**PROGRESS REPORT 2011**

I. Project Title: Non-*Saccharomyces* yeast on wine quality-part 2, aroma and flavor development

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III. Summary:

There is evidence that non-Saccharomyces yeast can contribute significantly to a wine's final flavor and aroma, because many non-*Saccharomyces* yeasts can produce significantly higher amounts of β-glucosidase than S. cerevisiae. This study isolated some native non-saccharomyces yeasts, and investigated their specific glycosidase activities and effect on final wine volatile composition in Pinot noir wine.

The first part of this project identified non-*Saccharomyces* species present during cold maceration and alcoholic fermentation of Pinot noir grapes from two different vineyards. These isolates were then screened for β-glucosidase activity and further characterized. The results revealed that high sugar content suppressed β-glucosidase activity for some non-*Saccharomyces* species but not all species. Furthermore, at cold maceration temperatures (8-10°C) the β-glucosidase activity of many of the non-*Saccharomyces* isolates increased dramatically possibly due to elevated production of the enzyme at cold temperatures.

Fermentation of Pinot noir grapes with the selected non-*Saccharomyces* species resulted in different volatile profiles in the wine. Cold soak with non-*Saccharomyces* species changed volatile alcohol, short chain fatty acids, and esters composition. In addition, cold soak with *Hansenula anomala* and Unknown Species #10 dramatically increased the release of β-citronellol in the wine. However, decreased trend was observed for some other terpene alcohols. In addition, the cold soak with selected non-*Saccharomyces* species infleunced β-damascenone concentration, but not increase the concentration. Cold soak with selected non-*Saccharomyces* species did not affect β-ionone concentration. The data demonstrated that non-*Saccharomyces* species have enzyme systems that are active at grape conditions, and can alter terpene alcohols and β-damascenone composition in wine. It is possible to find non-*Saccharomyces* species to enhance the production of β-damascenone and β-ionone, the most important compounds in red wine.

IV. Objectives and Experiments Conducted to Meet Stated Objectives:

**Objective 1.**
**Isolate non-*Saccharomyces* yeast species that have high glycosidase activities including α-L-rhamnopyranosidase, α-L-arabinopyranosidase and α-L-arabinofuranosidase and β-D-glucosidase activities from Oregon wineries (see detailed report from James Osborne).**

As in 2010, samples were taken from grapes undergoing pre-fermentation cold maceration and alcoholic fermentation in order to compare yeast populations and diversity from the same vineyards in two different years. Grapes from the same vineyard lots sampled in 2010 were sampled in 2011. Grapes from both vineyards were transported to the OSU winery and stored overnight at 4ºC before being processed the following day. Grapes were randomly allocated to 100 L stainless steel tanks with cooling jackets. Two fermentors per vineyard were prepared. During the pre-fermentation cold maceration tanks were maintained between 8-10ºC and sampled aseptically daily after mixing. After 7 days cold maceration the fermentations were warmed to approximately 25 ºC and alcoholic fermentations proceeded without inoculation. All samples were plated onto WL media and lysine media after appropriate dilutions and incubated @ 25 ºC for 2-3 days. Plates were then counted and colonies examined on WL media in order to identify unique colony types based on color, shape, consistency, and size. Unique colony types were restreaked and colonies purified on WL medium. These isolates were then stored on agar slants (potato dextrose agar) at 4ºC.

**Objective 2.**
**Investigate the behavior and ability of selected non-*Saccharomyces* yeast species of hydrolyzing grape-derived volatile glycosides on isolated grape glycoside substrate.**

Isolates from 2010 fermentations that demonstrated β-glucosidase activity on 4-MUG plates (Charoenchai et al. 1997) were tested for β-glucosidase activity using a liquid assay. Three different sugar concentrations and two different temperatures were tested. Media was adjusted to either 5 g/L glucose, 20 g/L glucose, or 100 g/L glucose and 100 g/L fructose (mimicking a grape juice containing 20 °Brix). The two temperatures tested were 25°C and 8°C. The conditions of 20 °Brix and 8°C were tested so as to model conditions present during a pre-fermentation cold maceration. Non-*Saccharomyces* species assessed were *Metschnikowia pulcherrim, Hanseniaspora uvarum, Kluveromyces thermotolerans, Hansenula anomala,* unknown #10 (suspected to be *Toralospora*), and a commercially available *Toralospora delbrueckii* (PRELUDE, Chr. Hansen).

Grape glycosides and other C13-norisprenoids precursors were isolated from both Pinot noir and Riesling grapes using solid phase extraction reported previously (*1, 2*), with some minor modifications. Destemmed grape samples were grinded to fine powder under liquid nitrogen to prevent oxidation and other enzymatic reactions. The grape power (50g) was mixed with 60 g sodium chloride and 50 ml 0.2 M citrate buffer (pH=3.2) in an aluminum foil wrapped centrifuge bottle to prevent light exposure. The mixture solution was shaken for 24 hours at room temperature. The mixture was then centrifuged at 10 000 rpm for 20 min at 4˚C. The supernatant was collected and then filtered through a filter paper under vacuum. Glycosides and other C13-norisprenoids precursors were isolated using C18 reversed-phase solid phase extraction

cartridges (10 x 500 mg each). Each cartridge was pre-conditioned with 10 ml of methanol, then with 10 ml of Milli-Q water. The clear grape extract (20 ml) was loaded onto the C18 cartridge at a flowrate of 1-2 ml /min. The cartridge was washed with 20 ml of Milli-Q water to remove sugar, acids and other water soluble components, the free volatile compounds were further washed off using 20 ml of dichloromethane. The glycosides and other precursors were finally eluted with 10 ml of Methanol. The glycosides were kept in methanol in freezer and ready for further research. The glycosides are being used as substrates to study the true glycosides hydrolysis behavior of the isolated non-*Saccharomyces* yeast strains. The research is still under progress.

**Objective 3.**
**Investigate volatile generation and bound flavor release in wine by selected non-*Saccharomyces* yeasts and their impact on wine flavor and aroma quality.**

Non-*Saccharomyces* yeast isolated from grapes undergoing cold maceration in 2010 were used to perform fermentations in Pinot noir. Pinot noir grapes (Woodhall Vineyard, Alpine, OR) were harvested at maturity and stored at 4°C overnight. 3 kg destemmed grapes were placed in food saver bags and 30 mg/L $SO_2$ was added. The grape were then treated by HHP (5 min @ 80,000 psi) to deactivate microbial cells and transferred aseptically to a 3L micro-fermentors and allowed to warm to room temperature before being inoculated with a non-*Saccharomyces* yeast at approximately 1 x $10^4$ CFU/mL. The yeast species inoculated were *Metschnikowia pulcherrim*, *Hanseniaspora uvarum*, *Kluveromyces thermotolerans, Hansenula anomala*, and an unknown non-*Saccharomyces* isolate (suspected to be *Toralospora*). One set of fermenters was not inoculated with any yeast (control) while another set was inoculated with all five non-*Saccharomyces* yeast species. All treatments were performed in triplicate. Fermenters were placed in a cold room at 10°C for seven days except treatment H, which was maintained at 27°C during the entire fermentation. At the completion of the cold maceration the fermenters were placed in a temperature controlled room at 27°C and inoculated with *S.cerevisiae* RC212 at approximately 1 x $10^5$ CFU/mL. All treatments were supplemented with a sterile solution of Fermaid K at 0.25 g/L. At the completion of the alcoholic fermentation the wines were pressed, a 30 mg/L $SO_2$ was made, and wines were settled at 4 °C for 120 hrs. The wines were then were taken for volatile analysis by GC-MS.

Table 1. Non-*Saccharomyces* yeast fermentation treatment (n=3)

| Treatment | Cold Soak | Fermentation |
|---|---|---|
| A | Cold Soak-No Microorganisms | RC212 |
| B | Cold Soak- *Metschnikowia pulcherrima* | RC212 |
| C | Cold Soak- *Hanseniaspora uvarum* | RC212 |
| D | Cold Soak- *Kluveromyces thermotolerans* | RC212 |
| E | Cold Soak- *Hansenula anomala* | RC212 |
| F | Cold Soak-Unknown Species #10 | RC212 |
| G | Cold Soak-Mixture of all non-sacc species above | RC212 |
| H | NO Cold Soak, 27°C | RC212 |

Wine samples (2 mL) were diluted with 8 mL saturated salt water in a clean 20ml auto-sampler glass vial and 20 µL of internal standard solution (containing 1.5mg/mL methyl propionate, 38.4 mg/L 3-heptanone, 47.5 mg/L hexyl formate, 43.9 mg/L 4-octanol and 41.7 mg/L octyl propionate) were added. The vials were tightly capped with Teflon-faced silicone septa. A SPME fiber coated with divinyl benzene /Carboxen/ polydimethylsiloxane phase (2 cm long, 50/30 µm) was used for the extraction of volatile compounds. The vials were placed in an automatic headspace sampling system and samples were pre-incubated at 50 ℃ for 30 min, and then extracted for 30 min at 50℃.

The volatile compounds extracted by SPME fiber were analyzed using a HP6890 gas chromatograph equipped with a 5973 mass spectrometry detector (MSD) system (Agilent Technologies, Palo Alto, CA) and fitted with a ZB-Wax capillary column (30 m X 0.25mm X 0.5 µm). Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The inlet temperature was 250 ℃. The desorption was performed in the split mode with a split ratio of 2:1. The oven temperature program was 35°C for 4 min, followed by an increase of 5℃/min to 230℃. The final temperature was held for 10 min. The MSD in scan mode was used. The electron impact (EI) energy was 70 eV, the MS transfer line and ion source temperature were 230 ℃ respectively. Electron impact mass spectrometric data from m/z 35-350 were collected.

V. Summary of Major Research Accomplishments and Results:

β-glucosidase activity of non-*Saccharomyces* isolates from 2010 was further characterized utilizing a liquid media assay. Initial experiments demonstrated that isolates that demonstrated β-glucosidase activity on MUG plates also had large levels of activity in media at wine pH containing 5 g/L glucose (see detailed report from James Osborne). Even at grape must conditions, β-glucosidase activity remained high for other species such as *Metschnikowia, Hansenula,* and unknown isolate #10. While the enzyme activity was reduced at cold temperature (8C) as observed for *Metschnikowia*, the enzyme activity was actually increased for other yeast isolates such as *Kluveromyces*, *Hansenula*, and *Torulospora* It appears that the high β-glucosidase activity observed for these yeast isolates is not due to elevated growth at cold temperatures but rather is likely due to increased production of β-glucosidase.

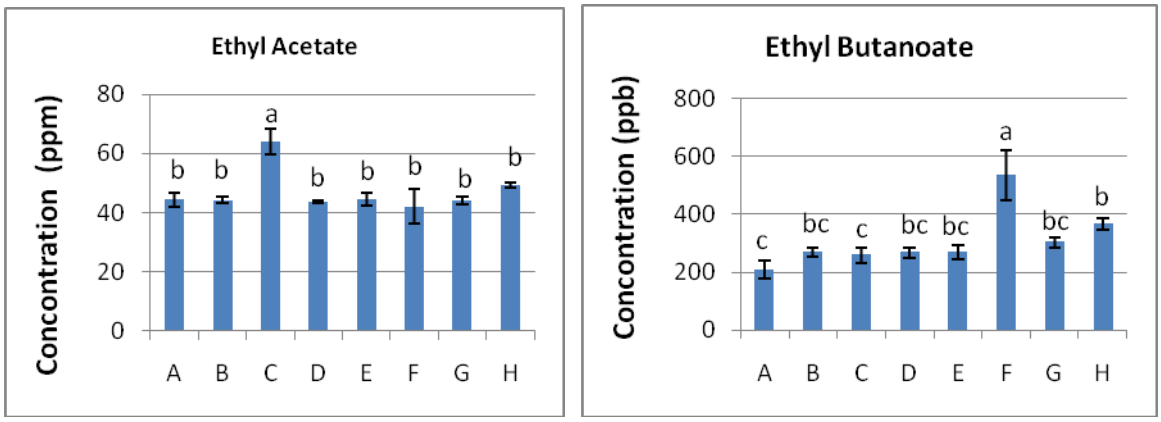
Yeast isolates demonstrating β-glucosidase activity were used to perform fermentations on Pinot noir grapes. When *S.cerevisiae* RC212 alone was inoculated alcoholic fermentation was completed in 13 days. The non-*Saccharomyces* isolates all grew well during the cold maceration, including the mixture. After inoculation of *S. cerevisiae* RC212 at the end of cold maceration, the growth of the non-*Saccharomyces* isolates differed. *Metschnikowia* and *Hanseniaspora* remained at high viable cell counts for 3 days prior to a rapid decline while *Kluveromyces thermotolerans* viable cell counts remained high throughout the alcoholic fermentation. In contrast, viable cell counts for *Hansenula anomala* and unknown isolate #10 declined below detectable limits after the inoculation of *S. cerevisiae* RC212. The length of alcoholic fermentation varied depending on the non-*Saccharomyces* isolate present. Fermentations containing *Hanesnula*, unknown isolate #10, and the combination of all isolates, were completed in 12 days. Fermentations containing *Metschnikowia* and *Hanseniaspora* were completed in 13 days while the fermentations containing *Kluveromyces* took 15 days to complete (see James

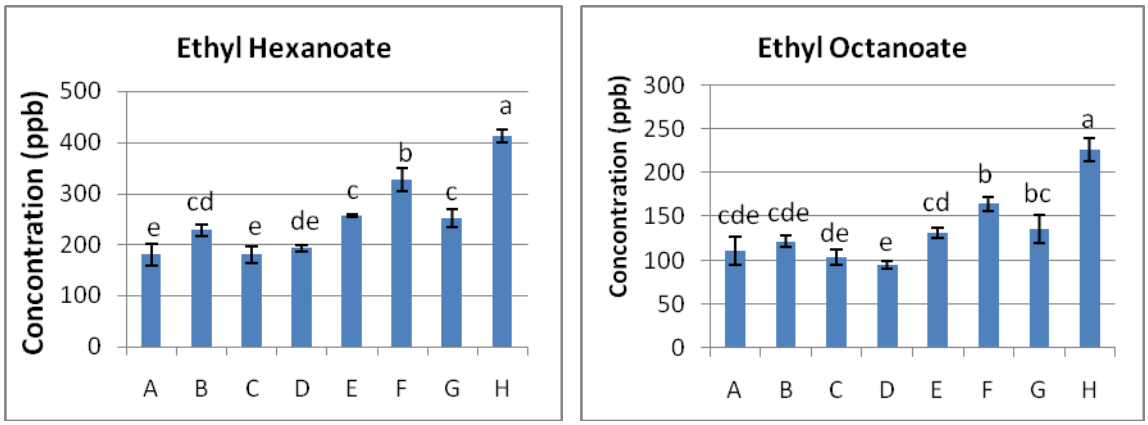
Osborne's report for detail). These fermentation differences affected the fermentation derived volatile composition as well as grape derived compounds.

A total of more than 50 volatile compounds were identified with the SPME-GC-MS method, among which, 40 compounds were quantified. The quantified compounds included fermentation esters, alcohols, acids, terpene alcohols, and $C_{13}$-norisoprenoids from all different fermentation treatments. A selection of some of the key aroma-active compounds was further discussed.

**Esters**

Ester was one of the major classes of aroma-active compounds analyzed in this study. Esters are the most abundant class of volatile compounds present in wine except alcohols and acids. The most abundant ethyl esters are ethyl acetate, butanoate, hexanoate, octanoate, and decanoate. Ethyl esters are important contributors to wine flavor because they are present in high concentrations and have low sensory thresholds (*3*, *4*).



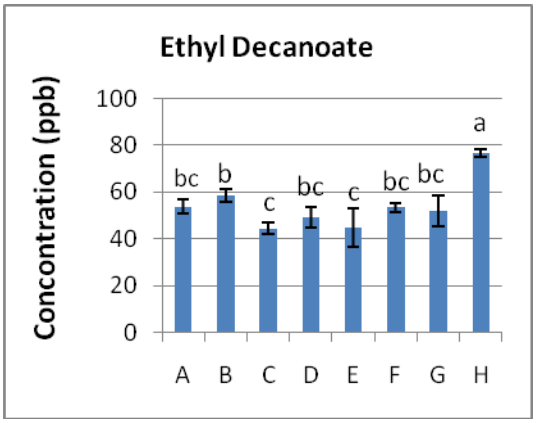



**Fig.1 Ethyl esters in Pinot noir wines by different fermentation yeast**

Ethyl acetate had the highest concentration ranging from 40 to 60 ppm. The results have shown that cold soak with *Hanseniaspora uvarum* (Treatment C) produced much higher ethyl acetate than other treatments (Fig. 1). Ethyl acetate had nail-polish aroma, and high concentration in wine can cause nail-polishing defect. For other ethyl esters, cold soak with the Unknown Species #10 (Treatment F) increased the concentration of ethyl butanoate in wine than other treatments. Meanwhile, the same treatment produced the highest ethyl hexanoate and ethyl octanoate among the cold soak treatments. Cold soak with species *Metschnikowia pulcherrima* (Treatment B) and *Hansenula anomala* (Treatment E) all produced higher ethyl butanoate and ethyl hexanoate than cold soak with no microorganism (Control), while cold soak with *Hanseniaspora uvarum (*treatment C*)* and *Kluveromyces thermotolerans* (Treatment D) had no effect on the production of these two esters. The Cold Soak with mixture of all non- Saccharomyce species (Treatment G) produced all these ethyl esters slightly higher than the cold soak with no microorganisms while usually lower than specific cold soak with single species.

In general, cold soak reduced the production of ethyl esters in most cases. As shown in Fig. 1, no cold soak (Treatment H) produced the highest amount of ethyl hexanoate, ethyl octanoate and ethyl decanoate, and the second highest amount of ethyl butanoate only to the cold soak with the Unknown Species #10 (F). The calculated Odor Activity Value was listed in Table 2.

Branch-chained esters such as isoamyl acetate, 2-methylbutyl acetate, ethyl isobutyrate, isobutyl acetate were also highlighted because of their low sensory thresholds (*3, 4*). They normally contribute to the characteristic fruity aromas of the wine even at low ppb concentrations.

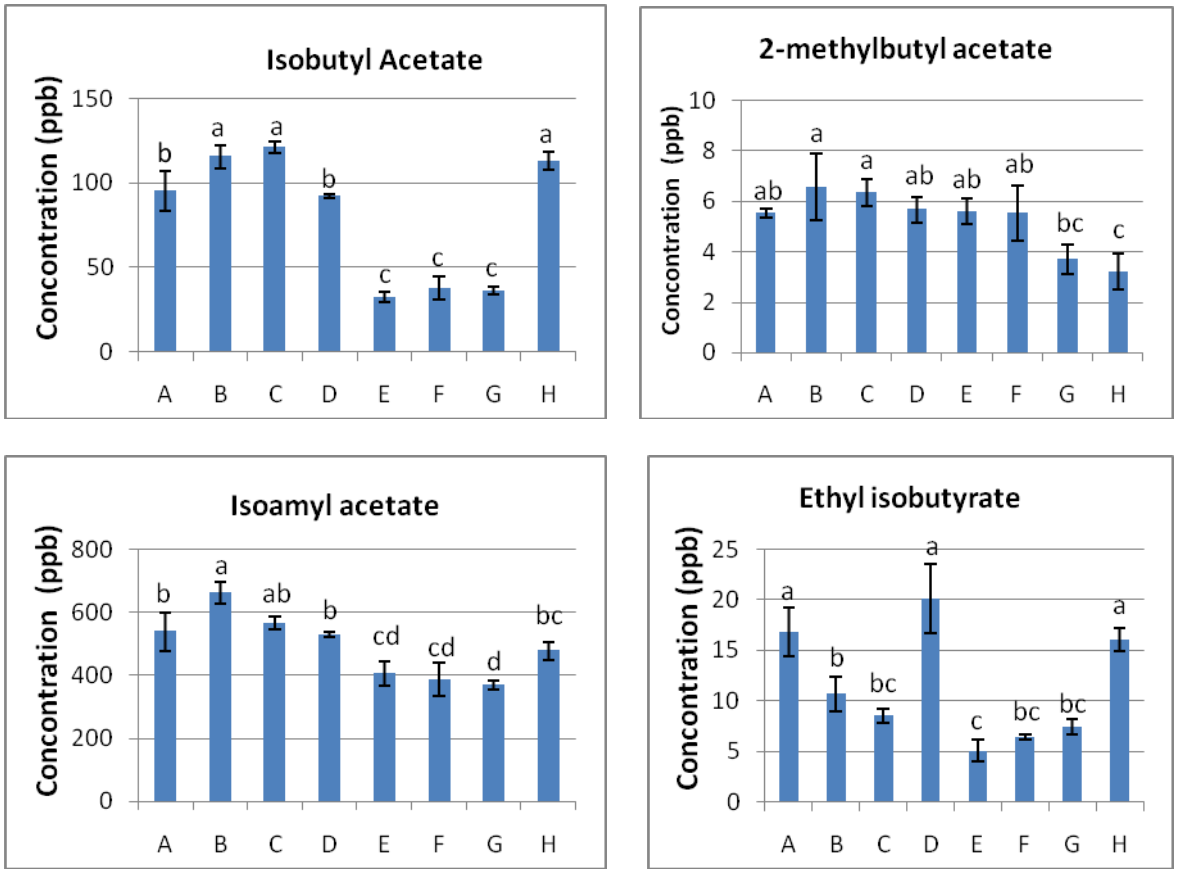



**Figure 2. Branch-chained esters in Pinot noir wines by different fermentation yeast**

The fermentation yeasts produced the branch-chained esters differently from the ethyl esters (Figure 2). Isoamyl acetate, which exhibits a banana-like aroma, had a high concentration (400-600 ppb) in the wines. Only cold soak with *Metschnikowia pulcherrima* (B) had 30% higher production of isoamyl acetate than cold soak with no microorganisms (control), whereas cold soak with *Hansenula anomala* (E), Unknown Species #10 (F) and mixture of all species (G) all produced less. Opposite to the result of yielding higher straight-chain esters, the cold soak with *Hansenula anomala,* Unknown Species #10 and mixture of all species yielded much lower isobutyl acetate than cold soak without microorganisms. The control (A), cold soak with *Kluveromyces thermotolerans* (D), and no cold soak (H) had higher amount of ethyl isobutyrate.

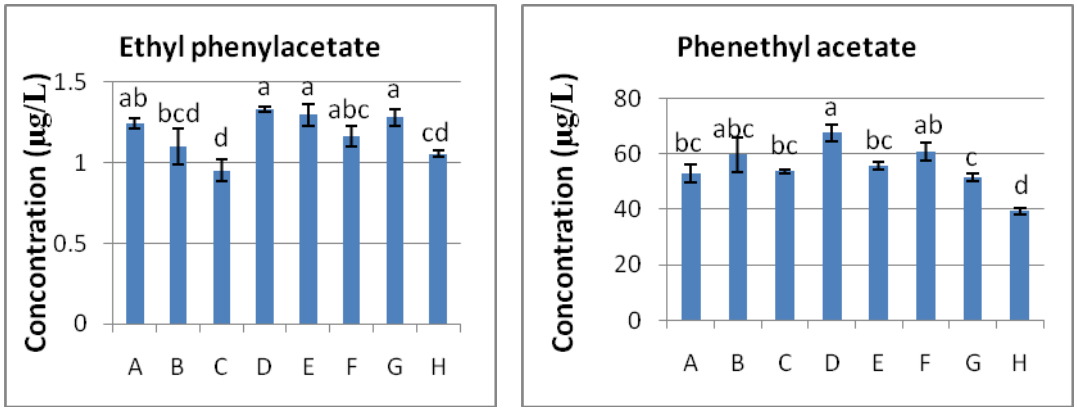
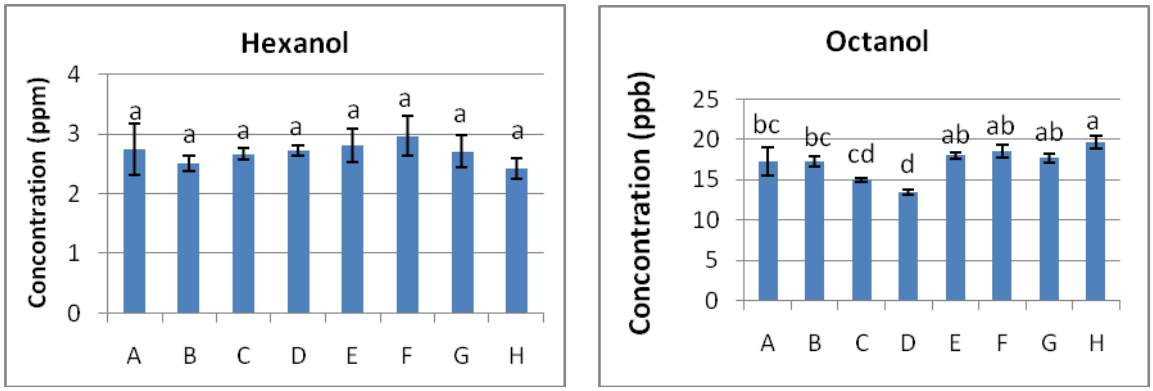



**Figure 3. Aromatic esters in Pinot noir wines by different fermentation yeast**

Aromatic esters such ethyl 2-phenylacetate and 2-phenethyl acetate were also compared among treatments. The concentration of 2-phenylethyl acetate ranged from 40 to 70 µg/L, below its sensory threshold of 250 µg/L and within the range reported in literature for red wines (*3*) . Ethyl phenylacetate and phenethyl acetate were all similar among cold soak treatments, but higher than no cold soak treatment (H).

**Alcohols**

Among the alcohols quantified, isobutyl alcohol, isoamy alcohol and 2-phenylethanol had very high concentration. Isobutyl alcohol had concentrations from 40 to 110 ppm, higher than its sensory threshold of 40 ppm(*5*). Isoamy alcohol had concentrations five times higher than its sensory threshold of 30 ppm (*5*). Both isobutyl alcohol and isoamyl alcohol should contribute to wine aroma. 2-Phenylethanol showed high concentration (25 to 35 mg/L) in the experimental wines. This compound has a sensory threshold of 1 mg/L in water(*6*), and exhibit rosy and honey aromas to the wine, and has been reported as a important aroma compound in Pinot noir wines (*7, 8*). Benzene alcohol, which was described as floral, also was present at concentration approaching mg/L level in the wine samples.



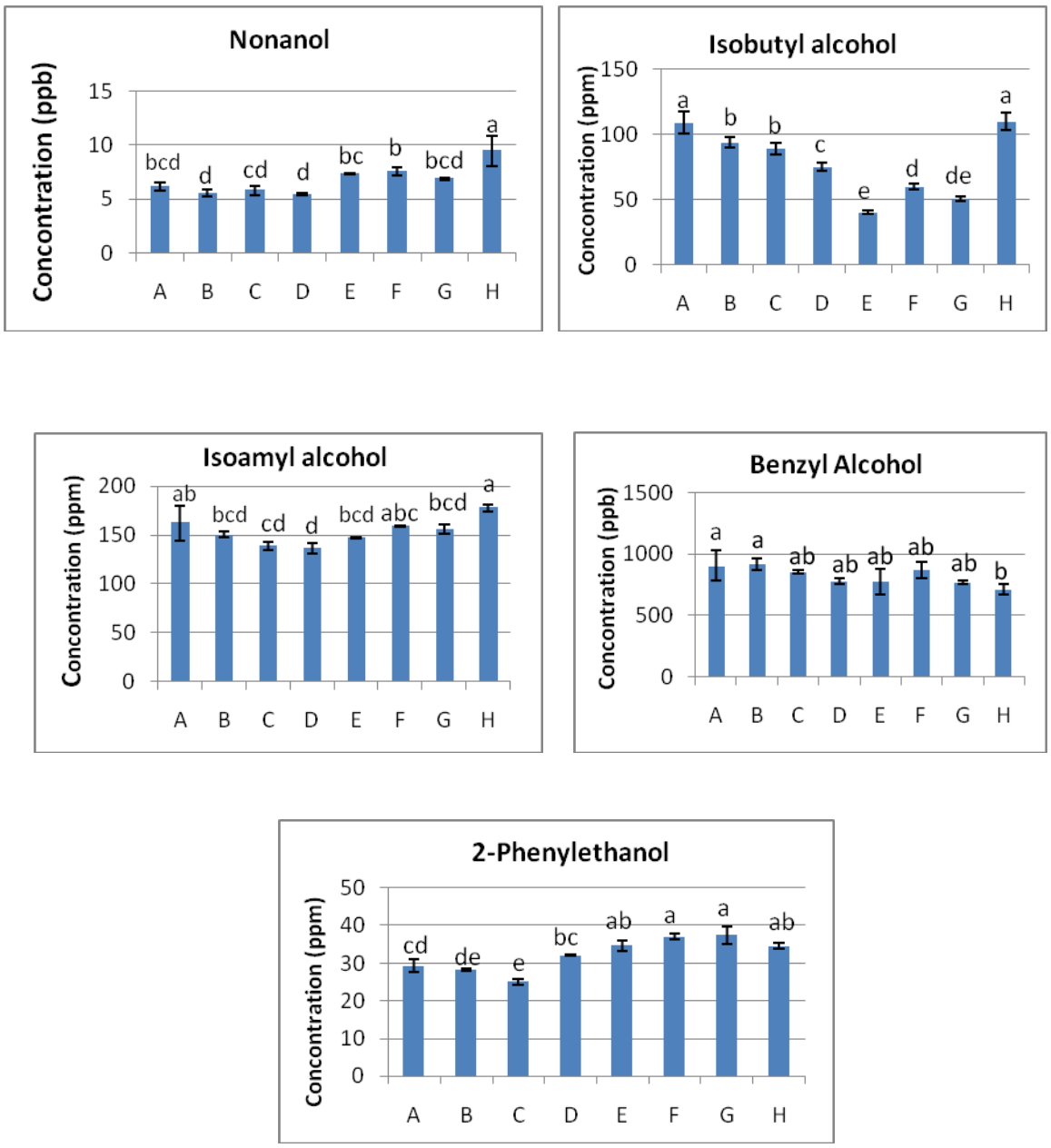



**Figure 4. Alcohols in Pinot noir wines by different fermentation yeast**

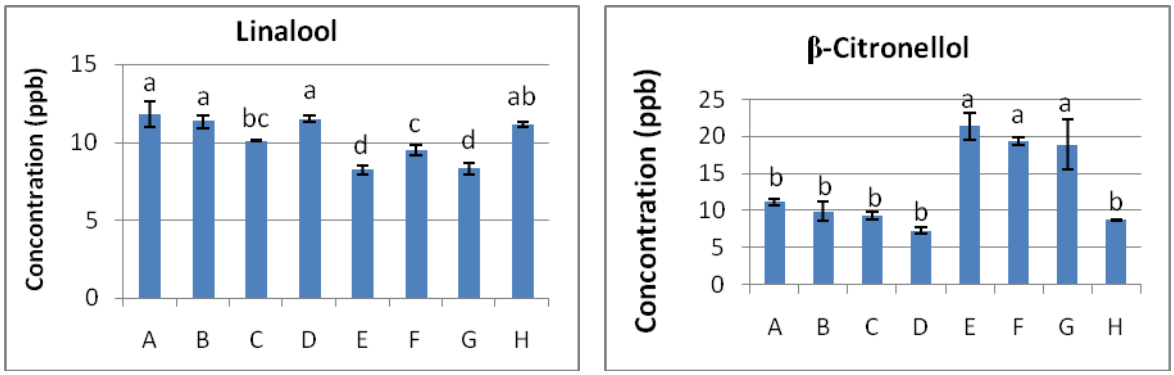
The results showed that the concentration of most alcohols were very similar among treatments (Figure 4). Again, the wines from the cold soak with *Hansenula anomala* (E), Unknown Species #10 (F) and mixture of all species (G) had much lower isobutyl alcohol than other treatments. In contrast, the wines from cold soak with *Kluveromyces thermotolerans, Hansenula anomala,* Unknown Species #10 and mixture of all species produced slightly higher 2-phenylethanol than other treatments.

**Terpene alcohols**

It had been widely reported that monoterpene alcohols are responsible for the characteristic floral aroma in grapes and wines. Monoterpenes belong to the secondary plant constituents, of which the biosynthesis begins with acetyl-coenzyme A (CoA) (9). They are largely present in the skin of the grapes, and glycoside precursors are the most abundant form (10), which varies with different varieties of grapes (11).

Enzymatic hydrolysis of terpene alcohol glycoside releases free terpene alchols to the wine. This hydrolysis involves two steps (*10*). In the first step, an α-L-rhamnosidase and an α-L-arabinofuranosidase or a β-apiofuranosidase (depending on the structure of the aglycone moiety) cleave 1,6-glycosidic linkages to give monoterpenyl β-D-glucosides. In the following step, β-glucosidase hydrolyzes the monoterpenyl β-D-glucosides to give monoterpene alcohols (*12*). Grapes have β-glucosidase activity but very low a-rhamnosidae, a-arobinosisade or β-apiosidase activities. In addition, grape β-glucosidase has very low activity at acidic pH (*13*). Wine yeasts have some β-glucosidase activities, but depending upon the origin of the yeasts, the β-glucosidase activities can be either inhibited by sugar or alcohol (*14-16*). The glycoside concentration and composition of grape must can also induce some yeast strains to generate β-glucosidase activites.

Besides enzymatic hydrolysis, acidic hydrolysis can also release the monoterpene alchols from their precursors. It has been confirmed that the progressive release of aroma with long periods of mild acid hydrolysis is reflected in the increase in intensity of the aroma attributes in wines undergoing natural aging (*17*).



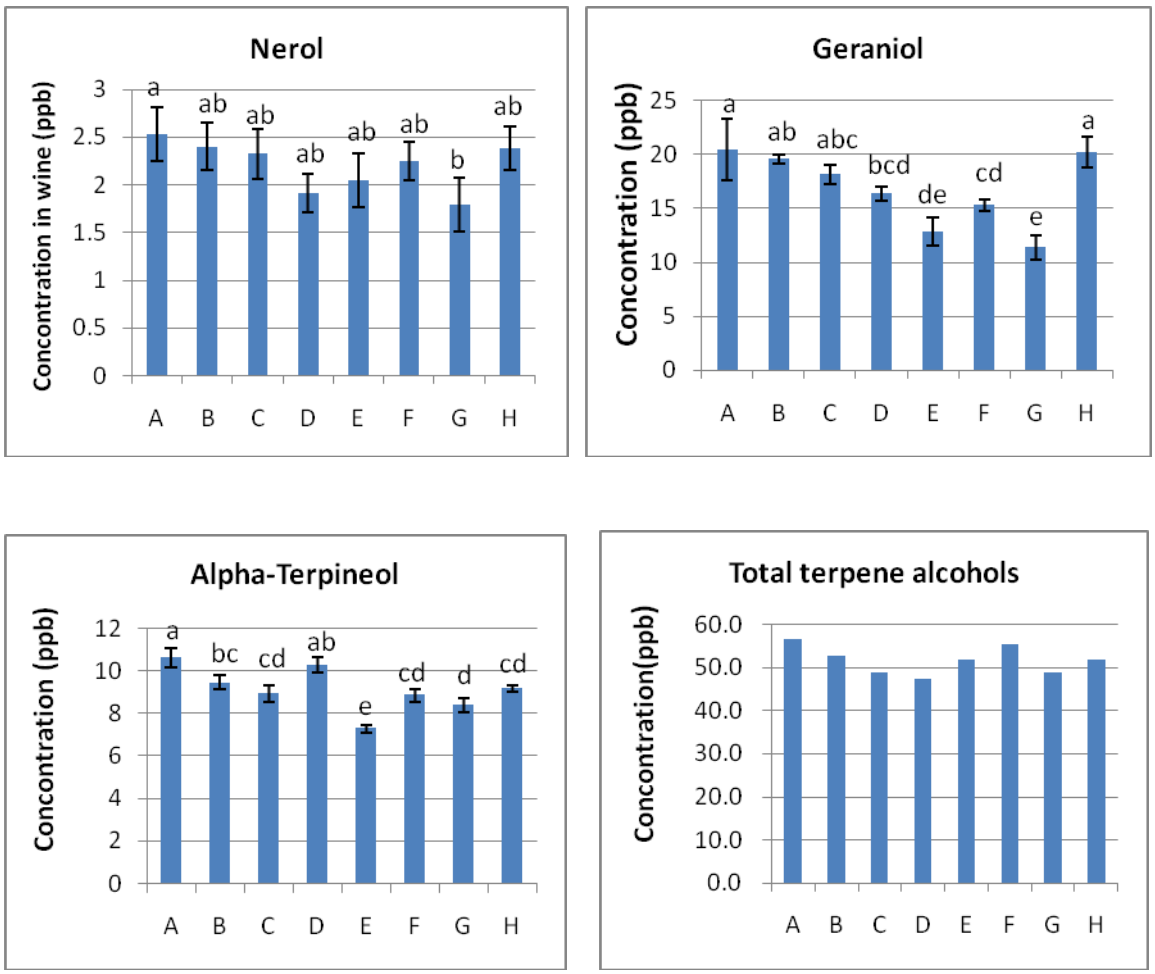



**Figure 5. Terpene alcohols in Pinot noir wines by different fermentation yeast**

In this study, five monoterpene alcohols including linalool, geraniol, citronellol, nerol and alpha-terpeniol were compared. The concerntration of linalool, nerol, geraniol, and α-terpineol among different treatments were not very different. In many cases, the wine with cold soak without microorganisms had the highest concentration, which was close to the wine with no cold soak treatment. However, the concentration of β-citronellol was highly affected by the cold soak treatment, the wines from cold soak with *Hansenula anomala (E),* Unknown Species #10 (F) and mixture of all species (G) had as much as twice of β-citronellol than the no cold soak and other treatments. This result support the enzyme activity data that β-glucosidase activity of some non-*sacchararomyces* yeasts decreased dramatically at grape composition, *Metschnikowia, Hansenula*, and unknown isolate #10 still retained high β-glucosidase activity. This data supports the hypothesis of this research that non-*Saccharomyces* yeast can contribute significantly to a wine's final flavor and aroma (*18-24*), because many non-*Saccharomyces* yeasts can produce significantly higher amounts of β-glucosidase than S. cerevisiae (*25-27*). However, the total amount of terpene alcohols did not differ much. The conversion of terpene

alcohol in wine is complicated, further studies with isolated glycosides and grapes with higher terpene alcohol contents will provide more insight about glycosides hydrolysis during wine making and aging process.

**$C_{13}$- Norisoprenoids**

β-Damascenone, which has a scent reminiscent of exotic flowers with a heavy fruity undertone, is variably described as apple, rose, and honey, with a sensory threshold reported as low as 0.002 µg/L (6). The concentrations of β-damascenone were very similar among the eight treatments except that the cold soak with Hanseniaspora uvarum and Kluveromyces thermotolerans, which had slightly lower concentration. Despite its low concentration, β-ionone is a significant contributor to the aroma of red wine due to its low sensory threshold (0.007 µg/L) (6). β-Ionone has a distinct berry and violet-like aroma, its concentration in the wine was 1.3 µg/L, much higher than its sensory threshold. The result showed that β-ionone concentrations were nearly the same among all the treatments regardless cold soak or not.

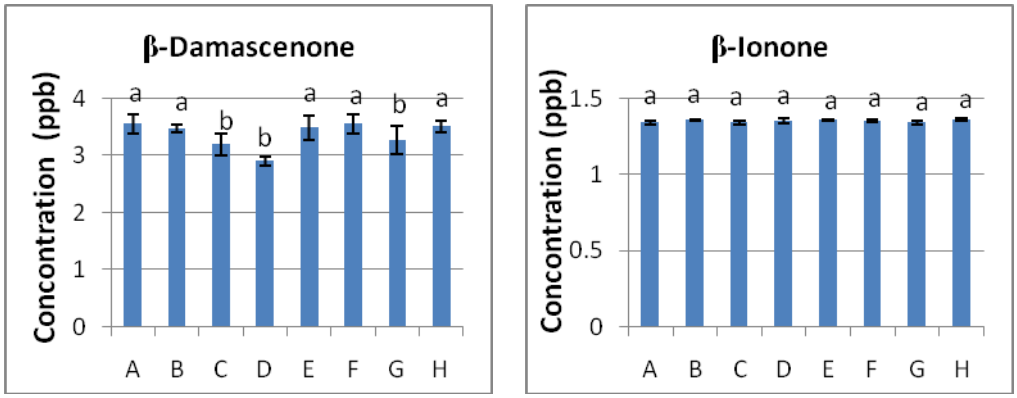
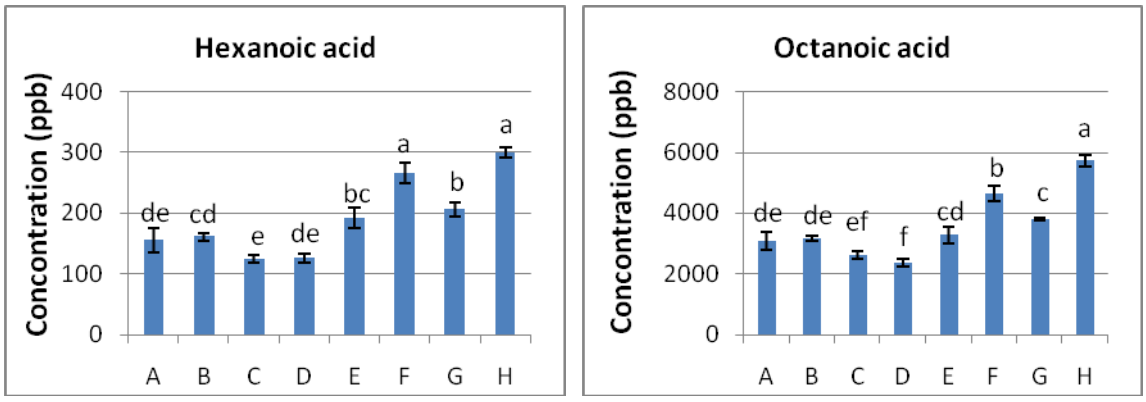



**Figure 6. $C_{13}$-norisoprenoids in Pinot noir wines by different fermentation yeast**

**Acids**



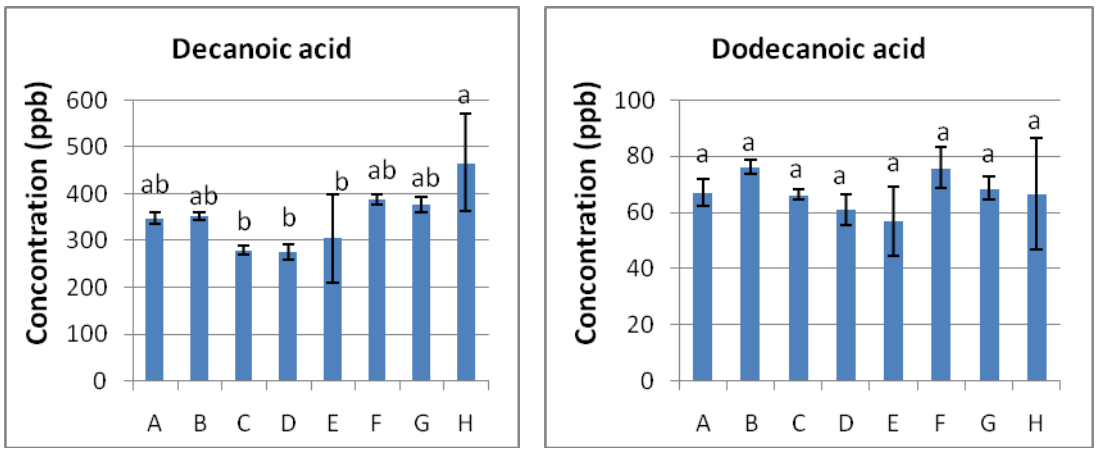



**Figure 7. Organic Acids in Pinot noir wines by different fermentation yeast**

The hexanoic acid and octanoic acid showed the same trend, in which the cold soak with Unknown Species #10 yielded highest concentration, followed by the cold soak with *Hansenula anomala,* and mixture of all species. Meanwhile, for hexanoic acid, octanoic acid and decanoic acid, the no cold soak treatment was all higher than the cold soak treatments.

**Conclusion**

The study was aimed to understand the effect of some non-S*accharomyces* species on volatile compound production in Pinot Noir wines. Through detailed volatile analysis, we conclude that different yeasts performed differently with volatile production. The cold soak with *Hansenula anomala* and Unknown Species #10 can produce more major ethyl esters, major alcohols, β-citronellol as well as some major acids. Meanwhile, they produce less branched-chain esters and monoterpene alcohols such as linalool, geraniol and nerol. Cold soak with non-S*accharomyces* species did not change β-damascenone and β-ionone concentration. Based on all the effect, the quality of wines from different treatments needs to be evaluated by a sensory test to confirm the effectiveness of *Hansenula anomala* and Unknown Species #10.

VI. Outside Presentations of Research:

Results from this research were presented at the 2011 OWRI Research Colloquium (August 25$^{th}$) and will be presented at the 2012 ASEV annual meeting in Portland (June 18$^{th}$ – 22$^{nd}$). Results have also been presented at Willamette Valley enology technical group meetings.

1. Qin Zhou, James Osborne, Michael C. Qian. Non-*Saccharomyces* yeast on Pinot noir wine volatile composition. 2011 OWRI Research Colloquium (August 25th)
2. Harper, H., Qian, M., and *Osborne, J.P. 2011. Impact of non-*Saccharomyces* yeast on wine quality: isolation of yeast with β-glycosidase activity. In: Proceedings of Oregon Wine Research Institute Viticulture & Enology Colloquium, Corvallis, OR. Aug 25.
3. Qin Zhou, James Osborne, Michael C. Qian. The effect of Non-*Saccharomyces* yeasts on Pinot noir wine volatile composition. 2012 ASEV national meeting (to be submitted).
4. Qin Zhou, James Osborne, Michael C. Qian. Selected Non-*Saccharomyces* yeasts affected terpene alcohol, b-damanscenone, but not b-ionone in Pinot noir wine. 2012 ASEV national meeting (to be submitted).

VII. Research Success Statements:

The first part of this project identified non-*Saccharomyces* species present during cold maceration and alcoholic fermentation of Pinot noir grapes from two different vineyards. These isolates were then screened for β-glucosidase activity and further characterized. The results revealed that high sugar content suppressed β-glucosidase activity for some non-*Saccharomyces* species but not others. Furthermore, at cold maceration temperatures (8-10°C) the β-glucosidase activity of many of the non-*Saccharomyces* isolates increased dramatically possibly due to elevated production of the enzyme at cold temperatures.
Fermentation of Pinot noir grapes with the selected non-*Saccharomyces* species resulted in different volatile profiles in the wine. Cold soak with non-*Saccharomyces* species changed volatile alcohol, short chain fatty acids, and esters composition. In addition, cold soak with *Hansenula anomala* and Unknown Species #10 dramatically increased the release of β-citronellol in the wine. However, the cold soak with selected non-*Saccharomyces* species did not increase β-damascenone and β-ionone concentration. The data demonstrated that non-*Saccharomyces* species have enzyme systems that are active at grape conditions, and can alter terpene alcohols and β-damascenone composition in wine. It is possible to find non-*Saccharomyces* species to enhance the production of β-damascenone and β-ionone, the most important compounds in red wine.

VIII. Fund Status:

A graduate student, Qin Zhou, has been working on this project. The requested budget was $34,275 with a full time student (0.49 FTE) working on the project. However, the project was heavily cut to $22,000. After the cost of chemicals, gases, supplies and other routine operation cost, the student can only be supported at 0.35 FTE level from this project due to the limited the budget. Regardless, the PI and the student have been stretching the fund to get the most out of it. All the remaining fund has been committed for the salary of the student.

VIV. References

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**Table 2.** Odor Active Value (OAV)[b] of Volatile Compounds in Pinot Noir Wines from 8 treatments

| Compounds | Odor threshold[a] | Description | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Ethyl Esters of fatty acid*** | | | | | | | | | | |
| Ethyl acetate | 7500[2] | Fruity , solvent | 5.9 | 5.9 | 8.5 | 5.8 | 5.9 | 5.6 | 5.9 | 6.6 |
| Ethyl propanoate | 2100[1] | Solvent, ripe strawberry | 0.03 | 0.03 | 0.04 | 0.04 | 0.03 | 0.03 | 0.03 | 0.02 |
| Ethyl butanoate | 20[2] | Strawberry, apple, banana | 10.4 | 13.4 | 12.9 | 13.4 | 13.4 | 26.7 | 15.2 | 18.4 |
| Ethyl Isobutyrate | 15[1] | Fruity | 1.1 | 0.7 | 0.6 | 1.3 | 0.3 | 0.4 | 0.5 | 1.1 |
| Ethyl pentanoate | 220[1] | Fruity, ester | 0.06 | 0.08 | 0.07 | 0.06 | 0.05 | 0.05 | 0.04 | 0.06 |
| Ethyl hexanoate | 14[2] | Pineapple, fruity, apple | 12.9 | 16.3 | 12.9 | 13.8 | 18.3 | 23.3 | 18.0 | 29.5 |
| Ethyl heptanoate | 220[1] | Pineapple, fruity | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Ethyl octanoate | 580[1] | Waxy, apple skin, fruity | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.3 | 0.2 | 0.4 |
| Ethyl decanoate | 200[1] | Waxy, soap, fruity | 0.3 | 0.3 | 0.2 | 0.2 | 0.2 | 0.3 | 0.3 | 0.4 |
| ***Acetates*** | | | | | | | | | | |
| Isobutyl acetate | 1600[1] | Waxy, fruity, apple, banana | 0.06 | 0.07 | 0.08 | 0.06 | 0.02 | 0.02 | 0.02 | 0.07 |
| 2-Methylbutyl acetate | - | Fruity, fatty,pleasant | - | - | - | - | - | - | - | - |
| Isoamyl acetate | 30[3] | Banana, fruity, sweet | 17.9 | 22.1 | 18.9 | 17.6 | 13.6 | 12.9 | 12.3 | 15.9 |
| Hexyl acetate | 670[3] | Pleasant fruity, pear,cherry | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Octyl Acetate | 800[1] | Waxy, fruity | 0.001 | 0.001 | 0.001 | <0.001 | 0.001 | 0.002 | 0.002 | <0.001 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Other esters*** | | | | | | | | | | |
| Ethyl phenylacetate | 73[1] | Flowery, rose, winy | 0.02 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 | 0.01 |
| Phenethyl acetate | 250[2] | pleasant, flowery | 0.2 | 0.2 | 0.2 | 0.3 | 0.2 | 0.2 | 0.2 | 0.2 |
| Diethyl succinate | 200,000[1] | Light fruity | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| ***Acids*** | | | | | | | | | | |
| Hexanoic acid | 420[2] | sweat, cheese | 0.4 | 0.4 | 0.3 | 0.3 | 0.5 | 0.6 | 0.5 | 0.7 |
| Octanoic acid | 500[2] | Rancid, harsh, cheese | 6.2 | 6.3 | 5.2 | 4.8 | 6.6 | 9.3 | 7.6 | 11.4 |
| Decanoic acid | 1000[2] | Fatty, unpleasant | 0.3 | 0.4 | 0.3 | 0.3 | 0.3 | 0.4 | 0.4 | 0.5 |
| Dodecanoic acid | 1000[1] | Dry, metallic, laurel oil flavor | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| ***Alcohols*** | | | | | | | | | | |
| Isobutyl alcohol | 40000[2] | Fusel, alcohol | 2.7 | 2.3 | 2.2 | 1.9 | 1.0 | 1.5 | 1.3 | 2.8 |
| Isoamyl alcohol | 30000[2] | Alcohol, harsh | 5.4 | 5.0 | 4.6 | 4.6 | 4.9 | 5.3 | 5.2 | 5.9 |
| Hexan-1-ol | 8000[2] | Green, grass | 0.3 | 0.3 | 0.3 | 0.3 | 0.4 | 0.4 | 0.3 | 0.3 |
| Heptan-1-ol | 1000[5] | Grape, sweet | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Octan-1-ol | 130[6] | Orange, Rose | 0.2 | 0.2 | 0.1 | 0.1 | 0.2 | 0.2 | 0.2 | 0.2 |
| Nonan-1-ol | 600[4] | Green | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 |
| Decan-1-ol | 400[4] | Orange flowery, special fatty | 0.003 | 0.003 | 0.003 | 0.002 | 0.003 | 0.003 | 0.003 | 0.004 |
| 2-Ethyl hexanol | 8000[4] | Mushroom, sweet fruity | 0.001 | <0.001 | <0.001 | 0.001 | 0.001 | 0.001 | <0.001 | 0.001 |
| Benzyl Alcohol | 200,000[5] | Sweet fruity | 0.005 | 0.005 | 0.004 | 0.004 | 0.004 | 0.004 | 0.004 | 0.004 |
| 2-Phenylethanol | 14000[3] | Roses | 2.1 | 2.0 | 1.8 | 2.3 | 2.5 | 2.6 | 2.7 | 2.5 |
| ***Terpenoids*** | | | | | | | | | | |
| Linalool | 25.2[3] | Flowery, fruity, muscat | 0.5 | 0.4 | 0.4 | 0.5 | 0.3 | 0.4 | 0.3 | 0.4 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| α-Terpineol | 250[3] | root, grass,anise | 0.04 | 0.04 | 0.04 | 0.04 | 0.03 | 0.04 | 0.03 | 0.04 |
| β-Citronellol | 100[2] | Green lemon | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 | 0.2 | 0.1 |
| Geraniol | 30[2] | Citric | 0.7 | 0.7 | 0.6 | 0.5 | 0.4 | 0.5 | 0.4 | 0.7 |
| Nerol | 300[7] | sweet rose | 0.008 | 0.008 | 0.008 | 0.006 | 0.007 | 0.007 | 0.006 | 0.008 |
| ***C13-norisoprenoids*** | | | | | | | | | | |
| β-Damascenone | 0.05[2] | apple | 71.0 | 69.4 | 63.8 | 57.9 | 69.8 | 70.9 | 65.3 | 70.2 |
| β-Ionone | 0.09[3] | Balsamic, rose, violet | 14.9 | 15.0 | 14.9 | 15.0 | 15.0 | 15.0 | 14.9 | 15.1 |

[a] OTH, Odor threshold, µg/L.

[b] OAV, Odor activity value was calculated by dividing concentration by odor threshold value of the compound.

The reference from which the odor threshold has been taken is given in parentheses. 1. Antalick et al. (2010). 2. Guth (1997). 3. Ferreira et al. (2000). 4. Ricardo López et al. (1999). 5. E. Gómez García-Carpintero et al. (2011).

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