AN ABSTRACT OF THE THESIS OF

David G. Takush for the degree of Master of Science in Food Science and Technology presented on September 10, 2009.
Title: The Impact of *Saccharomyces* and non-*Saccharomyces* Yeast on the Aroma and Flavor of *Vitis vinifera* L. cv. 'Pinot Noir' Wine.

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James P. Osborne

The impact of yeast on the aroma and flavor of *Vitis vinifera* L. cv. 'Pinot Noir' wine was investigated. Due to the presence of naturally occurring yeast and bacteria on grapes and wine equipment, and the influence these microorganisms have on wine, a means of eliminating microorganisms from grape must was explored.

High Hydrostatic Pressure (HHP) processing was investigated as a means of inactivating wine microorganisms in grape must without impacting organoleptic properties. Table grape must adjusted to 23°Brix was inoculated with wine-relevant yeast and bacteria and treated for 5 and 10 minutes at 551MPa. At both 5 and 10 minutes of pressure treatment all microorganisms were reduced to below detectable numbers.

Next, wine was produced from HHP treated Pinot Noir grapes and the impact of HHP on the sensory properties of the wine was investigated. Descriptive analysis of the wines using a trained panel showed minimal significant differences between wines produced from HHP treated and untreated grape must. Chemical analysis showed no

significant differences in color; however, there was a 70% increase in total phenolics in the wine produced from HHP treated must.

Because the sensory effects of HHP treatment on grape must appeared to be minimal, the impact of specific yeast on Pinot Noir wine was investigated. HHP treated must was fermented in sterilized red wine micro-fermentors. The yeast strains and species studied were EC1118, RC212, Assmanshausen (AMH) (Lallemand, Montréal, Canada), MERIT.ferm, and Symphony (a blend of MERIT.ferm and *Kluyveromyces thermotolerans*) (Chr. Hansen, Hørsholm, Denmark). All *Saccharomyces* yeast strains were inoculated at approximately 10^6cfu/mL while the non-*Saccharomyces* yeast was inoculated at approximately 10^5cfu/mL. Each yeast strain was inoculated in triplicate, and a total of fifteen micro-fermentors, each containing 2.75kg of HHP treated grape must, were used. Fermentation profiles were similar between yeast strains with minimal variability between replicates. However, AMH replicates finished fermentation 24 hours after other yeast strains. After fermentation, analysis showed a significant difference in color at 520nm between the AMH and EC1118 wines. There were no other significant color differences between wines.

Descriptive analysis with a trained panel indicated that yeast strain had a significant effect on the sensory profile of Pinot Noir wine. Significant sensory attributes included overall fruity aroma, red fruit aroma, dark fruit aroma, and overall fruit flavor, among others. Principle Component Analysis results showed EC1118 and RC212 trending toward high overall aroma intensities and dark fruit and jammy characteristics. MERIT.ferm also produced wines with high aroma intensities;

however, they trended towards red fruit and floral characteristics. The wines produced from AMH and Symphony yeasts were not distinct and resulted in low aroma and flavor intensities in several descriptor categories.

The results show that HHP processing is a viable means of inactivating microorganisms from grape must without causing large alterations in the final aroma and flavor profile of wine. The data also demonstrates the feasibility of utilizing HHP processing in conjunction with autoclavable micro-fermentors to conduct experimental red wine fermentations without the influence of native yeast and bacteria. The sensory data from yeast strain trials indicate that yeast can have a significant impact on Pinot Noir wine aroma. This data could help improve control of wine aroma profiles and help improve wine quality in the Pinot Noir industry. However, more research is needed to profile a larger array of oenological yeast strains, including non-*Saccharomyces* species, and to examine the effects of co-inoculation.

The Impact of *Saccharomyces* and non-*Saccharomyces* Yeast on the Aroma and Flavor of *Vitis vinifera* L. cv. 'Pinot Noir' Wine

by
David G. Takush

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APPROVED:

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David G. Takush, Author

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CONTRIBUTION OF AUTHORS

Cindy Leaderer in the Food Science Sensory Lab at OSU provided invaluable support with wine sensory experiment design and statistical analysis. Scott Robbins at OSU's Woodhall Vineyard grew the Pinot Noir grapes for the project.

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INTRODUCTION

Sound winemaking involves intricacies in all aspects of vineyard management, grape harvest, processing, fermentation, aging, and bottling. It is often viewed as an involved and lengthy process. However, the most fundamental aspect of winemaking is the action of yeast converting grape sugars into ethanol and carbon dioxide. This fermentation by yeast, namely *Saccharomyces cerevisiae*, also contributes the majority of the total volatile aroma compounds in wine (Nykanen 1986; Rapp and Mandery 1986; Lambrechts and Pretorius 2000; Mateos, Perez-Nevado et al. 2006). Winemakers have traditionally relied on the spontaneous fermentation of grape must by yeast; however, beginning in 1906, commercial yeast preparations were recommended for California winemakers (Bioletti 1906).

Presently, winemakers may inoculate their grape must with one of the hundreds of commercially available *S. cerevisiae* strains. Yeast strains are selected based on a number of criteria. These criteria can include low volatile acidity and sulfide production, completeness of fermentation, temperature tolerance, alcohol and sulfite tolerance, glycerol production, enzyme activity, nutritional requirements, killer factors, and volatile aroma production (Zoecklein, Fugelsang et al. 1990; Degre 1992; Reynolds, Edwards et al. 2001).

However, there is still much debate regarding which yeast strains are best suited for specific wine varieties and styles. Questions also arise regarding the relevance of yeast strain on overall sensory contribution. In fact, among both researchers and winemakers, there is a lack of consensus regarding the impact of yeast strain on specific wine sensory characteristics and whether differences, if any, will fade over time (Zoecklein, Fugelsang et al. 1990; Egli, Edinger et al. 1998; Dubourdieu, Tominaga et al. 2006).

Previous research on the impact of yeast on wine flavor and aroma has been extensive, but has almost exclusively focused on white wine production (Egli, Edinger et al. 1998; Antonelli, Castellari et al. 1999; Reynolds, Edwards et al. 2001; Jolly, Augustyn et al. 2003; Clemente-Jimenez, Mingorance-Cazorla et al. 2004; Dubourdieu, Tominaga et al. 2006; Reynolds, Schlosser et al. 2007; Reynolds, Schlosser et al. 2007; King, Swiegers et al. 2008; Swiegers, Kievit et al. 2009). Research on the influence of yeast strain in red wine has been somewhat limited due to difficulties conducting sterile fermentations while keeping grape skins in contact with the must. Red grape must cannot be sterile filtered without removing the skins, which play an integral part of red wine flavor development. Other obstacles include replicating commercial techniques of cap management and inducing enough extraction of phenolic material, all while keeping the must free of microorganisms present on grapes, in the environment, and on winery equipment.

In this study, the new and novel technique of High Hydrostatic Pressure (HHP) processing was utilized in order to create a grape must free of microorganisms while minimizing the effect on flavor and sensory attributes (Ogawa, Fukuhisa et al. 1990;

Ogawa, Fukuhisa et al. 1992; Knorr 1993; Takahashi, Ohta et al. 1993; Cheftel 1995; Delfini and Conterno 1995; Shellhammer, Aleman et al. 2003). Along with HHP processing, fully autoclavable fermentors were developed to ensure fermentation by inoculated yeast strains without the influence of unwanted bacteria and native yeast. The fermentors, a similar size and shape of those used by Sampaio, Kennedy et al. (2007), and based on a design by Osborne and Edwards (2006), were used to make sufficient wine for both sensory and GC-MS analysis and to allow for cap management during fermentation. The HHP processing and the use of sterile vessels were key factors that allowed for research focusing on yeast strain impact *without* the influence of native yeast, bacteria, harsh chemicals, sterile filtration or thermal processing.

The specific objectives of this research were as follows:

1.) Investigate the use of High Hydrostatic Pressure processing for the elimination of microorganisms in grape must prior to fermentation.
2.) Develop autoclavable micro-fermentation vessels that allow for traditional red-wine production and sufficient wine for both sensory and chemical analysis.
3.) Conduct fermentations of *Vitis vinifera* cv. Pinot Noir grapes using different *S. cerevisiae* and non-*Saccharomyces* yeast strains and study the impact of yeast strain on the chemical and sensory properties of Pinot Noir wine.

LITERATURE REVIEW

Wine Aroma

Wine aroma is an integral part of wine quality and one of its most important distinguishing aspects (Lambrechts and Pretorius 2000; Mateos, Perez-Nevado et al. 2006). Research has shown that wine aroma is made up of hundreds of different compounds (Nykanen 1986; Rapp and Mandery 1986; Sefton, Francis et al. 1993; Fang and Qian 2005), some of which have individual aroma impacts, and others which may be present in low concentrations and may work together synergistically to contribute to the overall flavor profile (Rapp and Mandery 1986; Lambrechts and Pretorius 2000; Fang and Qian 2005; Loscos, Hernandez-Orte et al. 2007). These aroma compounds can include alcohols (ethanol and higher alcohols), volatile fatty acids, esters, carbonyl compounds (aldehydes and ketones), C_{13}-norisopreniods, volatile phenols, sulfur compounds, and terpenes and their alcohol derivatives, among others (Nykanen 1986; Rapp and Mandery 1986; Lambrechts and Pretorius 2000).

Wine aromas are usually classified into categories depending on their origins which can include: 1) grape derived or varietal aroma, 2) aroma derived from modifications during processing, 3) fermentative aroma produced by yeast and bacteria, and 4) post-fermentative aroma or, ‘bouquet’, which develops during aging (Schreier 1979; Rapp and Mandery 1986). While the post-fermentative and processing derived flavors are important in wine, this discussion will focus on fermentative aromas produced by yeast, and the action of yeast on grape derived flavors.

Grape Derived Flavors

Aroma and flavor compounds in the grape berry are secondary metabolites produced during growth and maturation (Jackson 2000; Kalua and Boss 2009). Many of the important aroma and flavor compounds are located in the berry juice and skins; however, distribution of these aroma compounds may differ throughout the berry (Rapp and Mandery 1986; Park, Morrison et al. 1991). Grape derived aroma compounds can include norisoprenoids, terpenes, thiols, pyrazines, C_6 alcohols and aldehydes, and ester compounds, and can change during berry maturation (Park, Morrison et al. 1991; Jackson 2000; Fang and Qian 2006; Kalua and Boss 2009).

C_6-aldehydes such as 2- hexenal and hexanal contribute many of the grassy and herbaceous notes found in grapes, and are especially prevalent in Grenache and Sauvignon Blanc cultivars (Stevens, Flath et al. 1969; Kalua and Boss 2009). These C_6-aldehydes are formed by enzymes during grape development, specifically those of the lipoxygenase pathway (see Figure 1.1) (Kalua and Boss 2009). This pathway involves the oxidation then subsequent cleavage of linoleic and linolenic acid, and may be induced enzymatically during processing when berry cell walls are ruptured in the presence of oxygen (Rapp and Mandery 1986; Chkaiban, Botondi et al. 2007). In Pinot Noir wine, these C_6-aldehydes are commonly found in their alcohol form rather than the oxidized aldehyde form (Brander, Kepner et al. 1980; Fang and Qian 2005). Another aldehyde of particular importance in Pinot Noir is benzaldehyde, which can be formed from a variety of metabolic pathways. Benzaldehyde consists of a benzene

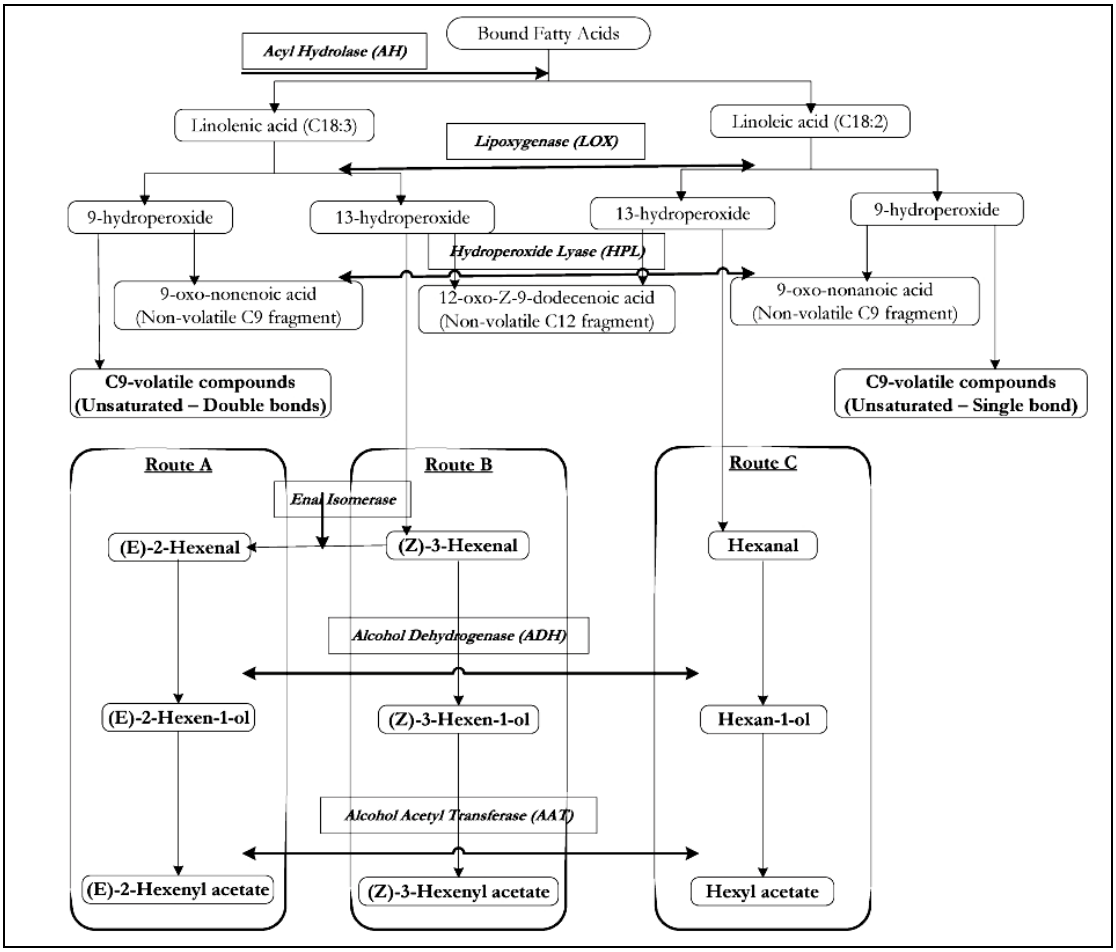

Figure 1.1: Formation of C_6-aldehydes and alcohols via the lipoxygenase pathway (Kalua and Boss 2009).

ring with an aldehyde subunit and can contribute either cherry or toasty almond aromas to wine, depending on concentration (Jackson 2000; Fang and Qian 2005; Dunlevy, Kalua et al. 2009).

Significant higher alcohols derived from grapes include 2-phenylethanol (rosy), benzyl alcohol (floral, dried fruit), and C_6-alchohols (grassy, herbaceous) (Gomez, Martinez et al. 1995; Jackson 2000; Fang and Qian 2005; Fang and Qian 2006). C_6-alchohols are characteristic of late berry-developmental stages and are closely related to the C_6-aldehydes produced by lipoxygenase pathway. C_6-alchohols

need only to be reduced to alcohol (by alcohol dehydrogenase) from their corresponding aldehyde form (see Figure 1.1), (Schreier 1979; Chkaiban, Botondi et al. 2007; Kalua and Boss 2009). In Pinot Noir, higher alcohols play a very important role. 2-phenylethanol can contribute significantly to the overall aroma of the wine, and may be one of the most important characteristic compounds of Pinot Noir (Mirandalopez, Libbey et al. 1992; Fang and Qian 2005; Fang and Qian 2006). 2-phenylethanol may be produced in fruits from phenylalanine by the removal of a carboxyl group and an amino group, and subsequently reduced to an alcohol (see Figure 1.2) (Tieman, Taylor et al. 2006). In addition, it has been shown that 3-octanol and 1-octen-3-ol or so-called 'mushroom alcohols' can contribute many of the mushroom, truffle and earthy notes noted in some styles of Pinot Noir (Brander, Kepner et al. 1980; Fang and Qian 2005).

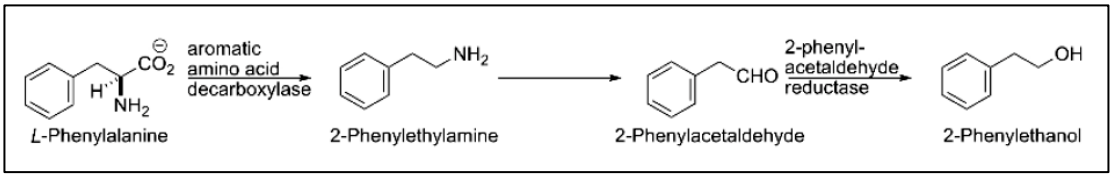

Figure 1.2: Enzymatic conversion of phenylalanine to 2-phenylethanol (Dunlevy, Kalua et al. 2009).

Ester compounds are not common in the grape berry with the exception of methyl and ethyl anthranilate which are phenolic esters found in some *Vitis labrusca* cultivars such as 'Concord' (Schreier 1979; Jackson 2000). Ethyl anthranilate and methyl anthranilate (and acetovanillone, a volatile phenol), have been reported in Pinot Noir. These phenolic compounds are described as having grapey, vanilla, sweet, and spicy/clove characters (Mirandalopez, Libbey et al. 1992). Although in most *V.*

vinifera wine methyl and ethyl anthranilate are usually present in concentrations well below threshold levels, new evidence also suggests that they may be of some aroma importance in Pinot Noir (Moio and Etievant 1995; Fang and Qian 2005).

Pyrazines are cyclic molecules that can contribute bell pepper and other vegetal/spicy notes to many cultivars. 2-Methoxy-3-(2-methylpropyl)pyrazine is an especially important contributor to the aroma of Sauvignon Blanc (Allen, Lacey et al. 1991; Lacey, Allen et al. 1991). Other isopropyl and isobutyl methoxypyrazines contribute many similar aromas to Cabernet Sauvignon as well Carmenere; however, these aromas are often viewed as off-flavors in high concentrations, indicating over-vigorous or over-shaded vines (Jackson and Lombard 1993; Parr, Green et al. 2007). Methoxypyrazines have also been found in Pinot Noir berries, but concentrations are much lower than those in Cabernet and decrease with ripening (Hashizume and Samuta 1999). Although the biosynthetic pathway that leads to the formation of methoxypyrazines in any plant species has yet to be revealed (Dunlevy, Kalua et al. 2009), it is agreed that the pathway involves an amino acid and a carbonyl compound forming an intermediate that is enzymatically methylated (Murray, Shipton et al. 1970; Leete, Bjorklund et al. 1992).

Norisoprenoids are compounds derived from the degradation of carotenoids in grapes, and are composed of C_5 isoprene precursors (Winterhalter and Skouroumounis 1997; Jackson 2000; Mendes-Pinto 2009). These precursors come from the methyl-erythritol-phosphate (MEP) pathway which is compartmentalized in the plant plastids. Norisoprenoid compounds contribute many of the fruity and floral flavors to wine and tend to increase during berry maturity. In general, C_{13}-norisoprenoids have been the

focus of much study; however, β-damascenone, β-ionone, and 1,1,6-trimethyl-1,2-dihydronapthalene (TDN) are three of the most important C_{13}-norisoprenoids found in wine (see Figure 1.3) (Fang and Qian 2006; Dunlevy, Kalua et al. 2009). TDN is common in many aged Riesling wines, and can impart a powerful 'kerosene-like' aroma. Interestingly, TDN has not been found in Pinot Noir wine (Fang and Qian 2005) and has only been found in very low amounts in glycosidically conjugated forms in the berry (Du, Ou et al. 2008). The two most important C_{13}-norisoprenoids found in Pinot Noir are β-damascenone and β-ionone due to their low threshold levels (2ng/L and 7ng/L) and relatively high concentrations (Buttery, Teranishi et al. 1988; Buttery, Teranishi et al. 1990; Fang and Qian 2006). β-damascenone has the scent of exotic flowers with heavy fruit undertones and can contribute many floral, honey, and apple aromas to wine, while β-ionone can contribute many of the important berry and violet aromas for which Pinot Noir is known (Fang and Qian 2006).

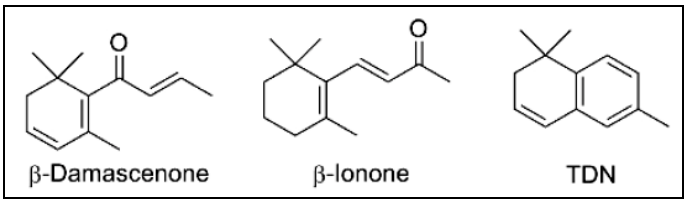

Figure 1.3: Structures of norisoprenoids commonly found in wine (adapted from Dunlevy, Kalua et al. 2009).

The biosynthetic pathway by which C_{13}-norisoprenoids are made begins with the formation of carotenoid compounds. Carotenoids are synthesized enzymatically in the berry plastid from the consecutive condensation of C_5 isopentenyl pyrophosphate (IPP) or dimethylallyl pyrophosphate (DMAPP) subunits. Once basic carotenoids are

synthesized, they undergo de-saturation, cyclization and hydroxylation to form a variety of carotenoids compounds depending on cultivar and environmental factors (Oliveira, Ferreira et al. 2004; Dunlevy, Kalua et al. 2009). The majority of carotenoids produced in fruit include β-carotene and lutein (Bureau, Baumes et al. 2000) among other minor constituents. Carotenoid cleavage dioxygenases then cleave carotenoid substrates to release volatile C_{13}-norisoprenoids, such as β-ionone (see Figure 1.4), while the formation of β-damascenone and TDN requires further chemical modification (Dunlevy, Kalua et al. 2009).

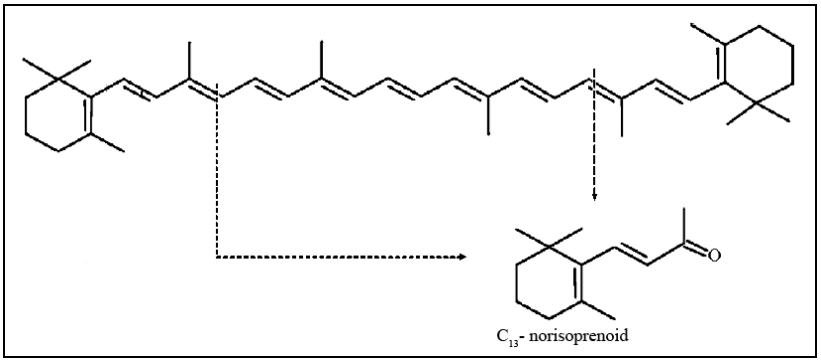

Figure 1.4: Formation of C_{13}-norisoprenoid compounds from β-carotene (adapted from Mendes-Pinto 2009).

Terpenes and their alcohol derivatives also play a large role in floral and fruity aromas in wine. Terpenes, like norisoprenoids are also derived from isoprene units; however, the isoprene precursors can be derived from the both the MEV pathway which is compartmentalized in the plastids, and the mevalonate (MVA) pathway which occurs in the cytosol (Dunlevy, Kalua et al. 2009). These pathways appear to be independently regulated, but there may be a unidirectional flow of substrates from the plastid to the cytosol via metabolite transporters (Bick and Lange 2003). The total

quantity of free monoterpenes varies in different cultivars, and classifications have been made depending on the levels present in the grape. 'Muscat-type' cultivars have high levels of free monoterpenes (up to 6mg/L) and the must of these varieties are extremely odiferous. Aromatic cultivars, or 'Riesling-types', contain 1-4mg/L of free monoterpenes, while the must aroma of neutral cultivars do not seem to be greatly influenced by free monoterpenes (Strauss, Wilson et al. 1986). Roughly 70 monoterpenes have been found in grapes and wine. The free monoterpene alcohols commonly found in grapes and must are citronellol, 3,6-dimethyl-1,5-octadien-1,7-diol, linalool, geraniol, nerol, and α-terpineol (Rapp 1998).

In Pinot Noir wine, some of the most important free monoterpenes alcohols include linalool, geraniol, and α-terpineol (see Figure 1.5) (Fang and Qian 2005). In Pinot Noir berries the concentration of free monoterpenes alcohols tend to increase with berry maturity, with the exception of linalool which decreases slightly (Fang and Qian 2006).

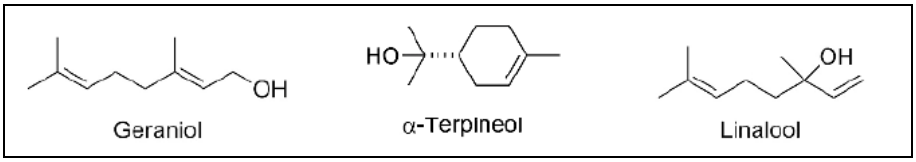

Figure 1.5: Important free monoterpenes alcohols in Pinot Noir wine (adapted from Dunlevy, Kalua et al. 2009).

It is also interesting to note that monoterpenes are not evenly distributed throughout the grape berry, and distribution varies depending on cultivar. In Muscat, free linalool is found in similar concentrations throughout the grape berry while geraniol and nerol tend to concentrate in the skin (Bayonove, Cordonnier et al. 1974; Cordonnier and

Bayonove 1978; Cordonnier and Bayonove 1981; Gunata, Bayonove et al. 1985; Wilson, Strauss et al. 1986). In contrast, in Pinot Noir free geraniol was found in both skin and grape must, yet free linalool was not found in the berry skin (Du, Ou et al. 2008).

The production of monoterpenes depends on the biosynthesis of geranyl pyrophosphate (GPP), a C_{10} compound formed from the condensation of two C_5 IPP precursors by geranyl pyrophosphate synthase. A variety of terpenoid synthases produce monoterpenes from the GPP substrate, and these enzymes, along with many subsequent modification enzymes, give rise to the final array of terpenes present (Dunlevy, Kalua et al. 2009). Additional research indicates that some strains of *S. cerevisiae* can also produce monoterpenes de novo, without grape precursors. These monoterpenes may be produced in amounts that may have a significant sensory impact (Carrau, Medina et al. 2005).

Terpenes and norisoprenoids in the grape berry can be found in both free (volatile) and glycosidically bound (odorless) forms (Cordonnier and Bayonove 1974; Gunata, Bayonove et al. 1986). These monoterpenes and norisoprenoids, along with aromatic alcohols such as benzyl and 2-phenethyl alcohol (Gunata, Bayonove et al. 1985), are generally conjugated to glucose as β-D-glucopyranosides (see Figure 1.6). The aglycones can also form more complex disaccharides when glucose is conjugated with a second sugar unit. These secondary sugar units can include α-L-arabinofuranose, α-L-rhamnopyranose, β-D-xylopyranose, and β-D-apiofuranose (see Figure 1.6) (Williams, Strauss et al. 1982; Sarry and Gunata 2004; Francis and Newton 2005). The majority of C_{13}-norisoprenoids and monoterpenes alcohols are

found as glycosidically bound conjugates in grapes; however, the ratios and concentrations vary significantly between cultivars (Williams, Strauss et al. 1982; Gunata, Bayonove et al. 1985; Park, Morrison et al. 1991; Du, Ou et al. 2008). It was also shown that glycosylated monoterpenes and norisoprenoids are distributed differently throughout the grape berry than their free counterparts (Wilson, Strauss et al. 1986; Park, Morrison et al. 1991; Du, Ou et al. 2008).

Volatile aglycones can be released during processing and fermentation by enzymatic hydrolysis from grape enzymes, yeast enzymes, bacterial enzymes, the addition of filamentous fungal derived enzymes or acid hydrolysis. The cleavage of non-volatile precursors by acid hydrolysis may have an impact on the sensory profile, yet it is important to note that the process of acid hydrolysis occurs quite slowly (Winterhalter and Skouroumounis 1997; Skouroumounis and Sefton 2000). The rate of acid hydrolysis depends on the pH, temperature, and the structure of the aglycone moiety (Sarry and Gunata 2004). For example, tertiary alcohols such as linalool are hydrolyzed more readily than moieties of primary alcohols such as nerol and geraniol (Gunata, Bayonove et al. 1986). While acid hydrolysis can be promoted by heating, the reaction may only target specific glycosides based on their aglycone moiety and can result in the cleavage of ether bonds and rearrangement of the released aglycone (Williams, Strauss et al. 1982; Skouroumounis, Massywestropp et al. 1992; Sefton 1998).

Enzymatic hydrolysis of monoglucosides requires the action of β-glucosidase. In contrast, the hydrolysis of disaccharide glycosides requires the sequential activity of a specific glycosidase to remove the outer sugar moiety, then the action of β-

glucosidase in order to liberate the aglycone from the remaining glucose (see Figure 1.6) (Gunata, Bitteur et al. 1988; Sarry and Gunata 2004). In grapes, low activities of glycosidases such as α-arabinosidase and α-rhamnosidase are generally found, although higher amounts of β-glucosidase have also been detected (Williams, Strauss et al. 1982; Aryan, Wilson et al. 1987; Gunata, Bayonove et al. 1990). There is also some evidence that suggests endo-glycosidases exist in grapes which alone can release volatile aglycones directly from their disaccharide conjugates (Gunata, Blondeel et al. 1998). Grape glucosidase activity in juice and must is virtually non-existent as low pH and glucose strongly inhibit the enzymes' activity (Gunata, Bayonove et al. 1990; Gunata, Blondeel et al. 1998). There is also evidence that suggests that limited hydrolysis occurs in the berry during maturation and ripening, although these enzymes are characterized by specificity to aglycone structure and are inhibited by glucose (Williams, Strauss et al. 1982; Aryan, Wilson et al. 1987).

Lactic acid bacteria may be a potential source of glycosidase enzymes. In a review by Matthews (2004), lactic acid bacteria were shown to have a variety of glycosidase activities, some of which may be relevant during winemaking. Although, these enzymes were shown to be functional and only partially inhibited by low pH and high glucose concentration, further research is necessary to determine the precise effect of these enzymes in must as opposed to synthetic media (Matthews, Grimaldi et al. 2004).

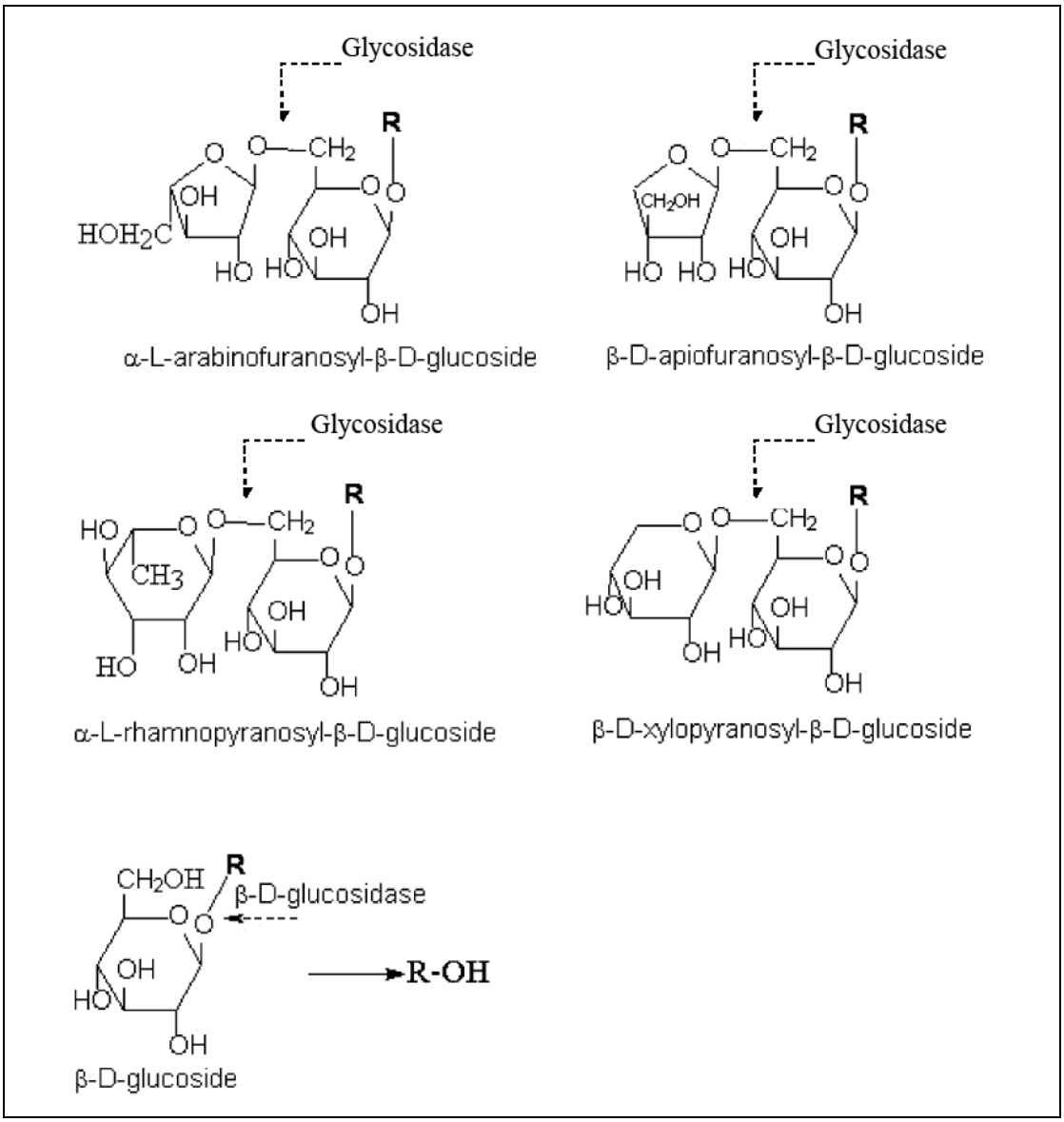

Figure 1.6: Glycosides found in grapes and their cleavage points via one-step and sequential enzymatic hydrolysis; R= monoterpenes, norisoprenoids, aromatic alcohols (adapted from (Sarry and Gunata 2004).

Exogenous enzymes produced by filamentous fungi can also release aroma compounds from their non-volatile bound forms (Gunata, Bayonove et al. 1990). Must from grapes infected with *Botrytis cinerea* have higher concentrations of β-glucosidases, as well as specific glycosidases; however, the must will also contain

compounds which strongly inhibit β-glucosidase (Gunata, Biron et al. 1989). Commercial enzyme preparations from *Aspergillus* species are commonly used for their pectinase and hemicellulase activities but they have also been shown to have β-glucosidase and glycosidase activities as well (Gunata, Bayonove et al. 1990). Although their relevance to winemaking has been questioned due to the inhibitory effect of glucose (Aryan, Wilson et al. 1987), after alcoholic fermentation the use of exogenous glycosidases has been shown to have a profound effect on increasing the total volatile content of wine (Cabaroglu, Selli et al. 2003).

The role of *S. cerevisiae* and its glycosidase activities in wine has been extremely contradictory. It has been reported that *Saccharomyces* yeast produce only very low levels of glycosidases that have little to no activity in grape must (Gunata, Bayonove et al. 1986; Leclerc, Arnaud et al. 1987; Rosi, Vinella et al. 1994; Winterhalter and Skouroumounis 1997). The activity of the *S. cerevisiae* glycosidases have also been called into question due to the inhibitory effects of must pH, glucose and ethanol concentrations, and temperature. Yet authors have more recently shown that strains of *S. cerevisiae* have significant glycosidase activity (Mateo and DiStefano 1997; Spagna, Barbagallo et al. 2002; Hernandez, Espinosa et al. 2003; Chassagne, Vernizeau et al. 2005). It has been reported that *S. cerevisiae* glycosidases are inhibited far less by glucose than fungal or plant enzymes (Aryan, Wilson et al. 1987), while SO_2 and ethanol have little to no inhibitory effect (Delcroix, Gunata et al. 1994; Spagna, Barbagallo et al. 2002; Hernandez, Espinosa et al. 2003). Although some studies revealed that ethanol (Mateo and DiStefano 1997), pH, and temperature (Delcroix, Gunata et al. 1994) may in fact inhibit the activity of *S. cerevisiae* derived

glycosidases, several other studies have shown the definitive hydrolytic ability of *S. cerevisiae* to release glycosidically bound volatiles during winemaking, under usual wine conditions, and its significant effect on wine aroma (Delfini, Cocito et al. 2001; Hernandez, Espinosa et al. 2003; Ugliano, Bartowsky et al. 2006). In fact, *S. cerevisiae* β-glucosidase activities and inhibitory parameters seem to vary between yeast strains. In particular, Spagna (2002) discovered a *S. cerevisiae* β-glucosidase whose optimal conditions included pH 3.5-4.0, 20°C, was not inhibited by glucose or fructose, was stable for 35 days in model must and wine solutions, and was even activated by ethanol.

Although yeast seem to play a significant role in releasing bound aroma compounds during fermentation, after fermentation much of the pool of bound volatiles remain (Gunata, Bayonove et al. 1986). Research has begun to investigate the role of non-*Saccharomyces* yeasts in the liberation of these additional volatile compounds. Studies have shown that many non-*Saccharomyces* yeast including *Pichia anomala, Candida pelliculosa* (formerly *Hansenula anomala*), *Hanseniaspora uvarum,* several *Debaryomyces* spp., and *Kloeckera apiculata,* produce significantly higher amounts of β-glucosidase than *S. cerevisiae* (Rosi, Vinella et al. 1994; Charoenchai, Fleet et al. 1997). This activity indicates that non-*Saccharomyces* can potentially play a greater role in wine aroma development, with finished wines showing greater 'varietal' expression. However, the ability of non-*Saccharomyces* to affect aroma profiles more than *S. cerevisiae* due solely to higher β-glucosidase activity is still under scrutiny due to the inhibitory effect of glucose among other factors (Charoenchai, Fleet et al. 1997). Much like the β-glucosidases produced by *S.*

cerevisiae, there is also a wide range of β-glucosidases produced by non-*Saccharomyces* species, some of which may function under winemaking conditions. *Debaryomyces hansenii* was shown to have an optimal pH range of 4.0-5.0 and was not inhibited by ethanol or glucose (Rosi, Vinella et al. 1994). β-glucosidase produced by *Debaryomyces pseudopolymorphus* was also shown to have functionality at wine pH, and resistance to glucose, ethanol, and sulfur dioxide. In addition, the *Deb. pseudopolymorphus* enzyme showed a high-substrate affinity and a large aglycone-substrate recognition, with the yeast significantly increasing the concentration of free volatiles in Chardonnay when co-inoculated with a *S. cerevisiae* yeast strain (Otero, Iranzo et al. 2003). It is important to note that intensity is not always the ultimate attribute of significance; the balance of aroma compounds in a finished wine may be the more accurate measure of quality to the winemaker.

Aromatic grape cultivars such as Muscat, Gewürztraminer and Riesling contain free volatile compounds, mainly monoterpenes alcohols. Thus, these aromatic cultivars have odoriferous musts and produce wines with similar flavor attributes (Strauss, Wilson et al. 1986). However, many of the major grape cultivars such as Cabernet Sauvignon, Chardonnay, Syrah and Pinot Noir do not have odiferous musts (Dubourdieu, Tominaga et al. 2006) yet still have recognizable varietal characteristics in the finished wine (Ugliano, Bartowsky et al. 2006). The varietal expression of these grapes is complex, lacking any single unique varietal compound, and may be determined by specific levels of many different compounds that can be present in a variety of cultivars (Lambrechts and Pretorius 2000; Fang and Qian 2005). It has been shown that yeast play an important role in the release of aroma compounds during

fermentation of these neutral cultivars (Abbott, Coombe et al. 1991; Francis, Sefton et al. 1992). It has also been demonstrated that other yeast enzymes (such as lyase) can hydrolyze thiol esters, releasing odors important to some cultivars that are characteristic of box-wood or black currant (Darriet, Tominaga et al. 1995; Tominaga, des Gachons et al. 1998). The release of odorless grape-derived precursors by yeast during fermentation indicates that yeast strain can play a significant role in determining the aroma profile and quality of wine from neutral cultivars.

Yeast Derived Flavors

Aside from the action of yeast on grape derived compounds, yeast themselves are another significant source of wine aroma. Flavors and aromas produced by yeast account for most of the basic constituents of wine and thus, are extremely important (Heard and Fleet 1986; Nykanen 1986; Rapp and Mandery 1986; Lambrechts and Pretorius 2000; Mateos, Perez-Nevado et al. 2006). Yeast produced aroma compounds are by-products of yeast metabolism of grape sugars and amino acids and can include alcohols (ethanol and higher alcohols), volatile fatty acids, esters, carbonyl compounds (diacetyl and acetaldehyde), volatile phenols, and sulfur compounds (Schreier 1979; Nykanen 1986; Rapp and Mandery 1986; Lambrechts and Pretorius 2000). Many of the most potent odorants in wine, based on their odor activity values, are derived from yeast metabolism (Cabaroglu, Canbas et al. 2002). The production of many of these metabolites is shown via pathways in Figure 1.7.

Ethanol is a major contributor to wine viscosity. It can affect mouth-feel and help the solubility of grape constituents in wine (Jackson 2000). Ethanol is formed as the end

product of glycolysis (Embden-Meyerhof pathway), the major route by which yeast produce energy. During glycolysis glucose is converted through a series of intermediates to pyruvate, which is then decarboxylated to an important aroma compound, acetaldehyde. Acetaldehyde is reduced to ethanol by NADH, which is oxidized back to NAD+ (Jackson 2000). The oxidation of NADH back to NAD+ is extremely important to maintaining a proper redox balance in the cell. NAD+ availability prevents the stalling of glycolysis and allows metabolism to continue (Lambrechts and Pretorius 2000). During yeast growth and reproduction, the need for NADH increases greatly, diverting reducing power to biosynthetic functions and away from the reduction of acetaldehyde, explaining its release from the cell and accumulation in the fermenting juice (Jackson 2000). The accumulation of acetaldehyde (threshold 100mg/L) can result in a sour/green apple aroma; however, in the later stages of fermentation, acetaldehyde can be transported back into the cell and reduced to ethanol (Lambrechts and Pretorius 2000). Within different oenological strains of *S. cerevisiae* there is a large variability of acetaldehyde production (Romano, Suzzi et al. 1994), from 13.1mg/L (Longo, Velazquez et al. 1992) to 120mg/L (Fleet and Heard 1993). Acetaldehyde can also be produced from the oxidation of ethanol in finished wine, and can be an indicator of oxidation state (Lambrechts and Pretorius 2000).

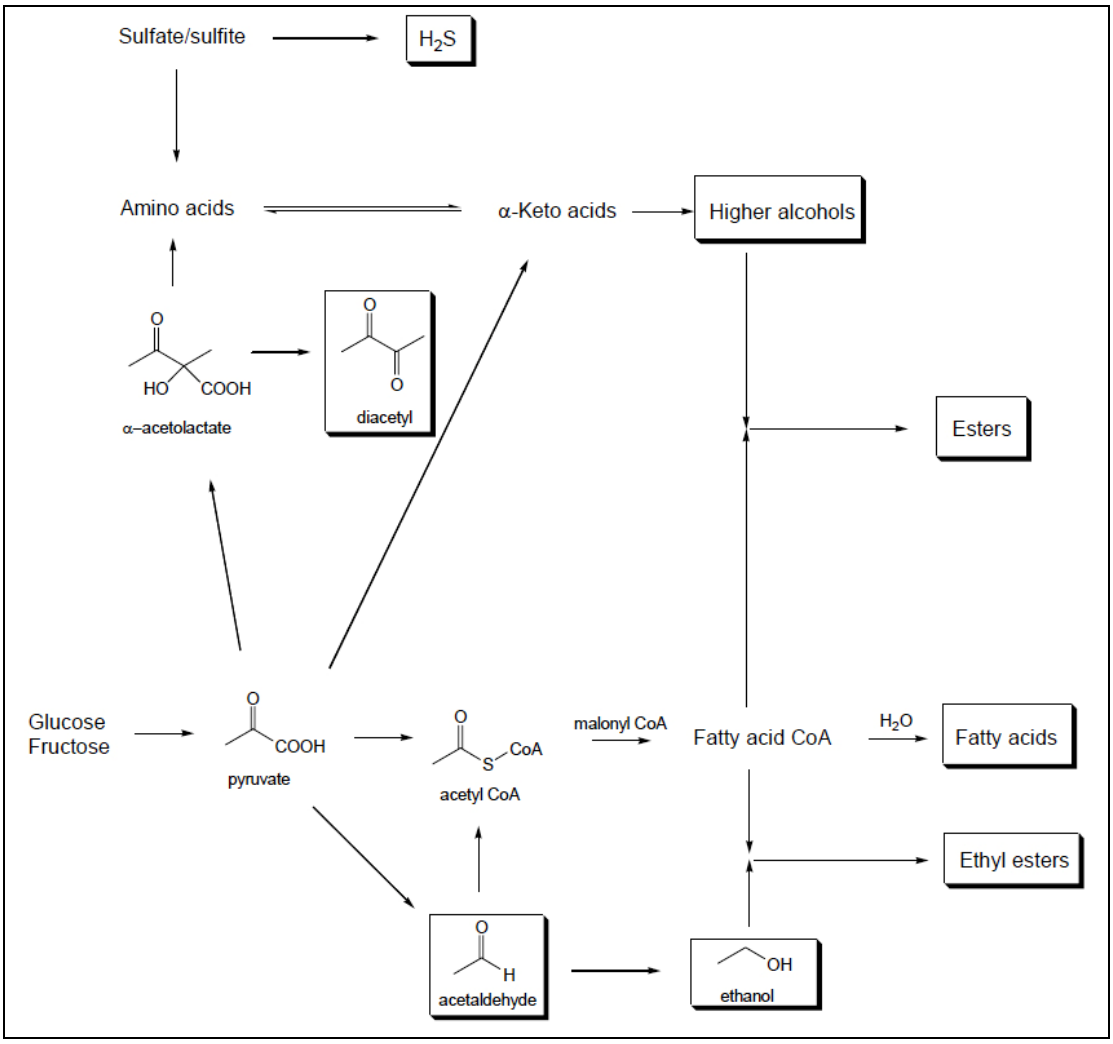

Figure 1.7: Production of some yeast derived aroma compounds (Eggers 2005).

In yeast, higher alcohols (fusel alcohols) are produced through Ehrlich reactions. During fusel alcohol formation amino acids are deaminated to α-keto acids, decarboxylated to aldehydes, and finally reduced to alcohols. Higher alcohols can contribute many of the sharp, harsh, and vinous characters to wine, and at low concentrations (below 300mg/L), add complexity (Jackson 2000; Lambrechts and Pretorius 2000). The reaction of higher alcohols with Coenzyme-A activated fatty acids (acetyl-CoA) can form acetate esters. This reaction is catalyzed by the enzyme

alcohol acetyltransferase, and the acetate ester products can contribute fresh fruit aromas to wine, including isoamyl acetate (banana) (Lambrechts and Pretorius 2000). The combination of ethanol with acetyl-CoA derived from fatty acid breakdown and synthesis can result in ethyl esters, another critical group of aroma compounds in wine. These ethyl esters are the source of many fruity and honey compounds in wine including ethyl butanoate, ethyl hexanoate, and ethyl octanoate (Jackson 2000; Lambrechts and Pretorius 2000).

While yeast produce a number and variety of esters through the addition of acids and alcohols, different yeast strains can have different esterase activities (Suomalainen 1981). Different yeast strains and species can produce different amounts of esters from the same starting material, and this has been well researched and reviewed in literature (Nykanen 1986; Lambrechts and Pretorius 2000). Results indicate that yeast strain can have a significant impact on wine aroma through ester production alone. It is also apparent that fermentation temperature, oxygen levels, and juice sediment can have an effect on ester production (Nykanen 1986; Jackson 2000).

Activated fatty acids, especially medium and long chain fatty acids, are usually found in ester form in wine, although volatile free fatty acids can be found as well. Fatty acid synthesis begins with the oxidative decarboxylation of pyruvic acid and the formation of acetyl CoA. Acetyl CoA carboxylase coverts acetyl CoA into malonyl CoA, which can undergo repetitive condensation by fatty acid synthase (Lynen 1967; Ratledge and Evans 1989; Paltauf, Kohlwein et al. 1992). The most important short chain volatile fatty acid that may be found in wine is acetic acid. Acetic acid can impart unpleasant vinegar aromas to wine at threshold levels and is considered a

serious off-aroma (Lambrechts and Pretorius 2000). Although high levels of acetic acid are usually indicators of acetic or lactic acid bacteria infections, yeast can also produce acetic acid in significant concentrations. In fact, within the same species different strains of wine yeast can produce different amounts of acetic acid (Delfini and Cervetti 1991). Monk and Cowley (1984) also confirmed the varying effect of pH, sugar, and nitrogen on acetic acid production by *S. cerevisiae* (Monk and Cowley 1984).

Hydrogen sulfide is another important off aroma caused by yeast during fermentation. H_2S and other sulfur containing compounds occur from the reduction of elemental sulfur (Schutz and Kunkee 1977) and the metabolism of sulfur-containing amino acids such as cysteine (Henschke and Jiranek 1991). H_2S production can be increased when nitrogen or other vitamins are deficient in the must (Vos and Gray 1979). Other classes of organic sulfur compounds are also produced during fermentation including mercaptans, which can contribute, fecal, cabbage, and skunky flavors to wine. Some of these compounds have been found in Pinot Noir (Fang and Qian 2005) and may have negative aroma impacts. Yet, other thiol-containing compounds are important to varietal aromas. 4-mercapto-4-methylpentan-2-one and other related compounds are liberated by yeast during fermentation and are key aroma compounds in Sauvignon Blanc wine, lending box-tree or passion fruit aromas to the aroma profile (Swiegers, Kievit et al. 2009). Additional thiols of importance include 3-mercaptohexan-1-ol (grapefruit/gooseberry/guava), 2-mercaptoethyl acetate (roasted meat) (Lavigne, Henry et al. 1998), and 3-mercapto-3-methylbutan-1-ol which can impart an odor of cooked leeks (Dubourdieu, Tominaga et al. 2006). Two thiol-

containing compounds were recently found in Oregon Pinot Noir. Both 3-ethythio-1-propanol and 3-methylthio-1-propanol were thought to play a significant part in the aroma profile of Pinot Noir based on the aroma extract dilution analysis values (FD ≥ 64). However, these two compounds may negatively correlate with positive aroma descriptors as both compounds have cooked potato-like aromas (Fang and Qian 2005).

In addition to *S. cerevisiae*, non-*Saccharomyces* yeast strains, commonly found on grapes and in the winery, can play a significant role in commercial fermentations. These yeasts, including *Kloeckera, Hanseniaspora, Candida,* and *Hansenula* tend to dominate the initial stages of un-inoculated fermentations and are present in high numbers even in musts inoculated with commercial wine strains (Barnett, Winch et al. 1972; Fleet, Lafonlafourcade et al. 1984; Heard and Fleet 1985; Heard and Fleet 1986; Egli, Edinger et al. 1998; Romano, Caruso et al. 2003). Other non-*Saccharomyces* yeast currently being researched for use in wine include *Pichia, Torulaspora, Metschnikowia, Zygosaccharomyces*, and *Kluyveromyces* (Pardo, Garcia et al. 1989; Ciani, Beco et al. 2006; Viana, Gil et al. 2008). The dominance of these yeasts is also increased during low temperature fermentations as they are more tolerant of cold temperatures (Egli, Edinger et al. 1998). Certain species of non-*Saccharomyces* yeast can even persist until the end of fermentation, contributing significantly to the overall aroma profile of the wine (Heard and Fleet 1988; Fleet 1990). That said, most non-*Saccharomyces* yeast die off early in the fermentation (1-3days) (Perez-Nevado, Albergaria et al. 2006). Past research has attributed the early death of non-*Saccharomyces* species due to low ethanol tolerance, reduction of nutrients in the must, or toxins produced by *S. cerevisiae* (Heard and Fleet 1987; Viegas, Rosa et al.

1989; Fleet and Heard 1993; Boulton, Singleton et al. 1996; Egli, Edinger et al. 1998; Lambrechts and Pretorius 2000). However, more recent studies found that the early deaths of non-*Saccharomyces* species could not be attributed to high alcohol levels, nutrient depletion, or the presence of toxic compounds. These studies indicated that the early deaths of non-*Saccharomyces* seems to be characterized by a cell-cell mediated contact mechanism which is dependant on the concentration of viable *S. cerevisiae* cells (Nissen and Arneborg 2003; Nissen, Nielsen et al. 2003).

Although the more vigorous *S. cerevisiae* will dominate the alcoholic fermentation, non-*Saccharomyces* yeasts can still have an impact on wine aroma (Fleet and Heard 1993; Ciani and Maccarelli 1998; Jolly, Augustyn et al. 2003). For example, a limited number of non-*Saccharomyces* yeast species may form terpenes in trace quantities (Drawert and Barton 1978; Fagan, Kepner et al. 1981; Hock, Benda et al. 1984). Also, in the early stages of fermentation many non-*Saccharomyces* species have been shown to produce large amounts of either positive or negative aroma compounds (Ciani and Maccarelli 1998; Egli, Edinger et al. 1998). Negative compounds produced by many non-*Saccharomyces* species can include ethyl acetate and acetic acid (Ciani and Maccarelli 1998). Acetic acid can impart a sour-vinegar taste to wine while ethyl acetate will impart nail polish and harsh chemical aromas. However, when ethyl acetate is found in small quantities (< 50mg/L) it may add complexity to the wine (Mateos, Perez-Nevado et al. 2006). Non-*Saccharomyces* species can also produce many positive floral compounds including esters, acetate esters, and higher alcohols (Romano, Suzzi et al. 1992; Rojas, Gil et al. 2001; Rojas, Gil et al. 2003). One important acetate ester produced by non-*Saccharomyces* yeast is

2-phenylethyl acetate, which contributes floral and fruity aromas to wine and has been found in Pinot Noir (Fang and Qian 2005; Viana, Gil et al. 2008). Certain non-*Saccharomyces* yeast strains have been characterized by high glycerol production, while in general, non-*Saccharomyces* yeasts also produce less acetaldehyde than many wine strains of *S. cerevisiae* (Fleet and Heard 1993; Romano, Suzzi et al. 1997; Viana, Gil et al. 2008).

High Hydrostatic Pressure Processing (HHP) and Micro-fermentors

While the contribution of different yeast species and strains to wine aroma and flavor is well documented, past research has almost exclusively focused on white wines. This is partially due to the importance that aroma plays in white wine quality, but also due to the difficulties associated with conducting experimental red wine fermentations. Two major problems arise when conducting yeast studies during red wine fermentations. The first is the presence of naturally occurring yeast and bacteria on the grape surface. For experimental white wine production this problem is solved through the sterile filtration of grape juice. However, sterile filtering red grape must is not an option, as red wine fermentations must take place in the presence of seeds and skins. Thus, it becomes extremely difficult to draw concrete conclusions in terms of yeast and their relation to specific aroma and flavor compounds due to the influence of naturally occurring yeast and bacteria. To try to overcome this problem previous red wine research has utilized high-inoculation rates of commercial yeast strains combined with: thermal processing (Romano, Fiore et al. 2003; Clemente-Jimenez, Mingorance-Cazorla et al. 2004), rehydrated juice (Pickering, Spink et al. 2008), or large amounts

of SO_2 and other chemicals (Mateos, Perez-Nevado et al. 2006; Gonzalez, Gallo et al. 2007; Reynolds, Schlosser et al. 2007; Del Prete, Costantini et al. 2009). However, some issues may arise with thermal processing and rehydrated juice due to the detrimental effects of heat on many aroma compounds. Previous research has even shown that thermal processing can increase aroma compounds and create aromas in concentrations that do not occur naturally (Girard, Kopp et al. 1997), while Jackson (2000) noted that heating of must or wine can form volatile phenolic aldehydes from fructose. It was also shown that while some yeasts strains and species are sensitive to sulfur dioxide, others are fairly resistant (Henick-Kling, Edinger et al. 1998) and that inoculation with a commercial strain of *S. cerevisiae* does not guarantee a monoculture of the inoculated yeast strain (Heard and Fleet 1988; Fleet 1990). In addition, high concentrations of SO_2 (>50ppm) may cause organoleptic changes due to color bleaching. The binding of colored anthocyanins to SO_2 can form anthocyanin-4-bisulfite, a colorless compound (Sims and Morris 1984). Thus, new techniques for the removal of microorganisms from grape must have been investigated.

Although High Hydrostatic Pressure processing is a relatively new form of food processing, it has been shown to be an effective means of inactivating spoilage organisms. The main benefit of HHP is the ability to kill most spoilage organisms with minimal effects on flavor and aroma compounds (Ogawa, Fukuhisa et al. 1990; Ogawa, Fukuhisa et al. 1992; Knorr 1993; Takahashi, Ohta et al. 1993; Cheftel 1995; Delfini and Conterno 1995; Velazquez, Gandhi et al. 2002; Shellhammer, Aleman et al. 2003; Balasubramaniam, Farkas et al. 2008; Oey, Lille et al. 2008). The limited impact of HHP on flavor and aroma compounds is due to covalent bonds remaining

unaffected during treatment, while non-covalent bonds can be broken or changed (Cheftel 1992; Lamela and Torres 2008). Pressure effects on weaker molecular interactions such as hydrogen, ionic and hydrophobic bonds can affect larger molecular weight compounds and the higher structures of proteins and enzymes, and this can interfere with microorganism metabolism and reproduction. HHP processing has also been shown to cause damage to microorganism cell wall structure resulting in leakage during processing, as well as cause cell conformational changes (Ogawa, Fukuhisa et al. 1992; Shimada, Andou et al. 1993; Takahashi, Ohta et al. 1993; Cheftel 1995; Smelt 1998; Lopez-Caballero, Carballo et al. 1999). It is thought that a combination of these factors leads to cell death during treatment.

Although some isocratic heating can occur during processing, temperatures can be kept low to avoid any thermal or sensory changes. In a study by Shellhammer et al. (2003), HHP treated juices and a control showed no perceptible sensory differences. In other studies the aroma, flavor, and color compounds of vegetable based foods were unaffected by high pressure processing (Oey, Lille et al. 2008).

The effectiveness of HHP on spoilage microorganisms is while documented yet some spores and pathogens, especially molds and heat resistant organisms, can be resistant to HHP treatment (Ogawa, Fukuhisa et al. 1990; Ogawa, Fukuhisa et al. 1992). However, these spores and pathogens are not relevant in wine making where high sugar, acid, alcohol, and low pH and low nutrients inhibit growth. A previous study by Delfini verified the ability of HHP to microbiologically stabilize grape must (Delfini and Conterno 1995).

The second issue that arises during experimental red wine making is the presence of microorganisms on the surfaces of the fermenting vessel. During experimental white wine making the fermenting vessel can be a simple autoclaved flask or carboy with a fermentation lock. However, during red wine making the fermenting grape must needs to be in contact with the grape skins. This requires some type of cap management. An experimental red wine fermentor needs to be sterilizable and have some way of managing the cap that does not allow contamination during the fermentation. Therefore, the fermentors used in this experiment were designed to be fully autoclavable and contain an internal punch-down device. The fermentors were based on those used by Sampaio et al. (2007) and Osborne and Edwards (2006). Research by Sampaio et al. (2007) showed that although anthocyanin content throughout fermentation was significantly less than that of commercial fermentations, after eight days contents were similar with minimal variability between replicates. This past work demonstrated that micro-scale fermentors may be used in wine research.

Summary

Wine aroma is an integral part of wine quality. Any increased ability to control the aroma and flavor profile of wine would greatly benefit winemakers and could increase wine quality. Previous research in white wine has shown that yeast strain and species can affect aroma profiles and may even lead to increased quality (Esteve-Zarzoso, Gostincar et al. 2000; Mateos, Perez-Nevado et al. 2006; Nikolaou, Soufleros et al. 2006). Other research has noted that different *S. cerevisiae* starter cultures could

be used to enhance fruity characteristics, while combined *S. cerevisiae* and non-*Saccharomyces* cultures could be used to enhance flavor profiles and produce more unique wines (Lambrechts and Pretorius 2000). Therefore, the aim of this research was to investigate the influence of yeast strain and species on Pinot Noir wine aroma.

To achieve this, this study utilized HHP processing to eliminate wine-relevant microorganisms from the must while having minimal effect on must flavor and aroma composition. Sterilized, sealed micro-fermentors with an internal punch-down device were used to prevent contamination of unwanted yeast and bacteria during fermentation while allowing cap management and mixing. Wines were then analyzed by a trained sensory panel to determine differences. This research fills a gap in red-wine aroma studies by allowing for controlled inoculated fermentations, simulated commercial winemaking techniques, and analysis by both sensory and chemical methods.

The Impact of *Saccharomyces* and non-*Saccharomyces* Yeast on the Aroma and Flavor of *Vitis vinifera* L. cv. 'Pinot Noir' Wine:
Part I. High Hydrostatic Pressure processing; removal of microorganisms in grape must

David G. Takush

ABSTRACT

The efficacy of High Hydrostatic Pressure (HHP) to eliminate microorganisms in grape must prior to fermentation was investigated. Table grape must adjusted to 23°Brix was inoculated with wine-relevant yeast and bacteria at approximately 10^5cfu/mL. The must was treated for 5 and 10 minutes at 551MPa. At both 5 and 10 minutes of pressure treatment all microorganisms were reduced to below detectable numbers.

The effect of HHP treatment (prior to fermentation) on the sensory properties of Pinot Noir wine was also investigated. The study utilized autoclavable, micro-vinification vessels to conduct replicate fermentations of HHP treated and untreated grape must. All micro-fermentors were inoculated with *S. bayanus* wine yeast. As a control, one vessel containing HHP treated must was not inoculated. The fermentation profiles of all wines were similar with minimal differences between replications. The HHP treated must that was not inoculated showed no signs of fermentation during the experiment. Descriptive analysis of the wine produced from HHP treated must showed a slight increase in the fruit aroma descriptor when compared to the wine made from untreated must. There were no other significant differences between the wines in any other descriptor attributes. Chemical analysis showed no significant differences in color; however, there was a 70% increase in total phenolics in the wine produced from HHP treated must.

The results of the two experiments show that HHP processing is a viable means of removing microorganisms from grape must while minimizing any influence

on the final aroma profile of wine. The data also shows the feasibility of utilizing HHP processing in conjunction with autoclavable micro-fermentors to conduct experimental red wine fermentations without the influence of native yeast and bacteria present on grapes and wine equipment.

INTRODUCTION

The aroma and flavor of wine is influenced by many aspects of winemaking. Vineyard management, grape harvest, processing, fermentation, aging, and bottling can all have a significant effect on a wine's final aroma and flavor. However, it is the action of yeast during fermentation that contributes the majority of the total volatile aroma compounds to wine (Nykanen 1986; Rapp and Mandery 1986; Lambrechts and Pretorius 2000; Mateos, Perez-Nevado et al. 2006). Although all wine yeast contribute similar basic constituents to wine (e.g. alcohols, volatile fatty acids, esters, carbonyl compounds, volatile phenols, and sulfur compounds (Schreier 1979; Nykanen 1986; Rapp and Mandery 1986; Lambrechts and Pretorius 2000)), there are important aroma differences that may arise due to yeast strain and species. Thus, it is advantageous for a winemaker to know what aromas specific yeasts will produce, and their impact on the final character of wine.

The impact of yeast on wine aroma has been well researched and documented in white wine (Nykanen 1986; Rapp and Mandery 1986; Lambrechts and Pretorius 2000; Mateos, Perez-Nevado et al. 2006) and has been facilitated by the relative ease of sterile filtering white grape juice prior to fermentation. For example, in white wine

yeast strains can vary significantly in the production of fermentation metabolites (Dubourdieu, Tominaga et al. 2006; Loscos, Hernandez-Orte et al. 2007; Reynolds, Schlosser et al. 2007; Swiegers, Kievit et al. 2009) and can add significantly to the wine's final character. It is also interesting to note that recent research has shown yeast strains can vary significantly in their ability to release volatile thiols of pre-fermentative origin. For example, it has been reported that yeast can modify the varietal characteristics of Sauvignon Blanc (Swiegers, Kievit et al. 2009), and that selection of yeast strain could be used to modulate the aroma profile of wine to a style desired by the winemaker and predetermined by consumer preference.

Despite evidence that yeast can impact white wine aroma, there is currently a lack of research regarding the impact of yeast on red wine, especially cool climate cultivars like Pinot Noir. The reasons for this are two fold. First, is the presence of naturally occurring yeast and bacteria on grapes resulting in contamination during fermentation. The influence of native grape microorganisms on wine has been well documented (Barnett, Winch et al. 1972; Fleet, Lafonlafourcade et al. 1984; Heard and Fleet 1985; Heard and Fleet 1986; Egli, Edinger et al. 1998; Romano, Caruso et al. 2003). Native yeast and bacteria have the ability to contribute significantly to fermentation and may even be present in must until fermentation is complete (Heard and Fleet 1988; Fleet 1990). They may add either positive or negative aroma compounds in significant quantities and may even release different grape secondary metabolites from their bound odorless forms (Rosi, Vinella et al. 1994; Charoenchai, Fleet et al. 1997; Ciani and Maccarelli 1998; Egli, Edinger et al. 1998). For experimental white wine production the presence of native micro-fauna is solved by

sterile filtration of the grape juice. However, sterile filtering red grape must is not an option as red wine fermentations are required to take place in the presence of skins and seeds. Thus, it becomes extremely difficult to draw concrete conclusions in terms of yeast and their relation to specific wine aroma and flavor compounds due to the influence of naturally occurring microorganisms.

To try to overcome this problem previous red wine research has utilized high-inoculation rates of commercial yeast strains combined with thermal processing (Romano, Fiore et al. 2003; Clemente-Jimenez, Mingorance-Cazorla et al. 2004), rehydrated juice (Pickering, Spink et al. 2008), or large amounts of SO_2 and other chemicals (Mateos, Perez-Nevado et al. 2006; Gonzalez, Gallo et al. 2007; Reynolds, Schlosser et al. 2007; Del Prete, Costantini et al. 2009). These studies have tentatively shown that different yeast strains may affect red wine aroma differently. For example, different commercial yeast strains were shown to have varied capacities to reduce 3-isopropyl-2-methoxypyrazine (green-pepper aroma) content in Cabernet Sauvignon wine (Pickering, Spink et al. 2008). Yeast strains were also shown to occasionally release varying amounts of volatiles in several red grape wines including acetaldehyde and ethyl acetate (Mateos, Perez-Nevado et al. 2006).

Unfortunately, issues arise with using rehydrated juice or removing microorganisms by means of thermal processing when conducting chemical and sensory analysis. Dehydration or thermal processing may influence many of the delicate volatile compounds in wine (Girard, Kopp et al. 1997). It was also shown that while some yeasts strains and species are sensitive to sulfur dioxide, others are fairly resistant (Henick-Kling, Edinger et al. 1998) and that inoculation with a commercial

strain of *S. cerevisiae* does not guarantee a monoculture of the inoculated yeast strain (Heard and Fleet 1988; Fleet 1990). In addition, some wine studies that claimed to have used sterilized must did not confirm the effectiveness of their must sterilization techniques (Del Prete, Costantini et al. 2009), while others studies do not report any effort to sterilize must at all (Nikolaou, Soufleros et al. 2006). Thus, new techniques for the removal of microorganisms from grape must have been examined.

High Hydrostatic Pressure processing (HHP) has been investigated as a means of destroying microorganisms present on the grape must while minimizing the effect on grape aroma and flavor compounds (Ogawa, Fukuhisa et al. 1990; Oey, Lille et al. 2008). Although HHP is a relatively new form of food processing, it has been shown to be an effective means of removing spoilage organisms from food. The main benefit of HHP is the ability to kill most spoilage organisms with little effect on flavor and aroma compounds (Ogawa, Fukuhisa et al. 1990; Ogawa, Fukuhisa et al. 1992; Knorr 1993; Takahashi, Ohta et al. 1993; Cheftel 1995; Delfini and Conterno 1995; Velazquez, Gandhi et al. 2002; Shellhammer, Aleman et al. 2003; Balasubramaniam, Farkas et al. 2008; Oey, Lille et al. 2008). It has been suggested that the limited impact of HHP on flavor and aroma compounds is due to covalent bonds remaining unaffected during processing. (Cheftel 1992; Lamela and Torres 2008).

During HHP processing, it is thought that the disruption or alteration of hydrogen or ionic bonds can impact larger molecular weight compounds and the higher structures of proteins and enzymes. This can interfere with microorganism metabolism and reproduction. It has also been shown that HHP causes damage to cell wall structure resulting in leakage during processing, as well as protein and cell

conformational changes (Ogawa, Fukuhisa et al. 1992; Shimada, Andou et al. 1993; Takahashi, Ohta et al. 1993; Cheftel 1995; Smelt 1998; Lopez-Caballero, Carballo et al. 1999). It is thought that a combination of these factors leads to cell death during treatment.

Although some isocratic heating can occur during HHP processing, temperatures can be kept low as to avoid any thermal or sensory changes. In a study by Shellhammer et al. (2003), HHP treated juices and untreated controls showed no perceptible sensory differences. In other studies, the aroma, flavor, and color compounds of vegetable based foods were unaffected by HHP processing (Oey, Lille et al. 2008). Some spores and pathogens, especially molds and heat resistant organisms, can be resistant to HHP. However, they are not relevant in wine making where high sugar, acid, alcohol, and low pH and low nutrients inhibit growth. As evidence, a previous study by Delfini verified the ability of HHP to microbiologically stabilize grape must (Delfini and Conterno 1995).

The second issue that arises during experimental red wine making is the presence of microorganisms on the surfaces of the fermenting vessel. During experimental white wine making the fermenting vessel can be a simple autoclaved flask or carboy with a fermentation lock. However, during red wine making the fermenting grape must needs to be in contact with the grape skins. This requires some type of cap management. An experimental red wine fermentor needs to be sterilizable and have some way of managing cap formation that does not allow contamination during fermentation. Therefore, sealed, autoclavable micro-vinification vessels, with

internal punch-down devices, have been developed in order to conduct replicated fermentations with the ability to manage cap formation.

The goal of this research was to assess the effectiveness of HHP as a means of removing microorganisms from grape must prior to fermentation, to investigate the effects on wine aroma, and to explore the viability of using HHP in conjunction with autoclavable micro-fermentors as a means of conducting future red wine research.

METHODS AND MATERIALS

High Pressure Processing: table grape trial

Grapes

Grapes used were Chilean red grapes purchased at Fred Myer, Corvallis, Oregon, USA. pH analysis was performed using a Mettler-Toledo Delta 320 pH meter (Shanghai, China). Titratable acidity (TA) was analyzed by titration with 0.1N NaOH and recorded as g/100mL tartaric acid. °Brix analysis was performed with an Anton-Paar DMA 35N Density Meter (Graz, Austria). The grapes were de-stemmed manually and crushed with a hand-powered crusher. Soluble solids were increased from 16.8 °Brix to 23°Brix with the addition of 50g/L glucose in order to simulate sugar content in wine grape must. Must TA was 0.675g/L (as tartaric acid) with a pH of 3.36. After inoculation the must was separated into four 1L aliquots and vacuum-sealed in Food Saver® bags (Jarden Corp., Boca Raton, Florida, USA).

Yeast and Bacteria

An active dry form of *S. bayanus* strain EC1118 (Lallemand, Montréal, Canada) was obtained. *Acetobacter aceti, Lactobacillus hilgardii and Brettanomyces bruxellensis* were obtained from Washington State University, Pullman, WA, USA and provided by Dr. Charles Edwards. The yeast were maintained on potato dextrose agar (Difco™, Franklin Lakes, NJ, USA) slants while the bacteria were maintained in de Man, Rogosa, and Sharpe (MRS) stabs (20g/L Tryptone, 5g/L peptone, 5g/L yeast extract, 5g/L glucose, 200mL apple juice, 1mL Tween 80 [5% w/w solution], 20 g/L agar, pH 4.5) and stored at 4°C.

Culture Preparation

Yeast were transferred from slants to YPD broth (10g/L yeast extract, 20g/L peptone, 20g/L dextrose, pH 7.0) and grown aerobically at 30°C for 48 hours. Bacterial cultures were transferred from stabs to MRS broth and grown aerobically at 30°C for five days. The cells were harvested by centrifugation at 4200rpm for 12 minutes, rinsed with 0.1% peptone solution, and centrifuged a second time. The cultures were decanted and mixed with 0.1% peptone. All microorganisms were inoculated into the grape must at approximately 10^5cfu/mL. After inoculation the must was separated into 1L aliquots and vacuum-sealed in Food Saver® bags.

HHP Processing

Two of the aliquots were HHP processed for 5 minutes at 551MPa (80,000psi) while two more replicates were processed for 10 minutes at 551MPa. The HHP unit

was custom made by the National Forge Company (Irvine, Pennsylvania) with a 22L maximum capacity and a 689MPa (100,000psi) maximum pressure. The high pressure intensifier pump had a maximum capacity of 620MPa (91,374psi) and was made by Flow International Corporation (model 7XS-6000, Kent, Washington).

Enumeration

Viable microbial populations were determined using diluents containing 0.1% peptone and plating using appropriate media. Yeast were grown on YPD agar while bacteria were enumerated using de Man, Rogosa, and Sharpe (MRSC) agar (20g/L Tryptone, 5g/L peptone, 5g/L yeast extract, 5g/L glucose, 200mL apple juice, 1mL Tween 80 [5% w/w solution], 20 g/L agar, pH 4.5, 100ppm cyclohexamide). Plates were incubated aerobically at 25°C for 48 hours (yeast) or 7 days (bacteria) prior to counting.

Pinot Noir: HHP and sensory trial

Grapes

The grapes used for this research were self-rooted *Vitis vinifera* L. cv. 'Pinot Noir'. The Pinot Noir clone was *Pommard*, and all grapes were harvested from the same block at Oregon State University's Woodhall Vineyard (Alpine, Oregon, USA) on October 15th, 2008. The harvest time was based on sugar levels and ripeness as determined by the vineyard manager. The grapes were stored at 2°C before being hand-sorted, crushed, and destemmed. The crusher/destemmer used was a Velo DPC 40 (Altivole, Italy). The grapes were pooled and divided into seven 2.75kg aliquots.

30mg/L SO_2 (in the form of potassium metabisulfite) was added to each aliquot. Each aliquot was vacuum-sealed in a Food Saver® bag and four bags were randomly selected and loaded into the HHP unit with ice. The remaining three bags were not HHP processed and were instead stored on ice until processing had finished.

HHP

The grapes were treated for 10 minutes at 551MPa. The grapes were 15°C (59°F) at the beginning of processing and 11°C (52°F) at the end of processing.

Micro-fermentors

The micro-vinification vessels were based on a design by Sampaio (2007) and Osborne and Edwards (2006). The base of the fermentors were fabricated from autoclave safe glass by Q Glass (Towaco, New Jersey, USA) with a 4L maximum capacity and were approximately 290mm tall with a diameter of 142mm. The lids were custom cut polycarbonate disks manufactured by Ridout Plastics (San Diego, California, USA), and the ring-gaskets were high-temperature, food-grade silicone (Applied Industrial Technologies, Cleveland, Ohio, USA). All bungs and airlocks used were also high-temperature, food-grade silicone (Vin Table, Ambler, Pennsylvania, USA), and the sample ports were constructed from food grade stainless steel tubing. The fermentors were equipped with a perforated polycarbonate punch-down (Ridout Plastics) attached by a stainless steel plunger (see Figure 2.1). The micro-fermentors were autoclaved at 120°C for 30 minutes at 15psi and allowed to cool prior to use.

Each aliquot of both processed and unprocessed grapes were loaded into the autoclaved fermentors under a laminar flow hood using aseptic technique.

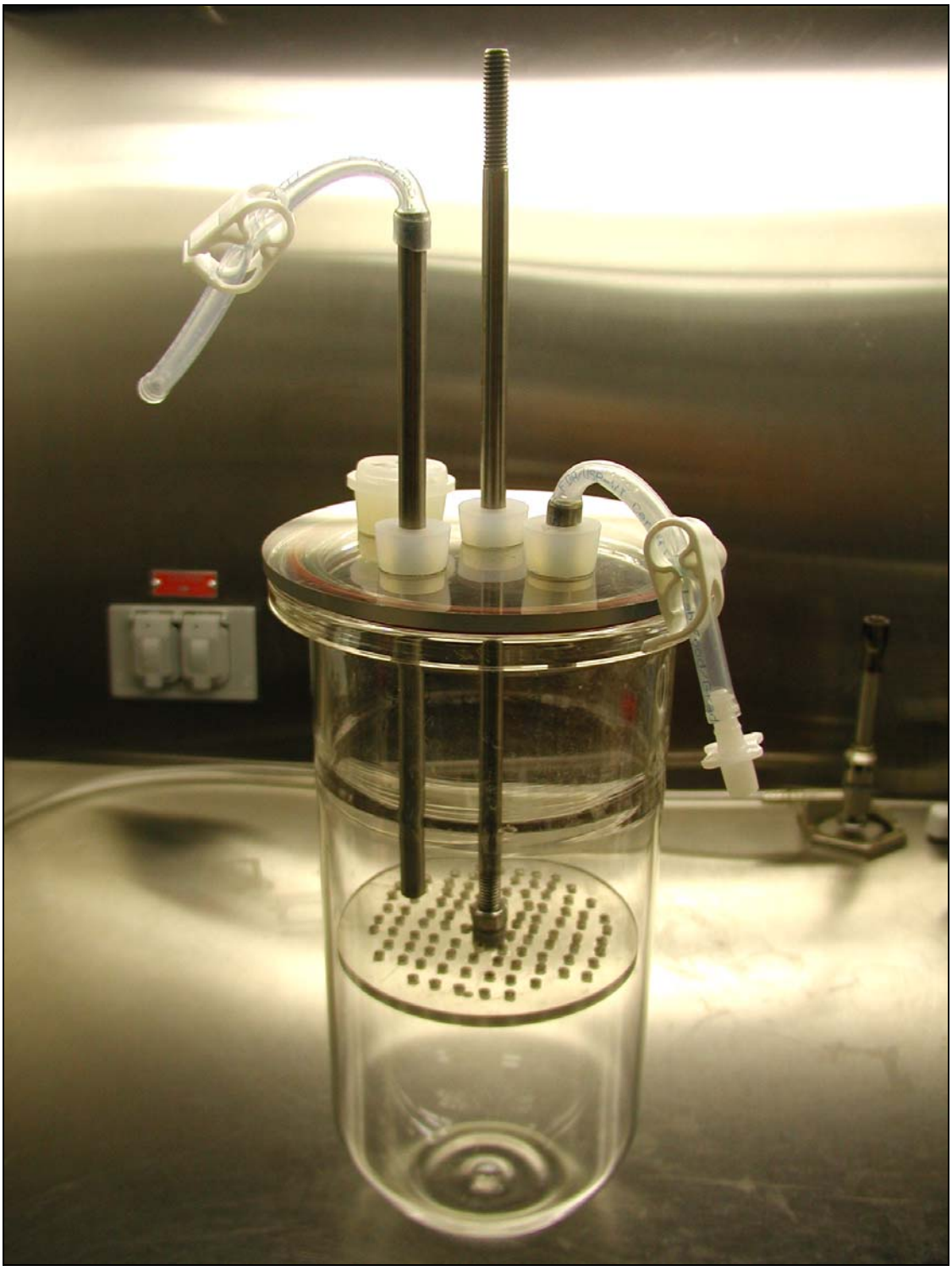

Figure 2.1: Autoclavable red wine micro-fermentor with internal punch-down

Yeast strain

An active-dry form of *Saccharomyces cerevisiae* MERIT.ferm (Chr. Hansen, Hørsholm, Denmark) was obtained. The yeast was maintained on potato dextrose agar (Difco, Franklin Lakes, NJ, USA) slants stored at 4°C.

Culture Preparation

A single isolated colony was transferred to YPD broth and grown aerobically at 30°C for 48 hours. The cells were harvested by centrifugation at 4200rpm for 12 minutes, rinsed with 0.1% peptone solution, and centrifuged a second time. The cultures were decanted then mixed with 0.1% peptone. Yeast were inoculated into each vessel at approximately 10^6cfu/mL at room temperature. In addition, an un-inoculated control was also prepared.

Fermentations

Fermentations were conducted in triplicate in a temperature-controlled room at 26.6°C (80°F). The cap punch-down device was pushed halfway down so that the grape skins were fully submerged during the fermentation. °Brix analysis was performed with an Anton-Paar DMA 35N Density Meter (Graz, Austria) and was monitored throughout the fermentation. Viable cell populations were also monitored throughout the fermentation using diluents containing 0.1% peptone and using appropriate media. Samples were taken using autoclaved pipettes after mixing with an internal punch-down. Yeast cells were grown on YPD agar while bacteria were enumerated using MRSC agar. Non-*Saccharomyces* populations were recorded by

plating onto Lysine media (Difco™, Franklin Lakes, NJ, USA), which is selective for non-*Saccharomyces* species. *Saccharomyces* and non-*Saccharomyces* populations were also enumerated on a differential WL media (Difco™, Franklin Lakes, NJ, USA). Plates were incubated aerobically at 30°C for 48h (yeast) or 7 days (bacteria) prior to counting. When all fermentations had finished (all replicates at 0.0°Brix for 4+ days), wines were pressed using a small basket press modified with a pressure gauge to apply constant pressure at 15psi for 1min. One minute was sufficient time to allow for all wine to press out. For each treatment the replicates were confirmed by the investigators to have no sensory differences and were blended together for sensory analysis. Prior to this, samples from each replicate were taken for chemical analysis. Wines were settled at 4°C for 48hrs before filtration and bottling. Bottling of each of the five wines was conducted under a laminar flow hood using a 0.45µm cartridge filter (Pall Corp., East Hills, New York). The wine was bottled into 375mL green wine bottles flushed with sterile nitrogen and sealed with a crown cap. Bottles were stored for four months at 12.8°C.

Color and Total Phenolics

Color was determined by spectrophotometric analysis (Thermo Scientific Genesys 10uv Spectrophotometer, Madison, Wisconsin, USA) at 420 and 520nm in 1mm pathlength cuvettes after pH adjustment to 3.60. Total phenolics were determined using the Folin-Ciocalteu (FC) assay. Color and total phenolic statistical analysis was performed using Minitab® 15 Statistical Software (State College, Pennsylvania, USA). Univariate Analysis of Variance (ANOVA) was used to

determine differences between treatment and control wines. Significant differences detected by ANOVA were subjected to post-hoc Tukey HSD multiple comparison at 0.05% significance levels.

Sensory Analysis

Panel Training

Fourteen panelists were recruited by email (see Appendix A) from the Oregon State University Food Science Department and from the Corvallis, OR community. Two panelists were absent during testing. Twelve panelists tested the wines produced from HHP treated must and untreated must. The panelists included five men and seven women who all were familiar with wine description or formal sensory analysis. The panelists were trained twice a week for three weeks during six hour-long sessions. During training sensory standards were used as described by Noble (1987), along with aroma intensity standards (see Appendix B). The aroma standards were presented in glass jars with plastic screw-on lids. At the beginning of training and testing sessions, panelists were encouraged to re-familiarize themselves with these aroma standards. Panelists were exposed to several different young Pinot Noir wines similar to the test wines, along with the actual test wines during training. Open discussion after sample evaluation allowed panelists to re-familiarize themselves with typical Pinot Noir descriptors, come to a consensus on descriptors, and as a group select appropriate descriptor categories for the ballot as guided by the investigators. The final evaluation ballot contained sixteen descriptors and utilized a sixteen-point intensity scale where the intensity of each descriptor was rated by: 0=none, 3=slight, 7=moderate, 11=large,

and 15=extreme (see Appendix B). Panelist scores from trial sessions were recorded on a community board and discussed to help judges correctly identify both category descriptor and intensity ratings in wines. Training was conducted until group discussions and recorded scores revealed panelist were in agreement on terms and intensity ratings. After final testing, two more panelists were dropped from the data. Examination of the data showed the removed panelists to be inconsistent with the rest of the panel in the use of some descriptor terms.

Training and Testing Area

During training and testing, the panelists were seated as a group at stainless steel food preparation benches. Panelists used paper ballots for training and the resulting ratings were entered on a board for group discussion. Testing was performed in the same area using the same paper ballots. Panelists were given access to water and a 0.1g/L pectin rinse solution during training and testing sessions.

Sample Preparation and Serving Procedures

The wines used were kept at 12.8°C until the day before testing. All wines were allowed to equilibrate to room temperature and poured 20 minutes before evaluation. 25mL samples were served in 240mL INOVA tulip glasses (St. George Crystal Ltd., Jeannette, Pennsylvania, USA) and covered with a plastic lid. A Balanced Complete Randomized Block Design (BCRBD) was used in this study. Over the testing sessions, each panelist tasted each wine treatment two times in random order. Panelist was treated as a blocking factor in the design. Each wine sample was

presented in each serving position equally and randomly across the panelists to minimize an order effect. Panelists were allowed three minutes to evaluate each wine and were given a mandatory one-minute break between samples.

Sensory Statistics

A univariate Analysis of Variance (ANOVA) was used to determine differences between the wine samples for each descriptor. The ANOVA was performed by General Linear Model (GLM) in SAS® release 9.1 (Cary, North Carolina, USA). The ANOVA model comprised two main effects (Panelist (PAN) and Wine (WINE)), a nested effect (Replication which was nested in PAN or (REP(PAN)) and a two-way interaction effect between PAN and WINE (WINE*PAN). The PAN, REP(PAN) and (WINE*PAN) were treated as random effects and WINE was treated as a fixed effect. Significant differences detected by ANOVA were subjected to post-hoc Tukey HSD multiple comparison to test least squares means of WINE (means) at the 0.05% significance level.

RESULTS

An initial study using Chilean table grapes investigated the ability of HHP to kill wine microorganisms. Before HHP treatment large populations of microorganisms were present in the grape must (Table 2.1). Counts on MRSC, YPD, and Lysine agar before HHP treatment were greater than 10^5cfu/mL. However, after both 5 and 10 minutes of HHP treatment, no growth was observed when samples were plated and

incubated. Samples were taken directly after HHP treatment. An example of growth on YPD from samples taken before and after HHP treatment is shown in Figure 2.2.

Table 2.1: Microorganism counts[1] (cfu/mL) in Chilean table grape must adjusted to 23°Brix, before and after HHP treatment.

		HHP TREATMENT @551MPa	
Media	BEFORE HHP TREATMENT	5 min	10 min
MRSC	$>10^5$	$<10^2$	$<10^2$
YPD	$>10^5$	$<10^2$	$<10^2$
LYS	$>10^5$	$<10^2$	$<10^2$

[1]values are means of triplicates

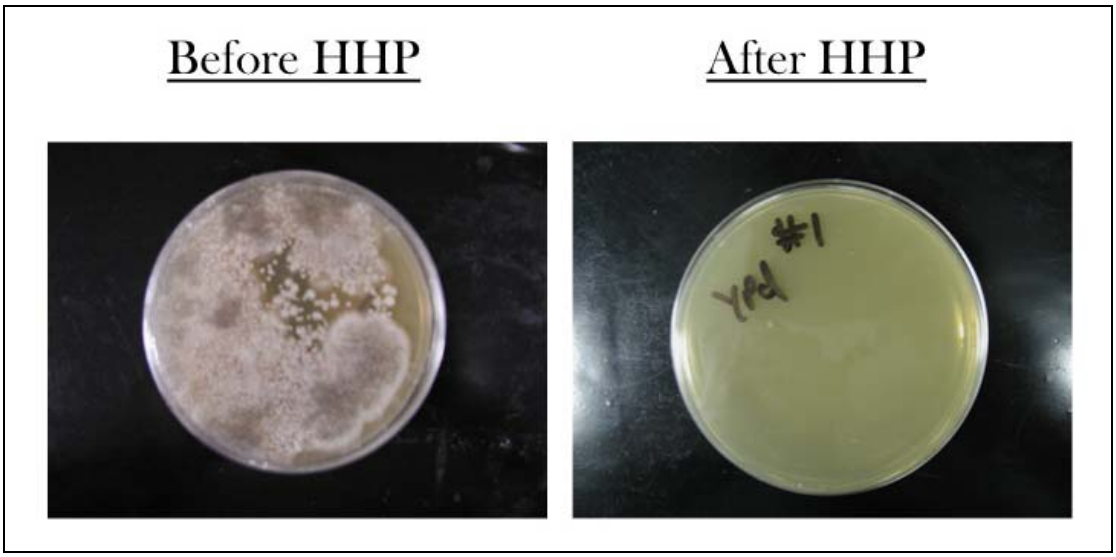

Figure 2.2: Microorganism growth on YPD plates before and after HHP treatment

Because HHP treatment appeared to be effective at eliminating wine microorganisms in grape must, an experiment investigating HHP treatment of Pinot

Noir grapes was conducted. In addition, wine was made from grapes that had received and had not received HHP treatment. HHP treatment of the Pinot Noir grape must did not affect the pH, TA, or °Brix (data not shown). Cell counts of samples taken before and after HHP indicated that HHP eliminated yeast and bacteria to below detectable limits (Table 2.2). Cell counts of Pinot Noir grape must that was not treated by HHP indicated $>10^5$cfu/mL for bacteria and yeast (Table 2.2).

Table 2.2: Microorganism counts[1] (cfu/mL) of Pinot Noir grape must treated and not-treated with HHP processing.

	No HHP	HHP 10min @ 551MPa
MRSC	$9.1x10^3$	$<10^2$
YPD	$5.8x10^5$	$<10^2$
Lysine	$4.7x10^5$	$<10^2$

[1]values are means of triplicates

During alcoholic fermentation, *S. cerevisiae* grew well in both HHP and non-HHP treated musts with maximum populations of $1.0x10^9$cfu/mL reached during fermentation (Figure 2.3). In the non-HHP treated wine, non-*Saccharomyces* populations initially increased to close to $1x10^8$cfu/mL before rapidly decreasing to below detectable limits by 150 hours into the fermentation (Fig 2.3). Fermentation curves for both HHP and non-HHP treated musts were almost identical with °Brix levels reaching zero 144 hours after inoculation (Figure 2.4). In addition, the HHP treated, un-inoculated control contained less than detectable amounts of microorganisms on MRSC, YPD, and Lysine agar (data not shown). Furthermore, the

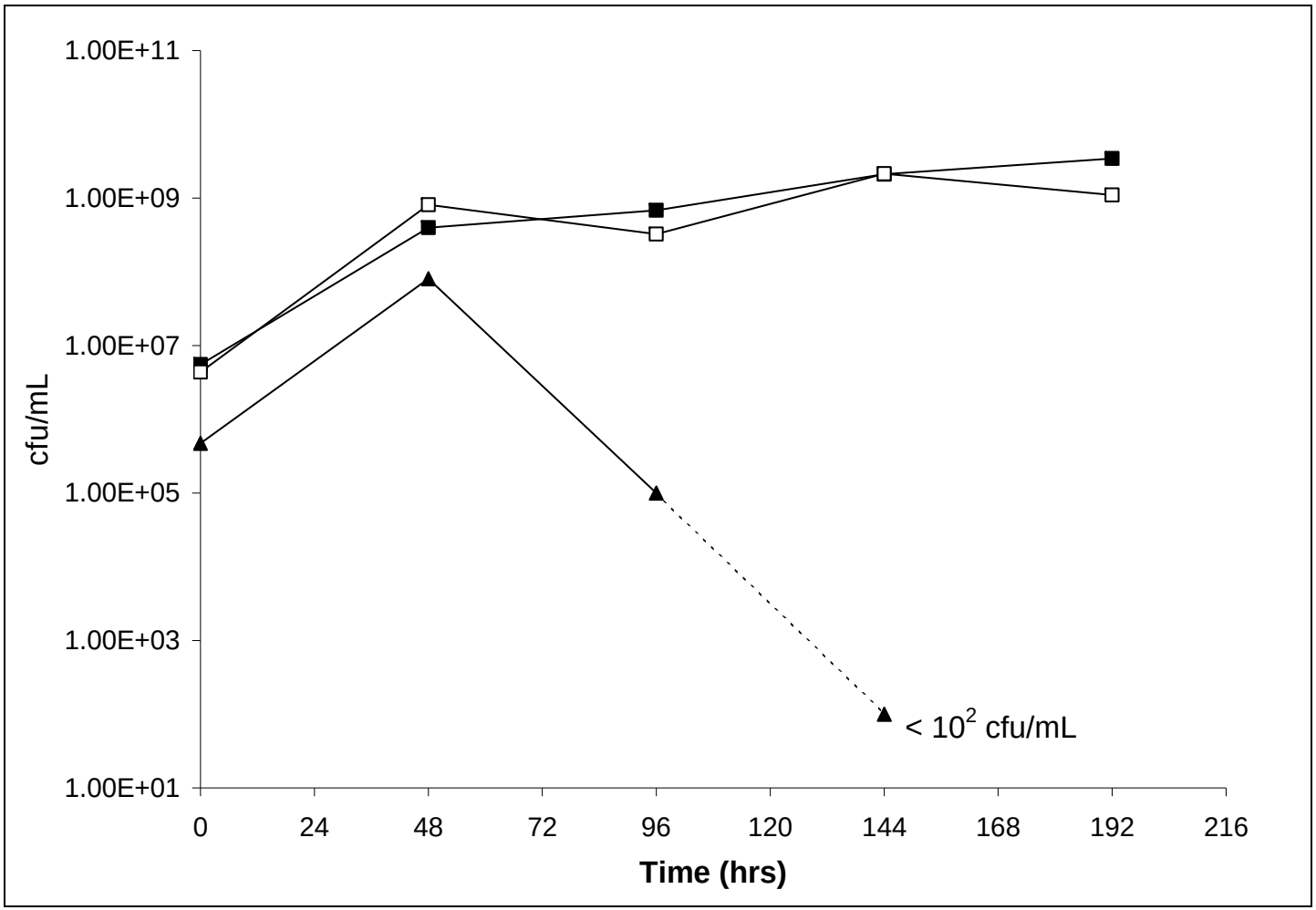

Figure 2.3: Yeast viable cell counts during alcoholic fermentation of: (■) HHP treated grape must, (□) Non-HHP treated grape must, by *S. bayanus* EC1118, and (▲) Non-*Saccharomyces* population in non-HHP treated grape must.

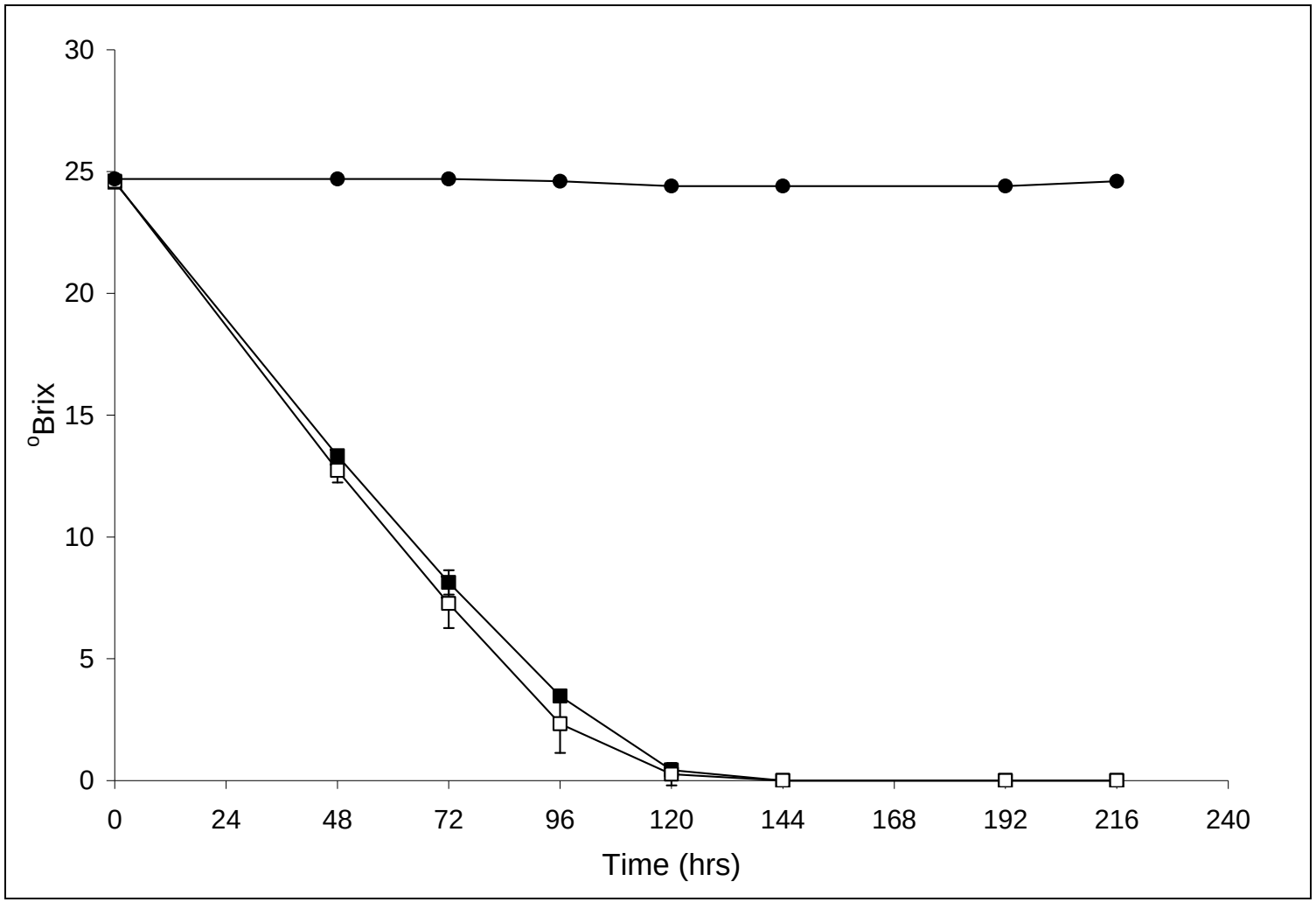

Figure 2.4: °Brix during alcoholic fermentation conducted by *S. bayanus* EC1118 of: (■) HHP treated grape must, (□) Non-HHP treated grape must, and (●) HHP treated un-inoculated control.

un-inoculated must did not undergo any fermentation during the experimental process as °Brix levels remained constant (see Figure 2.3).

After pressing and filtration, wine color was similar between wines produced with or without HHP treatment with no significant differences at 520nm or in hue (420nm/520nm) (see Table 2.3). However, total phenolic levels in the wines were significantly different (see Table 2.3). The HHP treated wine contained 2759.52mg/L (GAE), a 70% increase in total phenolic levels when compared to wine made from untreated grapes (1614.41mg/L [GAE]).

Table 2.3: Color, hue, and total phenolic levels[1] of wine made with HHP treated and non-HHP treated grapes; values with different letters within a column are significantly different at the $p<0.01$ level.

	Color (absorbance @ 520nm)	Hue (420nm/520nm)	Total Phenolics (mg/L Gallic acid)
No HHP (Control)	4.60^{a} (+/- 0.32)	0.60^{a} (+/- 0.00)	1614.41^{a} (+/- 9.41)
HHP Processed	5.34^{a} (+/- 0.35)	0.56^{a} (+/- 0.04)	2759.52^{b} (+/- 1.77)

[1]values are means of triplicates

HHP effect on sensory characteristics

Wines produced from HHP treated or non-HHP treated grapes were analyzed by descriptive sensory analysis. When wine made with HHP treated and non-HHP treated must were tested, descriptive analysis by ten trained panelists showed a slight increase in overall fruit aroma for wine made from HHP treated must. No other significant flavor or aroma differences were found (see Table 2.4). For example, overall aroma and flavor intensity values for both wines were quite close, and the

mean value of aged flavors in both wines were exactly the same (see Table 2.5). In addition, no significant differences were found between the wines for secondary aroma descriptors such as citrus fruit, red fruit, dark fruit, and jammy fruit aromas.

Table 2.4: Analysis of variance F-ratio attribute ratings (10 judges) for wines produced from HHP treated and non-HHP treated grape must; (*) indicates significance at the $p<0.05$ level, (**) indicates significance at the $p<0.01$ level; (***) indicates significance at the $p<0.001$ level.

Descriptors	Treatment	Panelist	Rep (Panelist)	Panelist x Treatment
Overall Aroma	0.53	2.94	1.00	1.15
Overall Floral Aroma	1.29	2.19	1.30	0.57
Overall Fruity Aroma	6.61*	0.86	10.47*	0.73
Citrus Aroma	0.31	4.30	2.18	0.57
Red Fruit Aroma	0.02	62.36	0.62	0.43
Dark Fruit Aroma	0.74	2.68	1.68	0.52
Jammy/cooked Fruit	0.44	3.26	4.54*	1.38
Overall Spicy-Earthy	1.76	13.10	0.74	0.33
Overall Aged	2.83	9.22	0.59	0.79
Musty/chalky	0.45	2.42	1.16	1.06
Overall Flavor	0.51	47.50	0.33	0.75
Fruit Flavor	0.27	53.65	0.35	0.78
Herb Spice Flavor	1.21	1.89	1.21	1.39
Aged Flavor	0.00	0.92	3.00*	8.67*
Sour	4.18	8.17	0.94	0.80
Astringent	3.66	4.80*	1.00	1.70
Degrees of Freedom	4	13	28	52

Table 2.5: Sensory description values[1] for wine made from HHP treated and non-HHP treated grapes; values with different letters within a row are significantly different; (*) indicates significance at the $p<0.05$ level.

Descriptor	HHP 10min@ 551MPa	*(SD)*	No HHP	*(SD)*
Overall Aroma Intensity	8.9[a]	*(1.2)*	8.6[a]	*(1.4)*
Overall Floral Aroma	5.5[a]	*(0.9)*	5.1[a]	*(1.8)*
Overall Fruity Aroma*	6.8[a]	*(1.9)*	6.2[b]	*(2.0)*
Citrus Aroma	1.7[a]	*(1.7)*	1.5[a]	*(2.1)*
Red Fruit Aroma	4.4[a]	*(2.2)*	4.3[a]	*(1.6)*
Dark Fruit Aroma	4.4[a]	*(1.7)*	4.2[a]	*(1.5)*
Jammy/Cooked Fruit Aroma	4.2[a]	*(2.0)*	4.4[a]	*(1.8)*
Spicy/Earthy Aroma	5.0[a]	*(1.1)*	4.7[a]	*(1.1)*
Aged Aroma	4.6[a]	*(1.8)*	4.1[a]	*(1.4)*
Musty Aroma	1.8[a]	*(1.5)*	1.5[a]	*(1.8)*
Overall Flavor Intensity	9.1[a]	*(1.3)*	9.3[a]	*(1.2)*
Fruity Flavor	7.4[a]	*(1.5)*	7.6[a]	*(1.5)*
Spicy Flavor	5.9[a]	*(0.9)*	5.5[a]	*(1.5)*
Aged Flavor	5.1[a]	*(1.3)*	5.1[a]	*(1.0)*
Sour	5.9[a]	*(2.1)*	5.1[a]	*(1.6)*
Astringency	8.3[a]	*(2.0)*	7.4[a]	*(1.8)*

[1]values are means of duplicates

DISCUSSION

HHP effect on wine microorganisms

The results from the table grape study clearly show the ability of HHP treatment to eliminate microorganisms from grape must, even when microorganisms are present in concentrations over 10^5cfu/mL. In this study *S. bayanus, A. aceti, Lb. hilgardii, and B. bruxellensis*, were all inoculated into the grape must. However, after only five minutes of processing at 551MPa, no microorganisms were detected in the treated must. In previous HHP studies conducted on citrus juice, yeast, bacteria, and

mold vegetative cells were destroyed to below detectable limits with 5 min of treatment at 400MPa (Ogawa, Fukuhisa et al. 1990; Ogawa, Fukuhisa et al. 1992). One study even showed the effectiveness of HHP at 400MPa at sugar concentrations of 25°Brix (Takahashi, Ohta et al. 1993). These studies also showed the effectiveness of HHP processing on both *S. cerevisiae* and non-*Saccharomyces* yeasts including *Hansenula* spp., *Pichia* spp., and *Candida* spp. at concentrations between 10^4 and 10^6cfu/mL. Similar results were found using 10 minutes of processing time. The effectiveness of HHP processing, specifically on grape must, is further supported in this study by elimination of microorganisms to less than significant numbers in Pinot Noir must (Table 2.2). In addition, the data from this study is in agreement with previous research performed by Delfini (1995), which showed HHP processing can be used as a viable means of removing vegetative microorganisms from grape must.

Micro-fermentors

The fermentation of HHP of non-HHP treated grapes was performed in micro-vinification vessels based on a design by Sampaio (2007) and Osborne and Edwards (2006). The fermentors were easy to autoclave as a whole, sealed unit, and were large enough to produce wine in sufficient quantity for chemical and sensory analysis. Variability was minimal between replicates for chemical parameters further supporting research by Sampaio (2007), justifying the application of micro-fermentors in wine research. However, vigorous mixing applied to the fermentors before sampling may have contributed to the wines having high total phenolic concentrations. Future

research should investigate less rigorous cap management in order to more accurately replicate phenolic extraction in commercial fermentations.

HHP effect on wine sensory profile

Although large differences in total phenolic content were observed, only minimal sensory differences were found between the wines made from HHP processed and unprocessed grapes. While a slight increase in fruit aroma was recorded, it is interesting to note that of the secondary descriptors (citrus fruit, red fruit, dark fruit, jammy fruit), none were significantly different between wines. In addition, no other sensory descriptors showed any significant differences between the wines. The minimal differences seen between the two wines are likely due to the fact that many of the primary flavor and aroma compounds in wine are produced or released during fermentation (Nykanen 1986; Rapp and Mandery 1986; Lambrechts and Pretorius 2000; Mateos, Perez-Nevado et al. 2006), which in this study occurred after HHP processing. Furthermore, grape derived aroma and flavor compounds are relatively unaffected by HHP processing. It has been indicated in research literature that HHP processing only affects the higher structures of molecules that are held together by ionic bonds or van de waal forces, and does not effect covalent bonds (Cheftel 1992; Balasubramaniam and Farkas 2008; Balasubramaniam, Farkas et al. 2008; Oey, Lille et al. 2008; Yaldagard, Mortazavi et al. 2008). Thus, aroma and flavor compounds in grapes, such as norisoprenoids, terpenes, and esters, remain relatively unaffected. Using GC analysis, previous studies have shown that HHP processing (400MPa, 10min) does not affect the volatile profile of fruit juice (Takahashi, Ohta et al. 1993).

For example, Takahashi et al. (1993) showed that two important volatile compounds found in wine, linalool and α-terpineol, did not significantly change during HHP treatment of juice.

Many other studies have shown no significant sensory differences between HHP treated juice and fresh untreated products (Ogawa, Fukuhisa et al. 1990; Ogawa, Fukuhisa et al. 1992; Knorr 1993; Takahashi, Ohta et al. 1993; Cheftel 1995; Delfini and Conterno 1995; Velazquez, Gandhi et al. 2002; Shellhammer, Aleman et al. 2003; Balasubramaniam, Farkas et al. 2008; Oey, Lille et al. 2008). A recent study in 2006 using a trained sensory panel showed no sensory differences between red wine treated at 350MPa for 10min and a control (Mok, Song et al. 2006). The results from the present study, along with previous HHP research, are strong indicators that HHP processing does not affect final wine aroma or flavor.

The panelists used in this study were trained in aroma evaluation; however, no formal training was conducted for sourness or astringent mouthfeel descriptors. Evidence of incomplete astringency training might be indicated by the lack of significant differences between the HHP treated and non-HHP treated wines. The two wines contained extremely different amounts of phenolic material, yet sensory analysis showed no significant differences. A difference in astringency between the wines would be expected given the chemical analysis. However, while phenolic levels in wine have been associated with astringency (Arnold and Noble 1978), human perception of astringency is complicated and correlations between chemical and sensory analysis can be difficult.

The increase in total phenol content in the wine made from HHP processed grapes could be due to cell wall breakdown (Balasubramaniam and Farkas 2008; Oey, Lille et al. 2008), which was observed in HHP treated spinach and cauliflower (Prestamo and Arroyo 1998). In previous studies, HHP has also been shown to effect particle size distribution in juice (Takahashi, Ohta et al. 1993). Smaller particle size and increased berry cell degradation can lead to higher phenol content in wine (Salinas, Garijo et al. 2003). However, HHP processing has not been shown to effect physiochemical properties such as sugar, TA, and pH, and this has been well established in literature (Ogawa, Fukuhisa et al. 1992; Takahashi, Ohta et al. 1993; Mok, Song et al. 2006).

The minimal sensory differences found in this study are in spite of the fact that during fermentation, the non-HHP treated must grew a large population of at least one species of non-*Saccharomyces* yeast. This growth may be one reason the wine made from non-HHP treated must had a slightly reduced overall fruity aroma, as non-*Saccharomyces* yeast are known to produce negative aroma compounds which may mask some positive aroma attributes. However, it would appear that the growth of resident non-*Saccharomyces* yeast did not have a large impact on the aroma and flavor profile of the wine (see Table 2.5). Any other changes in the wine profile due to non-*Saccharomyces* yeast growth may have been overpowered by the inoculated *Saccharomyces* yeast, or may have been too subtle for the sensory panel to discern. In addition, complexity and mouth-feel are two aspects of wine that were not specifically tested in this study and could have been affected native yeast populations.

HHP processing technology is currently used throughout the food-processing industry to provide fresh-tasting products with improved shelf-life, and is even used to create some novel products such as ‘fresh’ guacamole and shell-shucked oysters. Future studies should investigate the use of HHP in winemaking to help create novel vinification methods including extended cold-soaks, fast or high extraction fermentations, or long-term storage of grape must for post-harvest fermentations. HHP processing in conjunction with sterile micro-vinification vessels will be a valuable tool in future enology studies. The impact of yeast strain and the effects of co-inoculation can be studied in red wine without the influence of native yeast or bacteria, allowing for accurate profiling of yeast strains and their influence, singularly or combined, on red wine aroma, flavor, color, and physiochemical properties.

Conclusions

This study has shown that HHP is an effective and viable means for the inactivation of microorganisms from grape must prior to fermentation. HHP in conjunction with sterile micro-fermentors can be an extremely useful tool in future red wine research due to minimal physiochemical changes to grape must and wine aroma and flavor profiles. This technique could be directly applied to future red wine research examining yeast affect on red wine aroma, color, and the effects of co-inoculation of different yeast strains and species.

The Impact of *Saccharomyces* and non-*Saccharomyces* Yeast on the Aroma and Flavor of *Vitis vinifera* L. cv. 'Pinot Noir' Wine: Part II. The effect of yeast strain on Pinot Noir aroma and flavor

David G. Takush

ABSTRACT

The impact of yeast strain and species on the aroma and flavor of *Vitis vinifera* L. cv. 'Pinot Noir' wine was investigated. High Hydrostatic Pressure (HHP) treated grape must in conjunction with autoclavable micro-fermentors were used in order to conduct fermentations of inoculated yeast strains without the influence of native yeast and bacteria found on grapes and wine equipment. The yeast strains and species studied were EC1118, RC212, Assmanshausen (AMH) (Lallemand, Montréal, Canada), MERIT.ferm, and Symphony (a blend of MERIT.ferm and *Kluyveromyces thermotolerans*) (Chr. Hansen, Hørsholm, Denmark). All *Saccharomyces* yeast strains were inoculated at approximately 10^6cfu/mL while the non-*Saccharomyces* yeast was inoculated at approximately 10^5cfu/mL. Fermentation profiles were similar between yeast strains while chemical analysis showed a significant difference in color at 520nm between the AMH and EC1118 wines. Descriptive analysis was conducted using a trained panel of judges who were familiar with formal wine or sensory analysis. Results of descriptive sensory analysis indicate that yeast strain can have a significant effect on the sensory profile of Pinot Noir wine. Significant sensory attributes included overall fruity aroma, red fruit aroma, dark fruit aroma, and overall fruit flavor, among others. Principle Component Analysis results show EC1118 and RC212 trending towards high overall aroma intensities and dark fruit and jammy characteristics. MERIT.ferm also produced wines that with high aroma intensities; however, they trended towards red fruit and floral characteristics. The wines produced

from AMH and Symphony yeasts were not distinct and resulted in significantly lower intensities in several aroma descriptor categories.

INTRODUCTION

Wine aroma is one of the most important aspects of wine quality, and it has been shown that yeast contribute a significant amount, if not the majority, of volatile aroma compounds in wine (Nykanen 1986; Rapp and Mandery 1986; Lambrechts and Pretorius 2000; Mateos, Perez-Nevado et al. 2006). As such, it is important for the winemaker to understand the influence of specific yeast strains and species in wine. There are many factors by which a winemaker can base yeast selection. These criteria may include low volatile acidity and sulfide production, completeness of fermentation, temperature tolerance, alcohol and sulfite tolerance, glycerol production, enzyme activity, nutritional requirements, and killer factors (Zoecklein, Fugelsang et al. 1990; Degre 1992; Reynolds, Edwards et al. 2001).

Obviously, the production of off-flavors is a key factor when selecting yeast; however, many winemakers can now select yeast strains based on their positive volatile aroma profiles. It has been shown that different yeast strains can produce varying amounts of volatile aroma compounds, and this can have a significant effect on a wine's final aroma profile. For example, in white wine, yeast strains can vary significantly in the production of fermentation metabolites and can add significantly to the final wine character (Antonelli, Castellari et al. 1999; Dubourdieu, Tominaga et al. 2006; Nikolaou, Soufleros et al. 2006; Loscos, Hernandez-Orte et al. 2007; Reynolds,

Schlosser et al. 2007; Swiegers, Kievit et al. 2009). In addition, Nikolaou et al. (2006) showed that different wine strains of *S. cerevisiae* could produce statistically different amounts of volatiles and resulted in wines with varying aromas when characterized by sensory analysis. Furthermore, (Swiegers, Kievit et al. 2009)showed that yeast strains can vary significantly in their ability to release volatile thiols of pre-fermentative origin. It was indicated that yeast can modify the varietal characteristics of Sauvignon Blanc, and that selection of yeast strain can be used to modulate the aroma profile of wine to a style desired by the winemaker and predetermined by consumer preference.

Recently, researchers have also become interested in non-*Saccharomyces* yeast species and their ability to influence wine aroma and flavor. Studies have shown that in the early stages of fermentation many non-*Saccharomyces* species can produce large amounts of either positive or negative aroma compounds (Ciani and Maccarelli 1998; Egli, Edinger et al. 1998). Negative compounds produced by many non-*Saccharomyces* species can include ethyl acetate and acetic acid (Ciani and Maccarelli 1998); however, when ethyl acetate is found in small quantities (< 50mg/L) it may add complexity to the wine (Mateos, Perez-Nevado et al. 2006). Non-*Saccharomyces* yeast can also produce many positive floral compounds including esters, acetate esters, and higher alcohols (Romano, Suzzi et al. 1992; Rojas, Gil et al. 2001; Rojas, Gil et al. 2003), all which may have a positive effect on wine aroma. In addition, some studies have shown that many non-*Saccharomyces* yeast may produce significantly higher amounts of β-glucosidase than *S. cerevisiae* (Rosi, Vinella et al. 1994; Charoenchai, Fleet et al. 1997). Such production indicates that non-*Saccharomyces* can potentially play a greater role in wine aroma development, with finished wines showing greater

'varietal' expression. However, the ability of non-*Saccharomyces* to affect aroma profiles more than *S. cerevisiae* due solely to higher β-glucosidase activity is still under scrutiny.

Much of the previous research on the impact of yeast strain and species in wine has been conducted specifically on white grape varieties, with little research conducted on red wine grapes. Most likely, this has been due to the difficulties associated with conducting experimental red wine fermentations. The presence of naturally occurring yeast and bacteria on the grape surface is a major issue that has not been properly addressed in past research. For example, some studies have used extremely large amounts of SO_2 to eliminate native microflora yet have not verified the effectiveness of such treatments on grapes (Del Prete, Costantini et al. 2009). Other studies have utilized thermal processing (Romano, Fiore et al. 2003; Clemente-Jimenez, Mingorance-Cazorla et al. 2004), which may inadvertently alter aroma compounds found in wine (Girard, Kopp et al. 1997). Yet other studies fail to report any means of removing native microorganisms from must before producing wine for sensory and chemical evaluation (Nikolaou, Soufleros et al. 2006). For experimental white wine production, the problem of naturally present microorganisms is solved through the sterile filtration of grape juice. However, the sterile filtration of red grape must is not an option as red wine fermentations must take place in the presence of seeds and skins. Thus, it becomes extremely difficult to draw concrete conclusions in terms of yeast and their relation to specific aroma and flavor compounds due to the influence of naturally occurring yeast and bacteria.

To overcome this issue, the new and novel means of High Hydrostatic Pressure (HHP) processing, in conjunction with autoclavable micro-vinification vessels was used in this study. This allowed the specific impacts of one strain of *S. cerevisiae* on the aroma of a wine to be investigated. In this study, the impact of yeast on Pinot Noir aroma and flavor was investigated, including the impact of a non-*Saccharomyces* yeast.

METHODS AND MATERIALS

Grapes

Grapes used for this research were self-rooted *Vitis vinifera* L. cv. 'Pinot Noir'. The Pinot Noir clone was *Pommard*, and all grapes were harvested from the same block at Oregon State University's Woodhall Vineyard (Alpine, Oregon, USA) on October 15th, 2008. The harvest time was based on sugar levels and ripeness as determined by the vineyard manager. The grapes were stored overnight at 2°C before being hand-sorted, and crushed and destemmed. The crusher/destemmer used was a Velo DPC 40 (Altivole, Italy). The grapes were then pooled and divided into fifteen 2.75kg aliquots. 30mg/L SO_2 (in the form of potassium metabisulfite) was added to each aliquot. In addition, 0.34g of Fermaid K® (Lallemand, Montréal, Canada) yeast nutrient was added to the must. Each aliquot was vacuum-sealed in a Food Saver® bag (Jarden Corp., Boca Raton, Florida) and loaded into the HHP unit with ice.

High Hydrostatic Pressure

The grapes were treated for 10 minutes at 551MPa (80,000psi). The HHP unit was custom made by the National Forge Company (Irvine, Pennsylvania) with a 22L maximum capacity and a 689MPa (100,000psi) maximum pressure. The high pressure intensifier pump had a maximum capacity of 620MPa (91,374psi) and was made by Flow International Corporation (model 7XS-6000, Kent, Washington). All grapes stayed within a range of 6.9°C to 12.3°C during processing.

Micro-fermentors

The micro-vinification vessels were based on a design by Sampaio (2007) and Osborne and Edwards (2006). The base of the fermentors were fabricated from autoclave safe glass by Q Glass (Towaco, New Jersey, USA) with a 4L maximum capacity and were approximately 290mm tall with a diameter of 142mm. The lids were custom cut polycarbonate disks manufactured by Ridout Plastics (San Diego, California, USA) and the ring-gaskets were high-temperature, food-grade silicone (Applied Industrial Technologies, Cleveland, Ohio, USA). All bungs and airlocks used were also high-temperature, food-grade silicone (Vin Table, Ambler, Pennsylvania, USA) and the sample ports were constructed from food-grade stainless steel tubing. The fermentors were equipped with a perforated polycarbonate punch-down (Ridout) attached be a stainless steel plunger. The micro-fermentors were autoclaved at 120°C for 30 minutes at 15psi and allowed to cool prior to use. Each of the fifteen aliquots of HHP treated grapes were loaded into the autoclaved fermentors under a laminar flow hood using aseptic technique.

Yeast strains

Four different wine strains of *Saccharomyces* and one non-*Saccharomyces* yeast strain blended with *S. cerevisiae* were used to make five different Pinot Noir wines. Active dry forms of EC1118, RC212, Assmanshausen (AMH) (Lallemand, Montréal, Canada), MERIT.ferm, and Symphony (a blend of MERIT.ferm and *Kluyveromyces thermotolerans*) (Chr. Hansen, Hørsholm, Denmark) were obtained. For all yeast strains single colonies were isolated by streaking on YPD media. To isolate the *K. thermotolerans* from *S. cerevisiae* the Symphony yeast blend was plated onto Lysine agar (Difco™, Franklin Lakes, New Jersey, USA) that is selective for non-Saccharomyces species. A single colony of *K. thermotolerans* was selected from the lysine agar. The isolated yeast was further confirmed by green colony morphology on WL media (Difco™, Franklin Lakes, NJ, USA), which is differential for *K. thermotolerans* (see Appendix C). These single colonies were then transferred to and maintained on potato dextrose agar (Difco, Franklin Lakes, NJ, USA) slants stored at 4°C.

Culture Preparation

Yeast were transferred from slants to YPD broth (10g/L yeast extract, 20g/L peptone, 20g/L dextrose, pH 7.0) and grown aerobically at 30°C for 48 hours. The cells were harvested by centrifugation at 4200rpm for 12 minutes, rinsed with 0.1% peptone solution, and centrifuged a second time. The cells were decanted then mixed with 0.1% peptone. EC1118, RC212, AMH, and MERIT.ferm were each randomly assigned in triplicate to micro-fermentors and added at approximately 10^6cfu/mL.

Three fermentation vessels also received a blend of *S. cerevisiae* MERIT.ferm (10^6cfu/mL) and *K. thermotolerans* (approximately 10^5cfu/mL) to simulate the Symphony yeast blend (Blend).

Fermentations

Fermentations were conducted in triplicate in a temperature-controlled room at 26.6°C (80°F). °Brix was also monitored on a daily basis (Anton-Paar DMA 35N Density Meter, Graz, Austria) and final sugar levels were confirmed with Accuvin Residual Sugar Quick Tests™. Viable cell populations were also monitored throughout the fermentation using diluents containing 0.1% peptone and using appropriate media. Samples were taken using autoclaved pipettes after mixing with an internal punch-down. Yeast cells were grown on YPD agar while bacteria were enumerated using de Man, Rogosa, and Sharpe (MRSC) agar (20g/L Tryptone, 5g/L peptone, 5g/L yeast extract, 5g/L glucose, 200mL apple juice, 1mL Tween 80 [5% w/w solution], 20 g/L agar, pH 4.5, 100ppm cyclohexamide). Non-*Saccharomyces* populations were determined by plating onto Lysine agar. Plates were incubated aerobically at 30°C for 48 hours (yeast) or 7 days (bacteria) prior to counting. When all of the fermentations reached dryness (4+ days at 0 °Brix and <1.0g/L reducing sugars), wines were pressed using a small basket press modified with an air pressure gauge to apply constant pressure at 15psi for 1 min. One minute was sufficient time to allow for all wine to drain out. After pressing, no sensory differences were noted within replicates and consequently replicates were blended together for sensory analysis. Wines were settled at 4°C for 48 hours before filtration and bottling. Bottling

was conducted under a laminar flow hood using a 0.45 micron cartridge filter (Pall Corp., East Hills, New York). The wines were confirmed to have between 13 and 20 mg/L free SO_2, were bottled into 375mL green wine bottles flushed with sterile nitrogen, and were sealed with crown caps. Bottles were stored for four months at 12.8°C.

Color

Before blending and bottling, 50mL samples of wine from each fermentor were centrifuged for 8min at 9000rpm. Where appropriate, samples were diluted with pH-adjusted water. Color was determined by spectrophotometric analysis (Thermo Scientific Genesys 10uv Spectrophotometer, Madison, Wisconsin, USA) at 420 and 520nm in 10mm pathlength cuvettes. Color statistical analysis was performed using Minitab® 15 Statistical Software (State College, Pennsylvania, USA). Univariate Analysis of Variance (ANOVA) was used to determine differences between treatments. Significant differences detected by ANOVA were subjected to post-hoc Tukey HSD multiple comparison at the 0.05% significance level.

Sensory Analysis

Panel Training

Fourteen panelists were recruited by email (see Appendix A) from the Oregon State University Food Science Department and from the Corvallis, OR community. The panelists included six men and eight women who all were familiar with wine description or formal sensory analysis. The panelists were trained twice a week for

three weeks during six hour-long sessions. During training sensory standards were used as described by Noble (1987), along with aroma intensity standards (see Appendix B). The aroma standards were presented in glass jars with plastic screw-on lids. At the beginning of training and testing sessions, panelists were encouraged to re-familiarize themselves with these aroma standards. Panelists were exposed to several different young Pinot Noir wines similar to the test wines, along with the actual test wines during training. Open discussion after sample evaluation allowed panelists to re-familiarize themselves with typical Pinot Noir descriptors, come to a consensus on descriptors, and as a group select appropriate descriptor categories for the ballot as guided by the investigators. The final evaluation ballot contained sixteen descriptors and utilized a sixteen-point intensity scale where the intensity of each descriptor was rated by: 0=none, 3=slight, 7=moderate, 11=large, and 15=extreme (see Appendix B). Panelist scores from trial sessions were recorded on a community board and discussed to help judges correctly identify both category descriptor and intensity ratings in wines. Training was conducted until group discussions and recorded scores revealed panelist were in agreement on terms and intensity ratings.

Training and Testing Area

During training and testing, the panelists were seated as a group at stainless steel food preparation benches. Panelists used paper ballots for training and the resulting ratings were entered on the board for group discussion. Testing was performed in the same area using paper ballots. Panelists were given access to water and a 0.1g/L pectin rinse solution during training and testing.

Sample Preparation and Serving Procedures

The wines used were kept at 12.8°C until the day before testing. All wines were allowed to equilibrate to room temperature and poured 20 minutes before evaluation. 25mL samples were served in 240mL INOVA tulip glasses (St. George Crystal Ltd., Jeannette, Pennsylvania, USA) and covered with a plastic lid. Each wine was identified with a three-digit random number. A Balanced Complete Randomized Block Design (BCRBD) was used in this study. Each panelist tasted each wine treatment three times in random order over three testing sessions. Five wines were evaluated during each testing session, and the three testing sessions were held over the course of two days. Panelist was treated as a blocking factor in the design. Each wine sample was presented in each serving position equally and randomly across the panelists to minimize an order effect. Panelists were allowed three minutes to evaluate each wine and were given a mandatory one-minute break between samples.

Statistics

A univariate Analysis of Variance (ANOVA) was used to determine differences between the wine samples for each descriptor. The ANOVA was performed by General Linear Model (GLM) in SAS® release 9.1 (Cary, North Carolina, USA). The ANOVA model comprised two main effects (Panelist (PAN) and Wine (WINE)), a nested effect (replication which was nested in PAN or (REP(PAN)), and a two-way interaction effect between PAN and WINE (WINE*PAN). The PAN, REP(PAN) and (WINE*PAN) were treated as random effects and WINE was treated as a fixed effect. Significant differences detected by ANOVA were subjected to post-

hoc Tukey HSD multiple comparison to test least squares means of WINE (means) at the 0.05% significance level. The means with the same letter are not significantly different at the 0.05% level.

A multivariate statistical approach, Principle Component Analysis (PCA) was used to determine the relationship of the aroma descriptors to the samples. The PCA was performed with Varimax rotation, covariance, and eigenvalue more than 1 was used as a criterion for selecting principle components. Cut-off value of 0.4 was used to select relevant descriptors for each component. SPSS® release 17.0.0 (Chicago, IL) software was used.

RESULTS

Grape Must and Fermentation

After HHP treatment the Pinot Noir must contained 0.786g/100mL titratable acidity (in grams tartaric acid), 24.2°Brix, and a pH of 3.52. The fermentation profiles progressed quite similarly between the treatments and within the replicates. °Brix levels reached zero for all wines after six days; however, the AMH treatments finished fermentation 24 hours after the rest of the yeast strains (see Figure 3.1). *S. cerevisiae* populations began between $4.5x10^5$ and $3.4x10^6$ cfu/mL and grew to above 10^9 cfu/mL for all fermentations (see Figure 3.2). *K. thermotolerans* numbers first rose to levels above 10^7cfu/mL in the first 48 hours, and then by 144 hours the populations had dropped to less than detectable numbers. Before HHP treatment, microorganism growth was observed when must was plated on YPD, MRSC and Lysine plates.

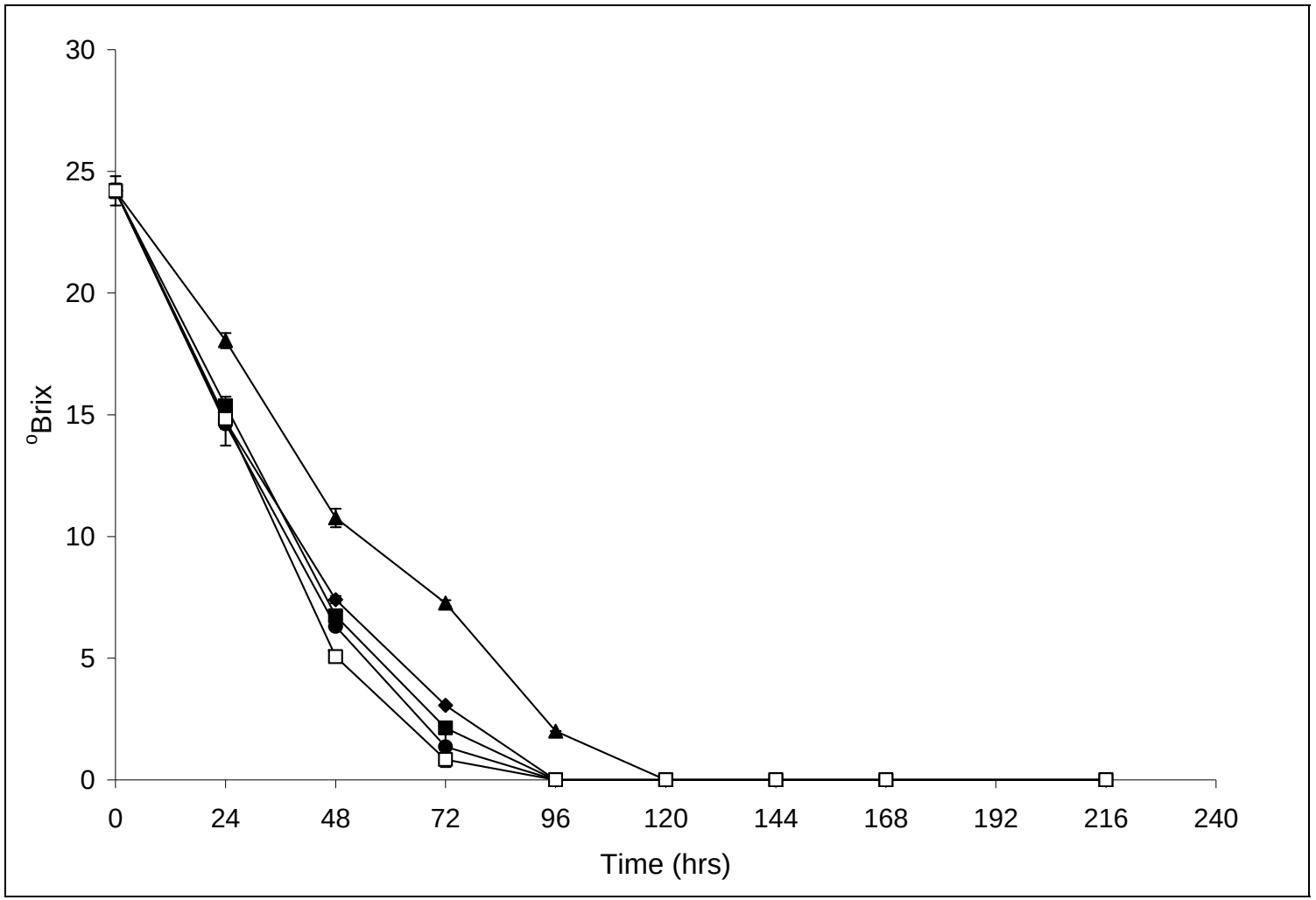

Figure 3.1: °Brix during alcoholic fermentation conducted by: *S. cerevisiae* strain (▲) AMH, (◆) RC212, (□) MERIT.ferm, and *S. bayanus* (●) EC1118, and a blend of *K. thermotolerans* and MERIT.ferm (■) Blend; values are means of triplicates.

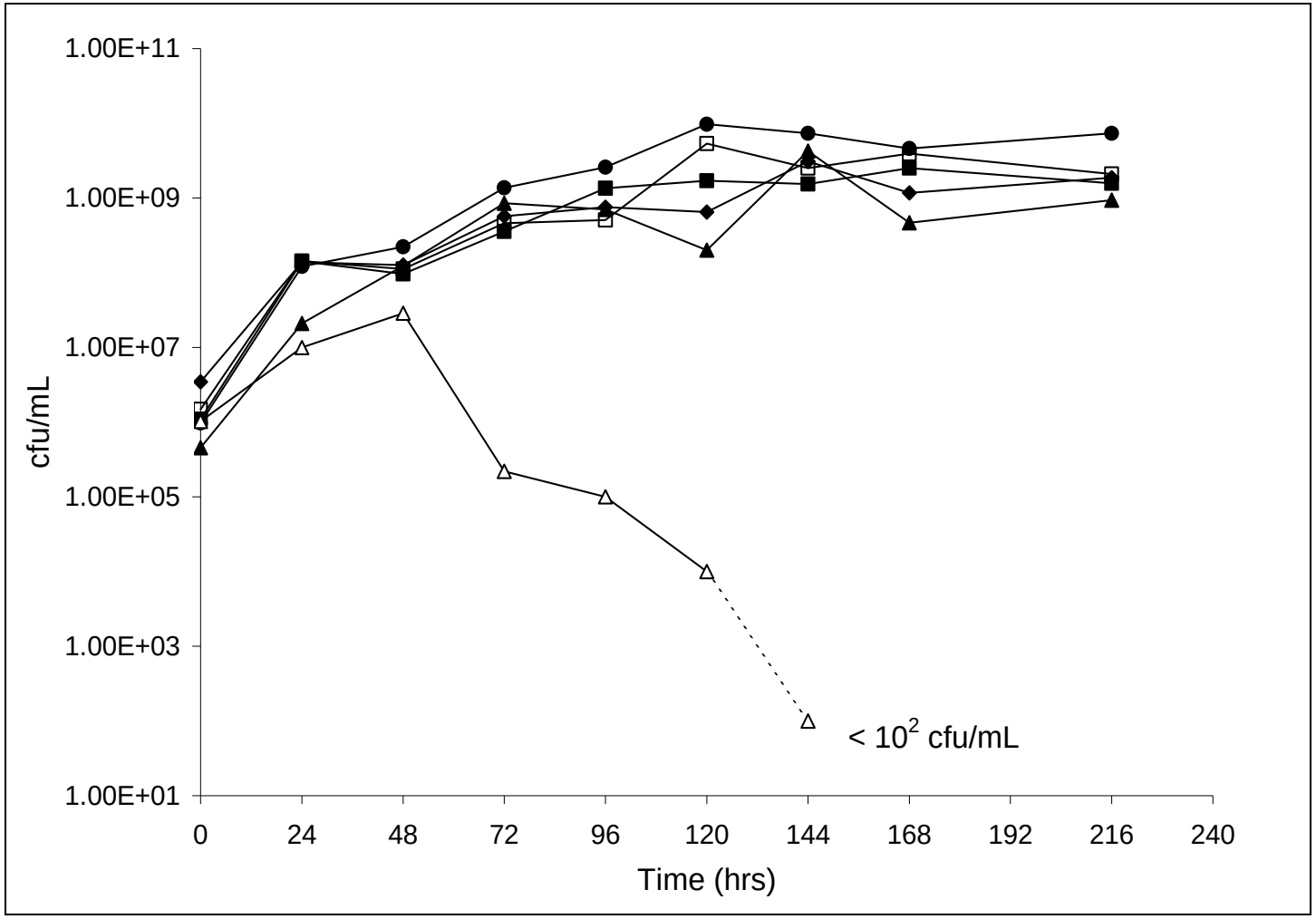

Figure 3.2: Viable cell counts during alcoholic fermentation of: *S. cerevisiae* (◆) RC212, (□) MERIT.ferm, (■) Blend, (▲) AMH, *S. bayanus* (●) EC1118, and (△) *K. thermotolerans*; values are means of duplicates.

However, after HHP treatment no microorganism growth was observed on media (data not shown). Wines fermented with EC1118 and AMH showed a significant difference in color at 520nm (see Table 3.1). The mean absorbance of the EC1118 wines was 5.239 and the mean absorbance of the AMH wines was 4.396, which were the highest and lowest absorbance values of all the treatments. There were no significant color differences between wines produced with any of the other treatment yeast strains (Table 3.1).

Table 3.1: Color and hue values[1] for wines fermented with different yeast strains; means with different letters within a column are significantly different at the $p<0.05$ level.

	Color (absorbance @ 520nm)	Hue (420nm/520nm)
AMH	4.40^{b} (+/- 0.45)	0.61^{a} (+/- 0.01)
Blend	4.62^{ab} (+/- 0.21)	0.57^{a} (+/- 0.01)
EC1118	5.24^{a} (+/- 0.20)	0.58^{a} (+/- 0.01)
MERIT.ferm	4.65^{ab} (+/- 0.27)	0.58^{a} (+/- 0.01)
RC212	5.07^{ab} (+/- 0.26)	0.55^{a} (+/- 0.01)

[1]values are means from triplicate fermentations

Sensory Analysis

As shown by the analysis of variance in table 3.2, significant differences were found between yeast strains in the following aroma and flavor attributes: overall aroma intensity, overall floral aroma, overall fruity aroma, citrus fruit aroma, red fruit aroma, dark fruit aroma, jammy/cooked fruit aroma, overall flavor intensity, and overall fruit flavor. Significant differences were also found between yeast strains in sour and astringent sensory categories. No significant differences were found between treatments in: overall herbal/spicy/earthy aroma, overall aged aromas, overall aroma

intensity, citrus aroma, overall flavor, herbal/spicy flavors, or aged flavors. Table 3.3 shows the means sensory description values for each yeast strain, and the significant differences between them.

Table 3.2: Analysis of variance F-ratios of attribute ratings (14 judges) for wines produced with different yeast strains; (*) indicates significance at the $p<0.05$ level, (**) indicates significance at the $p<0.01$ level; (***) indicates significance at the $p<0.001$ level

Descriptors	Treatment	Panelist	Rep (Panelist)	Panelist x Treatment
Overall Aroma	0.85	4.65***	0.54	2.05***
Overall Floral Aroma	2.90 *	5.74***	1.88**	1.6*
Overall Fruity Aroma	5.85***	8.11***	1.09	1.99**
Citrus Aroma	2.01	27.58***	0.80	1.61*
Red Fruit Aroma	5.86***	11.49***	1.98**	1.51
Dark Fruit Aroma	6.79***	7.93***	1.04	2.01**
Jammy/cooked Fruit	2.64*	25.81***	0.88	1.20
Overall Spicy-Earthy	2.11	4.71***	1.39	1.11
Overall Aged	0.40	11.51***	0.76	1.20
Overall Flavor	0.57	10.15***	1.10	1.95**
Fruit Flavor	8.06***	12.09***	1.03	2.34***
Herb Spice Flavor	0.22	5.08***	2.5***	1.81**
Aged Flavor	2.38	18.35***	1.38	0.72
Sour	3.06*	9.80***	1.51	1.41
Astringent	7.10***	31.37***	0.83	1.19
Degrees of Freedom	4	13	28	52

Table 3.3: Means sensory description values[1] for wine fermented with different yeast strains; values with different letters within a row are significantly different; (*) indicates significance at the p<0.05 level, (**) indicates significance at the p<0.01 level; (***) indicates significance at the p<0.001 level; (SD) standard deviation.

Descriptors	AMH	*(SD)*	BLEND	*(SD)*	EC1118	*(SD)*	MERIT	*(SD)*	RC212	*(SD)*
Overall Aroma^{NS}	9.0^{a}	*(1.3)*	8.8^{a}	*(1.4)*	9.3^{a}	*(1.2)*	8.8^{a}	*(1.2)*	9.1^{a}	*(1.0)*
Overall Floral Aroma*[2]	5.1^{a}	*(1.9)*	5.0^{a}	*(2.0)*	5.7^{a}	*(1.3)*	5.9^{a}	*(1.2)*	5.5^{a}	*(1.5)*
Overall Fruity Aroma***	5.7^{c}	*(1.6)*	5.8^{bc}	*(1.8)*	7.0^{a}	*(1.6)*	6.4^{abc}	*(1.5)*	6.6^{ab}	*(1.5)*
Citrus Aroma	1.8^{a}	*(1.9)*	1.5^{a}	*(1.7)*	1.9^{a}	*(2.0)*	2.0^{a}	*(1.8)*	2.2^{a}	*(2.0)*
Red Fruit Aroma***	3.5^{c}	*(2.1)*	3.8^{bc}	*(2.3)*	4.5^{ab}	*(2.4)*	4.9^{a}	*(1.9)*	4.5^{ab}	*(2.1)*
Dark Fruit Aroma***	3.4^{b}	*(1.7)*	3.5^{b}	*(1.8)*	5.0^{a}	*(1.6)*	3.9^{b}	*(1.6)*	4.2^{ab}	*(1.8)*
Jammy/cooked Fruit*[2]	3.7^{a}	*(1.9)*	3.9^{a}	*(1.9)*	4.5^{a}	*(2.3)*	3.6^{a}	*(2.3)*	4.2^{a}	*(2.1)*
Overall Spicy-Earthy^{NS}	4.8^{a}	*(1.1)*	5.2^{a}	*(1.9)*	4.8^{a}	*(1.2)*	4.9^{a}	*(1.0)*	5.3^{a}	*(1.3)*
Overall Aged^{NS}	4.5^{a}	*(1.2)*	4.4^{a}	*(1.5)*	4.2^{a}	*(1.7)*	4.1^{a}	*(1.6)*	4.3^{a}	*(1.4)*
Overall Flavor^{NS}	9.1^{a}	*(1.9)*	9.3^{a}	*(1.7)*	9.5^{a}	*(1.3)*	9.1^{a}	*(1.4)*	9.2^{a}	*(1.3)*
Fruit Flavor***	5.5^{c}	*(2.0)*	5.7^{bc}	*(2.1)*	7.2^{a}	*(1.7)*	6.6^{ab}	*(1.8)*	6.5^{abc}	*(1.6)*
Herb Spice Flavor^{NS}	6.1^{a}	*(2.0)*	6.1^{a}	*(1.8)*	6.1^{a}	*(1.4)*	5.9^{a}	*(1.4)*	6.0^{a}	*(1.4)*
Aged Flavor^{NS}	5.3^{a}	*(1.9)*	5.0^{a}	*(1.9)*	4.9^{a}	*(1.5)*	4.7^{a}	*(1.8)*	4.7^{a}	*(1.9)*

[1]values are means from triplicates
[2]a significant difference between means was found using ANOVA; however, Tukey's HSD could not separate means

EC1118

EC 1118 stood out as consistently having the highest mean values for overall fruity aroma, dark fruit aroma, jammy/cooked fruit aroma and overall fruit flavor. EC1118 produced significantly higher overall fruit aromas than Blend or AMH wine treatments. Table 3.3 shows that the mean intensity rating for EC1118 in overall fruit aroma was 7.0, while the scores for AMH and Blend were 5.7 and 5.8 respectively. It is interesting to note that while there were no significant differences between EC1118

and RC212, there was a significant difference between EC1118 and MERIT.ferm in dark fruit aroma. In addition, large differences were also found between EC1118 and AMH and Blend, in dark fruit aroma. The dark fruit descriptor rating for EC1118 was 5.0 while the AMH and Blend treatments scored 3.4 and 3.5, respectively. Similar results showed EC1118 producing higher fruit flavors than AMH and Blend (Table 3.3). In terms of jammy/cooked fruit aroma descriptors, EC1118 was not significantly different by Tukey's HSD. However, EC1118 did have the highest mean value for jammy/cooked notes, and the mean values were shown to be significantly different at the $p<0.05$ level, as seen in table 3.2.

RC212

RC212 was shown to be remarkably similar to EC1118 in all aroma and flavor attributes. Although RC212 had similar descriptor intensity ratings to EC1118, it did not show the same distinction from other yeast strains in many aroma and flavor attributes. RC212 did, however, produce wine with significantly higher overall fruity descriptors than AMH (6.6 and 5.7, respectively). RC212 was also significantly higher than AMH in red fruit aroma (4.5 vs. 3.5).

MERIT.ferm

The data means from the MERIT.ferm yeast strain showed some interesting differences from EC1118. While there were no significant differences between MERIT.ferm and RC212, MERIT.ferm showed a significantly less dark fruit aroma when compared to EC1118. It is also important to note that MERIT.ferm differed

significantly from the Blend treatment, which contained the same yeast strain plus the addition of *K. thermotolerans*. The floral (5.9) and red fruit (4.9) aroma descriptors in MERIT.ferm were higher than the Blend treatment (floral 5, red fruit 3.8). Although for overall floral aroma, MERIT.ferm was not significantly separated from Blend by Tukey's HSD (Table 3.3), ANOVA showed a significant difference in means between the treatment groups (Table 3.2). MERIT.ferm also scored high in the fruit flavor category (6.6), which was significantly higher than the AMH treatments (5.5).

Blend and AMH

The Blend and AMH yeast treatments did not produce wines significantly different from each other in any of the sensory descriptor categories. While both the Blend and AMH yeast were significantly different than EC1118 and RC212 in overall fruit aromas and dark fruit aromas, AMH and MERIT.ferm showed significant differences in fruit flavor. Also, AMH showed significantly lower aroma and flavor intensities in several descriptor categories when compared to EC1118.

Principle Component Analysis

Trends arose showing differences in flavor profiles between different yeast strains. The overall differences between yeast strains can be seen on the principle component analysis (Figure 3.3). The horizontal axis accounts for 35% of the total variability and is described by floral, red fruit, citrus fruit, dark fruit, and overall fruit aromas on the right, and aged aroma on the left. The vertical axis is described by

jammy/cooked fruit, dark fruit, overall fruity aromas, and overall aroma intensity at the top, while aged aromas characterize the bottom of the vertical axis. The vertical

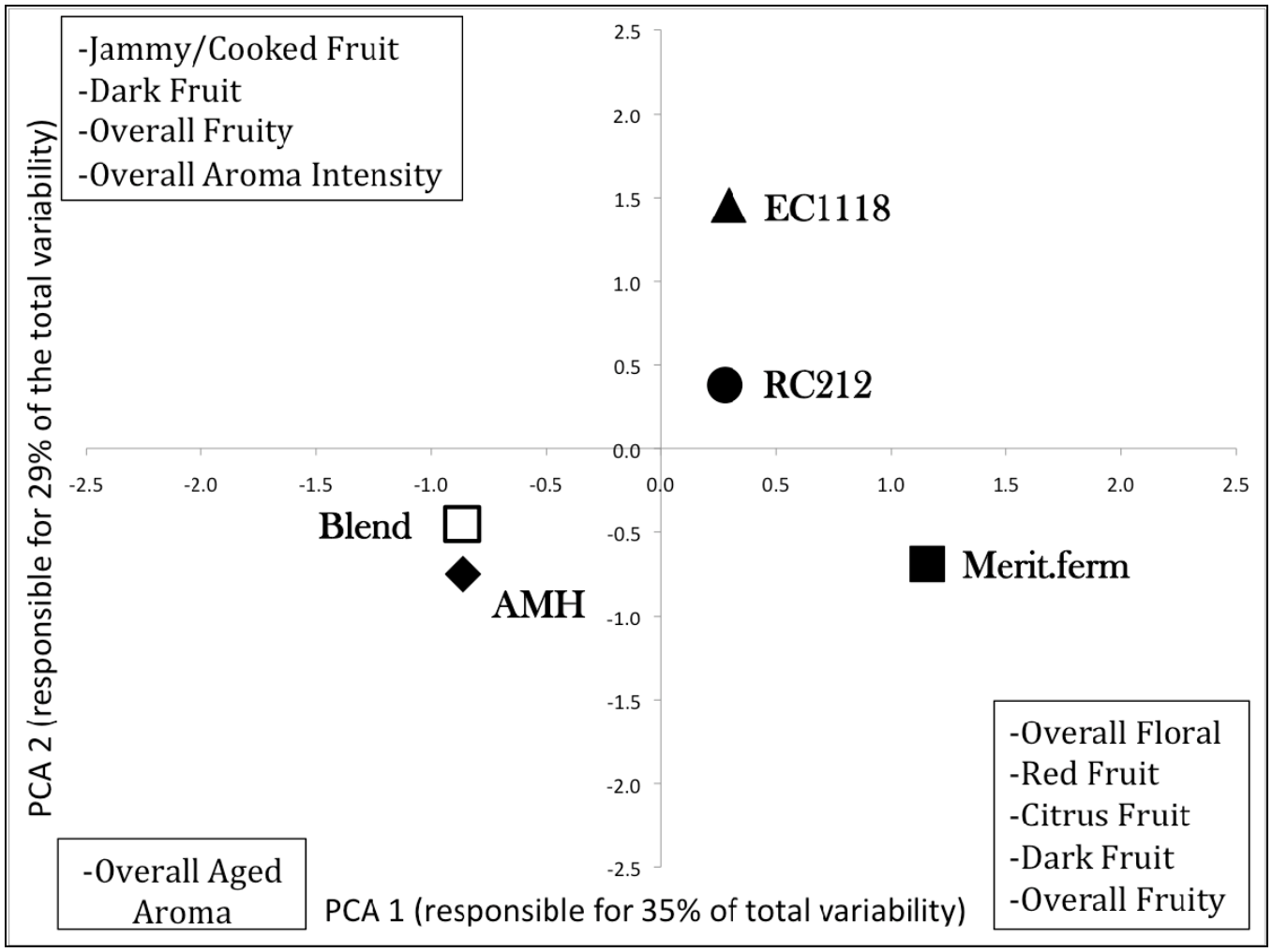

Figure 3.3: Principle Component Analysis (PCA) of wines fermented with different yeast strains[1]

[1]aroma descriptors only.

axis describes 29% of the total variability between treatments. The aroma profile of the Pinot Noir produced with EC1118 shows a strong trend toward the top of the PCA graph and the jammy/cooked fruit, dark fruit, overall fruit aroma, and overall aroma intensity descriptors. RC212 also shows a similar trend, however not as strongly. The aroma profiles of the wines fermented with MERIT.ferm, Blend, and AMH yeast strains all showed similar orientation on the vertical axis, trending toward the bottom of the vertical axis and away from the jammy/cooked fruit, dark fruit, overall fruit aroma, and overall aroma intensity descriptors. On the horizontal axis, MERIT.ferm clearly trends toward the right side of the graph and the floral, red fruit, citrus fruit, dark fruit, and overall fruit aroma descriptors. Although RC212 and EC1118 also trend in this direction, the Blend and AMH wine aroma profiles trended in the opposite direction away from the floral, red fruit, citrus fruit, dark fruit, and overall fruit aroma descriptors. Both the AMH and Blend yeast strains were remarkably similar in both their PCA profiles and mean values for all aroma and flavor descriptors, showing no significant differences. While the MERIT.ferm, RC212, and EC1118 yeast strains produced similar aroma profiles in the finished wine, unlike the Blend and AMH, they differed in several different descriptor categories.

DISCUSSION

Wine aroma is primarily derived from the action of yeast, either through the production of volatile compounds such as esters or through their action on grape derived flavor and aroma compounds. While it has been well documented that yeast

strain can impact white wine aroma, this study is one of the few that has documented the impact of different yeast strains on a red wine.

Important differences arose between yeast strains for many sensory descriptors, yet fruit descriptors played a key part in differentiating the wines produced from different yeast strains. It is possible that many of these differences are due to variation in yeast secondary metabolite production. The findings in this research are supported by previous studies that indicate yeast strains can differ not only in the production of fruity and floral compounds such as esters or higher alcohols (Antonelli, Castellari et al. 1999; Romano, Fiore et al. 2003; Nikolaou, Soufleros et al. 2006), but more importantly they may differ in the amount of off-aromas they produce (Mateos, Perez-Nevado et al. 2006). Such activities could possibly mask floral and fruit aromas in wine.

Some sensory distinctions between the wines may also be explained by a difference between various yeast's abilities to liberate grape-derived aroma compounds. Norisoprenoids, monoterpene alcohols, other aromatic alcohols, and thiols can be liberated during fermentation and are responsible for many fruity and floral aroma characteristics in wine. However, it should be cautioned that there is very little evidence that these differences, if any, can make a significant sensory impact. The presence and activity of glycosidases in the yeast strains and species studied in this research has yet to be confirmed and more research in this area is needed.

Overall fruit intensity ratings differed significantly between the wines produced from EC1118 and the AMH yeast. It is also interesting to note that wine produced from the Blend yeasts had lower red fruit aroma than wine from

MERIT.ferm, and a lower fruit aroma and fruit flavor intensity than many of the other yeast strains. While the low floral attribute rating of the wine produced by Blend yeasts is not significantly different by Tukey's HSD, there is a significant difference between means in this attribute category as shown by ANOVA (Table 3.2), and MERIT.ferm can be separated from Blend by using Fisher LSD (not shown). Symphony (Blend) yeast is marketed as a mixed starter culture in which the *K. thermotolerans* is intended to increase 'flavor impact', which in this research, it did not. Previous research studies investigating non-Saccharomyces yeast have found that some specific species including *Hanseniaspora osmophila, Candida stellata,* and *Torulaspora delbrueckii* are well suited to producing wine due to positive impacts on wine aroma (Ciani and Maccarelli 1998; Viana, Gil et al. 2008). While a limited amount of research has been performed on *K. thermotolerans* showing its potential use in mixed starter cultures with *S. cerevisiae*, and it's low volatile acid production (Kapsopoulou, Kapaklis et al. 2005; Kapsopoulou, Mourtzini et al. 2007), its affect on red wine aroma has not been reported. In fact, considering the reduction of fruit flavor and floral aroma observed in the present study, *K. thermotolerans* appeared to cause a negative impact on the wine. Whether this was due to an actual reduction of flavor and aroma compounds, or masking of these characters by other volatiles may be discovered by future GC-MS research. It should also be noted, although it was not investigated in this study, that there may be other, more subtle, flavors and tastes associated with using *K. thermotolerans* or other non-*Saccharomyces* yeasts. This issue of 'complexity' or 'uniqueness' may warrant further future research.

PCA analysis shows some important trends in the aroma profiles of the wines produced with different yeast strains. For example, the wine produced by MERIT.ferm was characterized by a trend toward floral and red fruit descriptors, which, combined with its purported high ethanol tolerance, makes it a suitable yeast for general red wine fermentation. These floral and red fruit descriptors can come from a variety of compounds, but in Pinot Noir wine it is likely that floral notes come from 2-phenethyl acetate or 2-phenethanol (Fang and Qian 2005; Fang and Qian 2006). Although 2-phenethyl acetate and 2-phenethanol are found in grapes, they are also produced in significant quantities by yeast. The level of 2-phenethyl acetate and 2-phenethanol production in yeast can vary significantly, with up to a four-fold difference between some yeast strains (Rankine and Pocock 1969; Patel and Shibamoto 2003). Fruit descriptors (and sometimes floral notes) can come from ethyl esters of butanoate, hexanoate, octanoate, or decanoate, branched chain esters, or acetate esters. The reaction of ethanol or higher alcohols with Coenzyme-A activated fatty acids (acetyl-CoA) can form these esters. This reaction is catalyzed by the enzyme alcohol acetyltransferase (Lambrechts and Pretorius 2000) and any increase in the activities of these pathways could result in higher concentrations in wine.

For wines produced with EC1118, a *S. bayanus* yeast, the PCA trended toward higher overall aroma intensities. This trend toward high aroma intensities was also discovered in previous chemical studies where *S. bayanus* strains showed higher secondary metabolite production than their *S. cerevisiae* counterparts (Antonelli, Castellari et al. 1999). In contrast, Reynolds, Edwards et al. (2001) reported that many commercial yeast strains produce more intense wine aromas and flavors than EC1118.

It should be noted that both of these studies were performed with cold-settled white grape juice rather than red grape must. In addition, either no verification of removal of microorganisms after using large amounts of SO_2 was performed (Reynolds, Edwards et al. 2001), or no effort was made to remove native yeast whatsoever (Antonelli, Castellari et al. 1999). Thus, future research that controls for the influence of natural grape micro-flora may be able to better confirm the influence of *S. bayanus* vs. *S. cerevisiae,* especially on red wine.

There is commonly a great deal of information provided by yeast companies which illustrate the specific effects of a yeast strain on wine. However, not all wines in this study matched their commercial descriptions or previously described aroma profiles. Wine produced from AMH yeast is purported to enhance both spicy and fruity flavors, specifically in Pinot Noir wine. This might include compounds such as eugenol, 4-vinylphenol, and guaiacol (or its derivatives), which are released or modified by yeast from grape precursors during fermentation, and have been found in Pinot Noir wine (Chatonnet, Dubourdieu et al. 1989; Chatonnet, Dubourdieu et al. 1992; Lambrechts and Pretorius 2000; Fang and Qian 2005). In this study, the wine made from AMH yeast failed to distinguish itself as a spicy wine by trained panel analysis and had low overall fruitiness when compared to EC1118 and RC212. A potential reason why AMH may not have distinguished itself as producing a more spicy wine is that these compounds were not present in the grapes to begin with. It is possible that under different growing conditions, these spicy precursors or bound compounds could have been increased, and thus could have been acted upon in different ways by various yeast strains. Another possibility is that AMH does not

actually have the ability to produce more spicy characters than any other *S. cerevisiae* yeast strain. Further profiling of oenological yeast for their ability to produce/release spicy aroma and flavor compounds should be investigated in future research.

Non-significant descriptors

Overall herbal/spicy/earthy aroma, aged aromas, herbal/spicy flavors, and aged flavor descriptor categories showed no significant differences between any of the treatment groups and were of little importance in the PCA. Many of these aroma descriptors may be derived from aging on oak barrels, or may develop over time in the bottle. Also, as noted earlier, many of the precursor compounds that can contribute to spicy flavors can be grape derived. Thus, it was expected to see no significant differences in some common aroma descriptors such as aged, earthy or spicy since all fermentations began with the same starting material.

Limitations and future work

While this research was unique in its ability to ferment red wine starting with a must free of microorganisms, the HHP processing used to treat the grape must did have some influence on the wine. HHP treatment of grape must can lead to grape cell breakdown, which may have resulted in the increased extraction of compounds, most notably phenolic material, into the must. However, it should be noted that the extraction of grape constituents into the wine during fermentation is a natural process that occurs during winemaking. In some cases increased extraction of compounds (such as phenolics) is desired and actively sought by the winemaker. Extended cold

soaks, the use of enzyme treatments, saignée, and extended maceration are all examples of techniques used to improve the extraction process. So, while HHP treatment can lead to increased extraction during fermentation, increasing extraction is not a practice foreign to commercial winemaking and can be used in experimental research. Another limitation of this study was the size of the fermentation vessels, and the overall scale of the must used and wine produced. While the use of micro-fermentors has been previously discussed in literature (Sampaio, Kennedy et al. 2007), and has been shown to be applicable to large-scale winemaking, scaling these HHP and autoclavable micro-vinification vessel techniques up to larger batch quantities should be considered for future investigations. Producing greater quantities of wine could facilitate more in-depth sensory research as well as more accurately replicate commercial winemaking conditions.

Future red wine research utilizing HHP processing should include GC-MS analysis of wine linked with sensory research. Studies should delve deeper into what specific volatile compounds are produced by yeast, in what quantities, and their relation to sensory profiles. Also, as previously noted, a greater range of yeast strains and species and their impacts on wine aroma should be studied using HHP processing techniques to ensure a starting must free of microorganisms. This should include not only yeast sensory description profiles, but also their interaction effects, strain-domination, ability to affect wine color, ability to release grape derived compounds (including spicy characters), and the ability to create ‘complexity’ and ‘mouth-feel’. Similar studies should investigate a range of non-*Saccharomyces* yeast common to winemaking, and the properties of non-*Saccharomyces* yeast that can be isolated from

region-to-region. In addition, comparing enological strains of *S. cerevisiae* with their *S. bayanus* counterparts may lead to interesting results concerning both aroma profiles and strain dominance. Finally, future red-wine research using HHP techniques should be expanded to include other red-grape varieties besides Pinot Noir.

Conclusions

Yeast strains had a significant effect on the aroma and flavor profile of Pinot Noir wine. Some important trends were shown, varying between yeast strains. EC1118 and RC212 showed strong overall aroma intensity profiles with high values trending toward dark fruit and jammy characteristics. MERIT.ferm also showed high overall aroma intensity; however, the aroma profile trended more toward red fruit and floral characteristics. Lower aroma intensities were shown in many descriptors for both the AMH and Blend (Symphony) yeast strains. Wine made with a *K. thermotolerans* developed low floral aroma values and was not distinct from the wine produced with the AMH yeast strain. In addition, wine made with *K. thermotolerans* differed from wine produced from MERIT.ferm alone, with significantly lower red fruit and fruit flavor. More research should be conducted with a greater variety of non-*Saccharomyces* and *Saccharomyces* yeasts, using a greater number of red grape cultivars, in order to develop a comprehensive profile of the many enological strains of yeasts available. Such research could benefit winemakers as future profiling of wine yeast could directly improve winemaker's ability to control or develop wine aroma profiles and thus improve overall wine quality.

SUMMARY

Wine aroma and flavor are important aspects of wine quality. Any increased ability to control the aroma and flavor profile of wine would greatly benefit winemakers and the industry. It has been well established through previous research that yeast strains and species can significantly alter the aroma of white wine; however, little research has been conducted regarding the impact of yeast on red wine aroma and flavor. The lack of research can be attributed to the complexities of red wine fermentations, including the influence of native yeast and bacteria in the environment. The contamination by native yeast and bacteria during research, and their possible influence on wine flavor and aroma, makes attributing specific aroma and flavor compounds to one yeast strain extremely difficult. In much of the previous research the methods used to control native yeast and bacteria (or lack thereof) has been inadequate or unsuited to sensory analysis. Thus, this study investigated new methods of removing microorganisms from red grape must prior to fermentation.

High Hydrostatic Pressure (HHP) processing was investigated as a means of removing microorganisms from grape must prior to fermentation. First, HHP processing was proven to be effective at removing several different organisms including *Saccharomyces bayanus, Acetobacter aceti, Lactobacillus hilgardii* and *Brettanomyces bruxellensis* from table grape must adjusted to 23°Brix. HHP processing was then shown to effectively remove native microorganisms from Pinot Noir must with minimal affects on aroma or flavor of finished wines as confirmed by a

trained sensory panel. HHP processing of the grape must did not affect color or hue of the finished wine but it did increase the phenolic content of the wine by 70%.

Because HHP processing, in conjunction with autoclavable micro-fermentors, was shown to be a viable method of removing microorganisms from must without affecting wine aroma and flavor, an investigation of different *Saccharomyces* and non-*Saccharomyces* yeast strains and their impact on wine aroma and flavor was conducted. The results of this study illustrated that yeast strain caused specific aroma and flavor differences in Pinot Noir wine. Specifically, yeast strains EC1118 and RC212 created more intense aromas trending toward dark fruit and jammy descriptors. MERIT.ferm also created intense aromas, yet these trended more toward floral and red fruit descriptors. The wine produced with the Blend yeast (MERIT.ferm combined with *Kluyveromyces thermotolerans*) resulted in a reduction of floral aroma and red fruit aroma. Although AMH yeast is purported to increase spicy notes in wine, the wine produced with AMH did not distinguish itself as having more intense spicy, earthy, or herbal characteristics than any of the other wines. However, the results of this study indicate that some yeast strains could be used to alter the aroma profile of Pinot Noir. Further research on both *Saccharomyces* and non-*Saccharomyces* yeast, and the effects of co-inoculation, is needed to create a comprehensive profile of oenological yeast strains.

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APPENDICIES

APPENDIX A
Wine panel recruitment email

Invitation to join a Pinot noir trained panel for winter term 2009

FROM: Cindy Lederer, James Osborne, and David Takush

Hello,

We will be starting up a descriptive panel for Pinot noir wines and we would like to invite you to participate. The panel begins _____ and ends _____. We are recruiting a panel of 14 panelists who have experience in trained panel and/or have experience (or interest) in tasting Pinot noir wines. This is sensory work to support Dave Takush's thesis study and Dave will be running the panel. Dave is a graduate student with James Osborne.

What do you get?
As a thank you for your participation, you will receive an $8 gift certificate (from Fred Meyer) per session awarded at the end of the panel. You will get experience in descriptive profiling of wine using descriptive analysis techniques.

What will you learn?
We will be looking at aroma, appearance, flavor-by-mouth, and mouth-feel qualities. We will be coming up with descriptors to describe what we smell and taste and then learn to use the 16 point intensity scale to rate the intensity of those aromas/flavors, etc. We will be using aroma and flavor references to help anchor our evaluations.

What your commitment will be?
Training will be 2 times a week for one hour each (on rare occasions, maximum 1.5 hr). We estimate about 6 to 8 training sessions.

Training begins _____ and below is a list of possible times and if you would like to participate. Please email me back with a yes or no if you can make the times given. Once I find out what times work best for everyone, I will send out a final schedule. If you are only going to be gone for a few sessions, no worries, I can work around your schedule with make-up sessions if needed.

Tuesday, Thursday
10:00 to 11:00
10:30 to 11:30
11:00 to 12:00
3:30 to 4:30
4:00 to 5:00
4:30 to 5:30

TESTING

Testing will be 2 times a week for one hour each time for a total of 4 to 6 testing sessions.

We would appreciate hearing back from you by _____. Reply to Dave and Cindy (takushd@onid.orst.edu; cindy.lederer@oregonstate.edu) Let us know if you cannot make the panel or if you are interested in being considered, and respond to the above training date availabilities.

Thanks,
Cindy, James, And Dave

APPENDIX B
Wine test ballot and aroma intensity standards

Name!!_____	***Date***_____		
16 point intensity scale:			
0 = None	6	11=Large	
1 = Just detectable	7=Moderate	12	
2	8	13=Large to Extreme	
3 = Slight (Safflower Oil)	9=Moderate to Large	14	
4	(Orange Drink)	15=Extreme (Cinnamon Gum)	
5 = Slight to Moderate	10		
1) Smell your standards			
2) Evaluate your WARM UP and note the anchored ratings below:			
Overall Aroma = 9			
Overall Floral=5			
Overall Fruity Aroma = 7			
Overall Herbal Aroma=5			
Overall Aged Aroma=4			
Overall Flavor = 10			
Sour = 5-6			
Astringency =6			
Descriptors	SAMPLE #_____	SAMPLE #_____	SAMPLE #_____
AROMA	AROMA	AROMA	AROMA
Overall Aroma			
Overall Floral			
Overall Fruity			
Citrus Fruit			
Red Fruit			
Dark Fruit			
Cooked/Jammy/Dried Fruit			
Overall Herbal/Spicy/Earthy			
Overall Aged Aroma ***(oak, leather, tobacco...)***			
Overall Musty/Chalky/Dusty			
FLAVOR	**FLAVOR**	**FLAVOR**	**FLAVOR**
Overall Flavor			
Overall Fruit			
Overall Herbal/Spice taste			
Aged Flavors			
Basic taste/mouth feel	**Basic taste/mouth feel**	**Basic taste/mouth feel**	**Basic taste/mouth feel**
Sour			
Astringent			

APPENDIX C

Examples of green and white colony morphology of *Kluyveromyces thermotolerans* and *Saccharomyces cerevisiae* on WL media