Key Constituents Affecting Wine Body – an Exploratory Study. *Journal of Sensory Studies*, *26*(1), 62–70. http://doi.org/10.1111/j.1745-459X.2010.00322.x

Sáenz-Navajas, M.-P., Campo, E., Fernández-Zurbano, P., Valentin, D., & Ferreira, V. (2010). An assessment of the effects of wine volatiles on the perception of taste and astringency in wine. *Food Chemistry*, *121*(4), 1139–1149. http://doi.org/10.1016/j.foodchem.2010.01.061

Schneider, V. (1998). Must Hyperoxidation: A Review. *American Journal of Enology and Viticulture*, *49*(1), 65–73.

Schüttler, A., Friedel, M., Jung, R., Rauhut, D., & Darriet, P. (2015). Characterizing aromatic typicality of Riesling wines: merging volatile compositional and sensory aspects. *Food Research International*, *69*, 26–37. http://doi.org/10.1016/j.foodres.2014.12.010

Sereni, A., Osborne, J., & Tomasino, E. (2016). Exploring Retro-Nasal Aroma's Influence on Mouthfeel Perception of Chardonnay Wines. *Beverages*, *2*(1), 7. http://doi.org/10.3390/beverages2010007

Vernhet, A., Pellerin, P., Prieur, C., Osmianski, J., & Moutounet, M. (1996). Charge Properties of Some Grape and Wine Polysaccharide and Polyphenolic Fractions. *American Journal of Enology and Viticulture*, *47*(1), 25–30.

Vidal, S., Courcoux, P., Francis, L., Kwiatkowski, M., Gawel, R., Williams, P., Cheynier, V. (2004). Use of an experimental design approach for evaluation of key wine components on mouth-feel perception. *Food Quality and Preference*, *15*(3), 209–217. http://doi.org/10.1016/S0950-3293(03)00059-4

GENERAL CONCLUSIONS AND SUMMARY

Specific non-antagonistic yeast and bacteria strains can be co-inoculated for AF and MLF to alter Chardonnay wine mouthfeel without risk of hindering fermentation kinetics. Our study demonstrated that co-inoculation took less time to complete AF and MLF while producing Chardonnay wines with significant differences in mouthfeel compared to sequentially inoculated treatments. *T. delbrueckii* did not negatively impact fermentation kinetics of co-inoculated must, and resulted in differences in mouthfeel compared to treatments without *T. delbrueckii*. This demonstrates another set of enological tools for winemakers to utilize in crafting wine style. Future research should investigate the influence of co-inoculation with and without *T. delbrueckii* on Chardonnay wine aroma and polysaccharide concentration to help in determining the role of these treatments in the generation of Chardonnay wine mouthfeel style.

Chapter three demonstrated through differences in sorting, and the use of descriptive terms between +R and –R that the volatile fraction of Chardonnay wine is important to the appreciation of mouthfeel. The contradictions between UFP groupings with and without retronasal aroma support that something pivotal is missing from each wine which would allow more consistent descriptive terms to be assigned by wine professionals. It is unclear if the influence of the volatile fraction is due to interactions between chemical groups, by indirect effects with the volatile fraction, or by associative learning. More research is needed to investigate these findings, but this work provides a first step in examining the intricacies and interactions at work for Chardonnay mouthfeel perception. Future research

should incorporate psychological imperatives of associative learning in respect to the flavor experience of Chardonnay wine, as well as research into the chemical interactions between the volatile and non-volatile wine fraction for specific influence on the perception of mouthfeel.

The pre-fermentation must treatments of hyper-oxidation, skin contact, skin contact + hyper-oxidation, and turbidity level did significantly alter the mouthfeel characterization of the resulting wines. Wine chemistry data was not strongly impacted by the pre-fermentation treatments. While this study further demonstrated enological techniques useful to winemakers for crafting Chardonnay style, more research is needed to determine the mechanism of these differences. Future research should investigate the specific differences in phenolic species impacted by pre-fermentation juice treatment, as well as correlation studies of mouthfeel and volatile fraction analysis. Once again, wine polysaccharide component qualification, and quantification may be of importance to elucidating the differences in mouthfeel between these treatments as well as the timing and temperature of MLF.

The chemical measurements utilized for this enological exploration into Chardonnay mouthfeel did not consistently correlate with the sensory results. We did demonstrate that the aromatic fraction influences Chardonnay mouthfeel, which emphasizes the importance of volatile analysis for a better understanding of Chardonnay wine style as a whole, and not simply to determine the olfactory potential. However, future research on the enological parameters of these studies should also include glycoprotein and

exopolysaccharides analysis. While this study investigated fermentation kinetics, possible differences in genetic expression, and the influence to yeast cell wall components, as well as bacterial exopolysaccharides, cannot be ruled out. One of the key points of this exploration in Chardonnay wine style is the importance of interactions which do not occur in a closed system experiment such as model wine solutions. Therefore, a broader inspection of the wine matrix should be emphasized. Our study provides valuable insight for future researchers when designing experiments, as findings from studies using reductionist techniques fail to include elements yet unknown to the influence of specific sensory experiences.

APPENDIX

Figure A.4 Correspondence Analysis of retronasal aroma, taste and mouthfeel attributes used to characterize different treatments (HT= high turbidity, HO=hyperoxidation, LT=low turbidity, MT=medium turbidity, SC=skin contact, SCHO=skin contact+hyperoxidation)

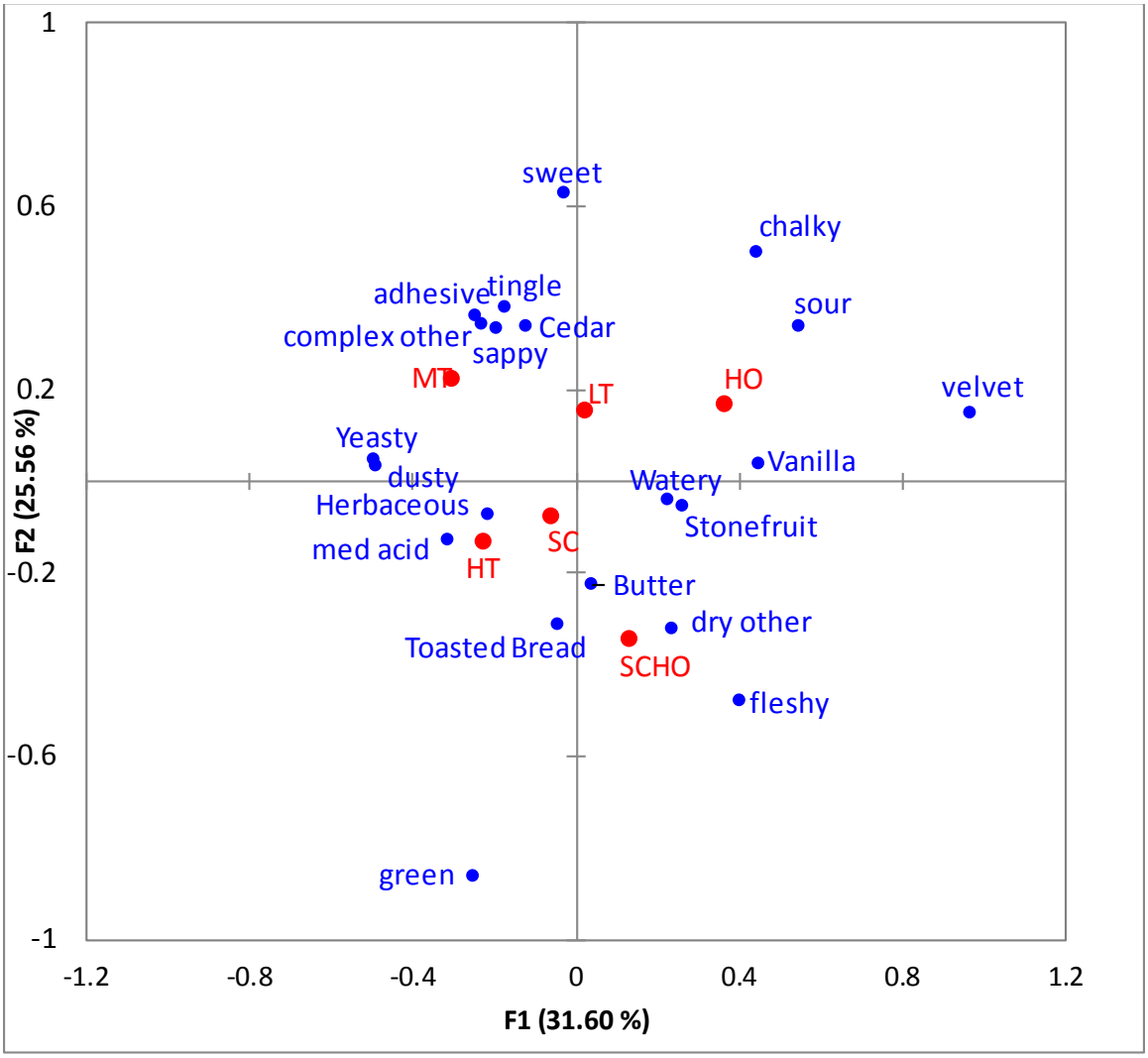


Table A.4 Contributions of retronasal aroma, taste and mouthfeel descriptors to each factor of correspondence analysis. Contributions above 0.02 are considered significant.

	F1	F2	F3	F4	F5
high acid	0.00	0.00	0.00	0.01	0.01
low acid	0.00	0.00	0.01	0.01	0.05
Citrus	0.00	0.00	0.00	0.00	0.00
Stonefruit	0.07	0.00	0.07	0.02	0.00
Apple	0.00	0.01	0.01	0.01	0.00
Nutty	0.00	0.01	0.04	0.01	0.00
Thin	0.01	0.00	0.00	0.00	0.08
Tropical fruit	0.01	0.01	0.06	0.06	0.05
Herbaceous	0.03	0.00	0.00	0.01	0.01
med acid	0.05	0.01	0.01	0.00	0.00
Green	0.00	0.07	0.04	0.04	0.01
Toasted Bread	0.00	0.06	0.00	0.02	0.05
warm	0.01	0.00	0.02	0.00	0.02
spritz	0.01	0.01	0.02	0.01	0.07
Spicy	0.02	0.00	0.02	0.01	0.08
syrup	0.01	0.00	0.02	0.00	0.00
Watery	0.02	0.00	0.01	0.05	0.01
Yeasty	0.11	0.00	0.00	0.01	0.04
Butter	0.00	0.03	0.02	0.05	0.01
pepper	0.02	0.00	0.06	0.01	0.00
Full	0.00	0.00	0.02	0.00	0.06
dry	0.00	0.01	0.02	0.00	0.00
sappy	0.01	0.05	0.01	0.01	0.00
Woody	0.00	0.01	0.09	0.01	0.14
Vanilla	0.07	0.00	0.00	0.01	0.05
adhesive	0.02	0.06	0.00	0.01	0.00
velvet	0.26	0.01	0.00	0.01	0.03
complex other	0.02	0.04	0.00	0.07	0.01
unripe-other	0.00	0.01	0.01	0.06	0.00
tingle	0.01	0.05	0.02	0.00	0.01
Coconut	0.00	0.00	0.02	0.02	0.00
rich	0.01	0.00	0.07	0.17	0.03
Cedar	0.00	0.03	0.01	0.00	0.00
Reduction	0.02	0.02	0.03	0.03	0.00
sour	0.07	0.03	0.01	0.03	0.00
fleshy	0.04	0.06	0.00	0.00	0.00

Table A.4 continued

dry other	0.01	0.03	0.13	0.03	0.02
Burnt	0.00	0.01	0.00	0.00	0.00
green	0.01	0.17	0.00	0.01	0.00
sweet	0.00	0.09	0.00	0.01	0.00
dusty	0.04	0.00	0.01	0.03	0.06
grainy	0.00	0.01	0.00	0.01	0.03
chalky	0.03	0.05	0.05	0.07	0.02
supple	0.00	0.01	0.00	0.01	0.00
mouthcoat	0.00	0.01	0.06	0.04	0.01