

OWB FINAL REPORT-2015

I. Project Title:

Formation of volatile sulfur compounds in Pinot noir post-fermentation -part 2: Lees level and contact time on volatile sulfur compounds in wine

II. Principal Investigator:

Michael Qian, Department of Food Science and Technology, Oregon State University, Corvallis, OR. 97331, ph 541-737-9114; email: michael.qian@oregonstate.edu

Co-Principal Investigator:

J.P. Osborne, Department of Food Science and Technology, Oregon State University, Corvallis, OR. 97331, ph 541-737-6494; email: James.Osborne@oregonstate.edu.

Industry Collaborators:

Tresider Burns, Assistant winemaker, Lemelson Vineyards; tresider@gmail.com

Elizabeth Clark, Winemaker, Airlie; elizabeth@airliewinery.com

Dave Paige, Winemaker, Adelsheim Vineyards; dpaige@adelsheimvineyard.com

Graduate student: Qin Zhou

III. Summary:

The effect of lees contact time during wine barrel aging on volatile sulfur compounds was investigated in this study. Pinot noir wines were made using grapes from the Oregon State University vineyard and fermented with two different commercial yeast strains. One set of wines was produced using the low/no H₂S producing yeast strain *Saccharomyces cerevisiae* P1Y2 , and the other set was produced using *Saccharomyces cerevisiae* RC212. Fermentations were conducted in triplicate. At the completion of alcoholic fermentation, wines were pressed and split into three different lees treatments based on settling time (0, 24 and 72 hrs) which were named as heavy, medium and light lees treatment respectively in this progress report. Samples were collected at 0, 2 weeks, and 1, 2, 3, 6, 9 months. Volatile sulfur compounds were analyzed by solid phase microextraction-gas chromatography-pulsed flame photometric detection (SPME-GC-PFPD) method.

The results showed that hydrogen sulfide (H₂S), methylmercaptan (methanethiol) and methyl thioacetate (sulfur containing ester) were the major sulfur compounds of the concern in the

wines. Moreover, the concentration of H₂S was directly influenced by the type of yeasts in the first month of storage. Wines made from *Saccharomyces cerevisiae* P1Y2 generally had lower concentration of H₂S than those from *Saccharomyces cerevisiae* RC212. In addition, lower H₂S concentration was observed in light lees load than in medium or heavy lees load treatment from *Saccharomyces cerevisiae* P1Y2 during the first two weeks of aging, whereas more H₂S were generated in higher lees loading samples from *Saccharomyces cerevisiae* RC212. H₂S accumulated with time during early aging, and reached to the maximum at one month, then decreased afterwards regardless of types of lees or the amount of lees loaded. Methanethiol also accumulated during aging, and reached its maximum at 2-3 months, then decreased slowly afterwards. High level of methyl thioacetate was detected in the experimental wines, wines from *Saccharomyces cerevisiae* RC212 had substantially higher level of methyl thioacetate than those from *Saccharomyces cerevisiae* P1Y2 regardless of lees level, and the concentration of methyl thioacetate stayed consistent during barrel aging. Heavy lees generally lead to more dimethyl sulfide (DMS) accumulation. During the aging storage, DMS reached to peak level at 2-3 months, decreased at 6 months, and then continued accumulating at 9 months. The levels of other sulfur compounds (carbon disulfide, dimethyl disulfide and dimethyl trisulfide) were very low for flavor contribution.

The samples from collaborating wineries were also analyzed, but the results were complicated due to various treatments and remedies performed at winery. H₂S was the major volatile sulfur compound in those winery samples, especially at the beginning of the barrel aging. Some winery samples also had high levels of methyl thioacetate and methanethiol. Although various remedies were performed in wineries to remove H₂S, the levels of methyl thioacetate were very high in many of the wines after 6 to 9 months of aging. High concentration of methanethiol was also observed in many wines. More winery samples were studied for 2014 harvest and high levels of hydrogen sulfide, DMS and methyl thioacetate were detected in most of those wines.

We collaborated with Dr. James Osborne further studied the effect of YAN on volatile sulfur formation. Detailed results were submitted separately as a single report. The study showed that the levels of YAN did not affect the generation of MeSH, CS₂, DMDS and DMDS. However, the amount of YAN and the type of YAN did affect the formation of thioacetates by *Saccharomyces cerevisiae* UCD522. DAP addition generated higher thioacetates.

We also studied the effect of elemental sulfur on volatile sulfur composition. Although the residual sulfur affected hydrogen sulfide production during fermentation (see the separate report), sulfur addition did not affect the volatile sulfur compounds in the final wine except for

methyl thioacetate. Sulfur addition generated more methyl thioacetate by both *Saccharomyces cerevisiae* P1Y2 and UCD522.

High levels of methyl thioacetate could be an important issue for winery. Methyl thioacetate impart sulfur off-flavor in wine, it can also be converted to methanethiol that has a very low sensory threshold. The results suggested the methyl thioacetate and methanethiol could be the major culprits for sulfur off-flavor development during barrel aging. This new finding is significant and needs to have further investigation in future.

IV. OBJECTIVES AND EXPERIMENTS CONDUCTED TO MEET STATED OBJECTIVES:

Chemicals and reagents

Dimethyl sulfide (DMS), ethyl methyl sulfide (EMS), diethyl disulfide (DEDS) were obtained from TCI America (Portland, OR, USA); carbon disulfide (CS₂) was from EMD Chemicals Inc. (Billerica, MA, USA); dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), and isopropyl disulfide (IsoPropylDS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Diethyl sulfide (DES), methyl thioacetate (MeSOAc), ethyl thioacetate (EtSOAc) and 3-methylthiopropanol (methionol) were supplied by Alfa Aesar (Ward Hill, MA, USA). A cylinder of gaseous methanethiol (MeSH) and hydrogen sulfide (H₂S) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol was obtained from Fisher Scientific (Waltham, MA, USA). Ethanol (200 proof, KOPTEC USP) was purchased from VWR (Radnor, PA, USA). Acetaldehyde was purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydroxide (NaOH) was obtained from Mallinckrodt Chemicals (St. Louis, MO, USA). Water was purified through a Milli-Q purification system (EMD Millipore, Billerica, MA, USA).

Sample Preparation and SPME Extraction

Two milliliters of samples were placed in a 20 mL autosampler vial, and then diluted with 8 mL of Milli-Q water. A 100 μL aliquot of internal standard solution (500 ppb EMS and 2 ppb Isopropyl DS) and 50 μL of 5% acetaldehyde (w/v) were added to each vial. Duplicate analysis was performed for each wine sample. An automatic headspace sampling system (CombiPAL autosampler, CTC Analytics AG, Zwingen, Switzerland) equipped with an 85 μm Carboxen-PDMS SPME fiber (SUPELCO, Bellefonte, PA, USA) was used for extraction of organic sulfur compounds. Before use, the fiber was pre-conditioned in a split/splitless GC injector port at 300 °C for 1 hour. For quantification of all the sulfur compounds except methionol, the samples

were equilibrated at 30 °C for 5 min with agitation rate of 500 rpm and then extracted with the SPME fiber at headspace for 20 min with 250 rpm agitation at the same temperature. The samples were prepared one more set for quantification of methionol in the same method mentioned above. The only difference was that the equilibration and extraction temperature were set up at 50 °C instead of 30 °C while all other conditions including sample preparation and chromatographic conditions were kept the same.

Chromatographic Conditions by GC-PFPD

The analysis was performed on a Varian CP-3800 gas chromatography equipped with a PFPD detector (Varian, Palo Alto, CA, USA). Volatile compounds extracted with the SPME fiber were desorbed at 300 °C for 5.5 min in the injector. The separation was achieved using a DB-FFAP capillary column (30 m × 0.32 mm I.D., 1.0 µm film thickness, Agilent Technologies, Inc, Santa Clara, CA, USA). The oven temperature program was set up as follows: initial 35 °C held for 3 min, increased to 150 °C at 10 °C /min and held for 5 minutes, and then increased to 220 °C at 20°C /min, the final temperature held for 3 min. The total GC run time was 26 min. Nitrogen was used as the carrier gas at a constant flow rate of 2 mL/min. GC injection port was in splitless mode for 5 min at 300 °C. The PFPD was operated in the sulfur mode at 300 °C. All sulfur compounds were identified by comparing their retention times with that of pure standards.

Quantification

Sulfur standards and internal standard preparation

The MeSH standard stock solution was prepared by bubbling pure MeSH gas directly into cold methanol (−18 °C) and the concentration was calculated by weight. The hydrogen sulfide (H₂S) standard stock solution was made by bubbling pure H₂S gas into 0.5 M NaOH solution and the concentration was calculated by weight. Individual standard stock solutions of CS₂, DMS, DES, DEDES, MeSOAc, EtSOAc, DMDS, DMTS and Methionol were prepared by weighing a certain amount into specific volume of pure ethanol. All of the standard stock solutions were stored at −18 °C until use. Individual standard stock solutions of EMS and IsopropylDS were made by dissolving certain amount of standard in pure ethanol. An internal standard solution was prepared by diluting and mixing to the proper concentration containing 500 µg/L of EMS and 2 µg/L of IsoPropylDS. The internal standard solution was kept at −18 °C until use.

Calibration of standard curve

Standard addition method was used for calibration to eliminate the matrix interference on the accuracy of calibration. Two milliliters of wine spiked with different concentrations of sulfur standards were placed in a 20 mL autosampler vials and then diluted with 8 mL of Milli-Q water.

A 100 μ L aliquot of internal standard solution (500ppb EMS and 2ppb Isopropyl DS) and 50 μ L of 5% acetaldehyde were added to each vial. The vials were tightly capped with Teflon-faced silicone septa and placed in the automatic headspace sampling system. The SPME extraction conditions and chromatographic conditions were set as described above. In the way of sample preparation, a calibration curve for methionol was prepared separately with the extraction temperature at 50 °C. The standard curve for individual sulfur compounds was built up by plotting the square root of response ratio of target compound and its internal standard against the concentration ratio.

V. Summary of Major Research Accomplishments and Results:

Volatile sulfur compounds (VSCs) are generally considered as negative contribution to wine aroma. They usually give off very strong and offensive odors such as rotten eggs, rotten cabbage and garlic (1). It is very challenging for winemakers to eliminate the existence of VSCs because of their extreme low sensory threshold and the difficulty of removing them from wine once it's formed.

Wine aging on the wine lees during post-fermentation is a well-known technique to improve wine quality (i.e. increasing color stability, decreasing astringency or bitterness) (2). Sulfur off-flavor problems have been observed during wine aging on lees among Oregon wineries, and these problems are often more serious in cooler years such as 2007 and 2010. The formation of VSCs is thought to be associated with yeast metabolism of nitrogen compounds, particularly sulfur containing amino acids, methionine and cysteine (3). Glutathione, a cysteine containing tripeptide, which accounts for about 1% of dry weight of *Saccharomyces cerevisiae* and is involved in cellular redox buffering (4), can be another potential source of VSCs. It is our hypotheses that the amount of wine lees and lees contact time may affect the release of volatile sulfur precursors (i.e. glutathione and sulfur-containing amino acids) from lees to the wine, and cause sulfur off-flavor during barrel aging. In addition, lees composition may be affected by grape nutrients (particularly amino acid composition), and it is the lees composition that resulted in the release of volatile sulfur compounds during aging.

Eight volatile sulfur compounds were analyzed in Pinot noir wine produced in OSU winery by solid phase microextraction-gas chromatography-pulsed flame photometric detection (SPME-GC-PFPD) method. The concentrations of volatile sulfur compounds in the wines fermented with both two yeasts were relative low after pressing (see 0 week) due to small fermenters used

in this experiment. Although the concentrations of the sulfur compounds were all very low in our experimental wines, the volatile sulfur formation and accumulation patterns were very obvious.

As is shown in Table 1, type of yeasts directly affected the concentration of hydrogen sulfide in the first month of barrel aging. Wines made from *Saccharomyces cerevisiae* P1Y2, a low H₂S-producing yeast, generally had lower H₂S concentration than the wines made from *Saccharomyces cerevisiae* RC212, a high H₂S-producing yeast. Light lees load from *Saccharomyces cerevisiae* P1Y2 led to lower H₂S concentration than the medium or heavy lees load treatment during the first two weeks of aging. Higher lees load from *Saccharomyces cerevisiae* RC212 definitely generated more H₂S in the wines, three times more H₂S was generated in heavy treatment compared with the light treatment of lees load from *Saccharomyces cerevisiae* RC212.

H₂S accumulated with time during early aging and reached to the maximum at one month of aging, then decreased afterwards regardless of the types or amount of lees (Table 1). Interestingly, more H₂S accumulated in the treatment with light lees load, compared to the heavy lees treatment. This trend was further confirmed after 2 months of aging time and beyond. The data supported the hypothesis that the lees in the wine would absorb H₂S from the wine during barrel aging.

Methanethiol is a very potent sulfur compound with low sensory threshold of 1.8 ppb and it has a rotten egg or rotten vegetable odor. The evolution of methanethiol was different from H₂S (Table 2). The high H₂S-producing yeast *Saccharomyces cerevisiae* RC212 generated slightly more than *Saccharomyces cerevisiae* P1Y2 yeast. Methanethiol also accumulated during the lees aging, and reached the maximum at 2-3 months. Compared with H₂S, the total amount of methanethiol accumulation was comparatively less, and it also decreased much slower, and some methanethiol could still be detected at 9 months of storage.

Heavy lees load generally lead to more DMS accumulation. DMS reached to a peak level at 2-3 months of aging (Table 4). After decreased at 6 months of aging, it continued to accumulate at 9 months. Although DMS can give an aged wine bouquet at low level, high concentration of DMS usually causes sulfury off-flavor, which would be difficult for removal in winery.

Wines from *Saccharomyces cerevisiae* RC212 had substantially higher level of methyl thioacetate than from *Saccharomyces cerevisiae* P1Y2 yeast regardless of the lees levels. The concentration of methyl thioacetate stayed unchanged during the lees aging. The data suggested

that this offensive compound was primarily generated during alcoholic fermentation, and we did not see the impacts of lees aging on this compound, and lee will not absorb it from the wine.

Methionol is not a typical off-flavor compound although it has an earthy raw potato odor. Due to the high sensory threshold in wines, it barely impacts the flavor of wines. The wines made from *Saccharomyces cerevisiae* RC212 wine contained more methionol than those made from *Saccharomyces cerevisiae* P1Y2, both of which were still far below the sensory detection limit though. A slightly decreasing of its concentration was also observed during the aging process.

Other sulfur compounds (i.e. CS₂, dimethyl disulfide and dimethyl trisulfide) did not contribute to any flavor due to their low levels.

1. Results on wines from OSU winery trail

Table 1 Concentration of hydrogen sulfide in wines with 3 different lees level during 9 months of aging time($\mu\text{g/L}$)

H ₂ S	0 week	2 weeks	1 month	2 months	3 months	6 months	9 months
<i>Saccharomyces cerevisiae</i> PIY1							
P1Y2 Light	1.94±0.18	1.88±0.21	6.83±1.14	3.75±0.53	2.98±0.54	1.31±0.22	N.D.
P1Y2 Medium	2.47±0.10	2.38±0.20	3.52±0.72	2.17±0.33	2.01±0.19	1.47±0.24	N.D.
P1Y2 Heavy	2.61±0.47	2.74±0.12	4.89±0.23	2.15±0.27	1.78±0.23	1.30±0.07	N.D.
<i>Saccharomyces cerevisiae</i> RC212							
RC212 Light	2.66±0.29	2.15±0.32	5.63±0.37	2.68±0.44	2.24±0.68	1.20±0.18	N.D.
RC212 Medium	2.33±0.11	3.64±0.68	4.23±0.10	2.91±0.41	2.45±0.45	0.95±0.11	N.D.
RC212 Heavy	7.06±1.3	7.46±0.95	4.92±0.89	1.87±0.25	1.51±0.14	1.35±0.64	N.D.

Table 2 Concentration of methanethiol in wines with 3 different lees level during 9 months of aging time($\mu\text{g/L}$)

Methanethiol	0 week	2 weeks	1 month	2 months	3 months	6 months	9 months
<i>Saccharomyces cerevisiae</i> PIY1							
P1Y2 Light	1.00±0.01	1.38±0.19	1.33±0.22	2.45±0.45	2.04±0.33	1.78±0.11	1.15±0.15
P1Y2 Medium	1.00±0.14	1.33±0.13	1.28±0.10	1.68±0.30	2.15±0.18	1.84±0.05	1.81±0.22
P1Y2 Heavy	0.84±0.13	1.89±0.26	1.39±0.08	1.08±0.08	1.22±0.13	0.85±0.12	0.67±0.14
<i>Saccharomyces cerevisiae</i> RC212							
RC212 Light	1.49±0.10	1.63±0.05	1.54±0.06	1.45±0.12	1.93±0.14	1.69±0.18	1.40±0.16
RC212 Medium	1.10±0.02	1.62±0.04	1.56±0.10	1.19±0.20	1.32±0.22	0.55±0.06	N.D.
RC212 Heavy	1.42±0.15	1.70±0.01	1.55±0.14	1.64±0.13	1.44±0.20	0.75±0.11	0.72±0.10

Table 3 Concentraion of carbon disulfide in wines with 3 different lees level during 9 months of aging time($\mu\text{g/L}$)

CS_2	0 week	2 weeks	1 month	2 months	3 months	6 months	9 months
<i>Saccharomyces cerevisiae</i> PIY1							
P1Y2 Light	0.040 \pm 0.007	0.035 \pm 0.004	0.032 \pm 0.003	0.048 \pm 0.005	0.048 \pm 0.002	0.023 \pm 0.000	0.036 \pm 0.007
P1Y2 Medium	0.038 \pm 0.010	0.049 \pm 0.006	0.038 \pm 0.003	0.051 \pm 0.001	0.051 \pm 0.004	0.030 \pm 0.001	0.043 \pm 0.000
P1Y2 Heavy	0.034 \pm 0.004	0.047 \pm 0.002	0.044 \pm 0.009	0.057 \pm 0.006	0.054 \pm 0.003	0.030 \pm 0.004	0.048 \pm 0.008
<i>Saccharomyces cerevisiae</i> RC212							
RC212 Light	0.050 \pm 0.002	0.049 \pm 0.002	0.042 \pm 0.001	0.065 \pm 0.004	0.066 \pm 0.003	0.029 \pm 0.001	N.D.
RC212 Medium	0.053 \pm 0.003	0.055 \pm 0.002	0.041 \pm 0.004	0.068 \pm 0.004	0.062 \pm 0.007	0.029 \pm 0.004	N.D.
RC212 Heavy	0.043 \pm 0.002	0.056 \pm 0.004	0.047 \pm 0.001	0.078 \pm 0.002	0.076 \pm 0.002	0.051 \pm 0.002	0.065 \pm 0.005

Table 4 Concentraion of dimethyl sulfide in wines with 3 different lees level during 9 months of aging time($\mu\text{g/L}$)

DMS	0 week	2 weeks	1 month	2 months	3 months	6 months	9 months
<i>Saccharomyces cerevisiae</i> PIY1							
P1Y2 Light	1.59 \pm 0.20	1.94 \pm 0.22	1.91 \pm 0.16	3.75 \pm 0.34	3.25 \pm 0.11	2.76 \pm 0.07	5.98 \pm 0.24
P1Y2 Medium	1.10 \pm 0.04	2.02 \pm 0.15	1.81 \pm 0.14	3.69 \pm 0.22	3.26 \pm 0.19	2.94 \pm 0.25	6.03 \pm 0.56
P1Y2 Heavy	1.97 \pm 0.05	2.95 \pm 0.08	2.60 \pm 0.05	4.77 \pm 0.10	4.65 \pm 0.21	3.69 \pm 0.02	6.95 \pm 0.36
<i>Saccharomyces cerevisiae</i> RC212							
RC212 Light	1.89 \pm 0.08	2.07 \pm 0.10	1.79 \pm 0.08	3.17 \pm 0.37	3.20 \pm 0.28	2.66 \pm 0.12	5.53 \pm 0.22
RC212 Medium	1.88 \pm 0.27	2.01 \pm 0.13	1.79 \pm 0.13	2.95 \pm 0.59	2.96 \pm 0.37	2.49 \pm 0.20	5.70 \pm 0.53
RC212 Heavy	1.82 \pm 0.29	2.70 \pm 0.14	2.71 \pm 0.18	4.62 \pm 0.12	4.51 \pm 0.18	3.77 \pm 0.24	7.10 \pm 0.86

Table 5 Concentraion of methyl thioacetate in wines with 3 different lees level during 9 months of aging time($\mu\text{g/L}$)

Methyl Thiolacetate	0 week	2 weeks	1 month	2 months	3 months	6 months	9 months
<i>Saccharomyces cerevisiae</i> PIY1							
PIY2 Light	N.D.	0.67 \pm 0.01	0.58 \pm 0.03	0.66 \pm 0.04	0.94 \pm 0.10	0.66 \pm 0.05	0.35 \pm 0.03
PIY2 Medium	N.D.	0.44 \pm 0.15	0.58 \pm 0.09	0.55 \pm 0.09	0.52 \pm 0.05	0.74 \pm 0.07	0.33 \pm 0.02
PIY2 Heavy	0.40 \pm 0.02	0.83 \pm 0.05	0.56 \pm 0.04	0.56 \pm 0.10	0.49 \pm 0.08	0.64 \pm 0.08	0.35 \pm 0.03
<i>Saccharomyces cerevisiae</i> RC212							
RC212 Light	3.61 \pm 0.01	4.09 \pm 0.10	3.23 \pm 0.07	4.12 \pm 0.17	4.06 \pm 0.17	3.66 \pm 0.03	3.24 \pm 0.07
RC212 Medium	3.35 \pm 0.14	3.75 \pm 0.66	3.36 \pm 0.13	4.69 \pm 0.07	3.97 \pm 0.07	3.66 \pm 0.09	3.61 \pm 0.15
RC212 Heavy	3.25 \pm 0.51	4.54 \pm 0.09	3.10 \pm 0.08	3.73 \pm 0.11	3.48 \pm 0.11	2.98 \pm 0.06	2.37 \pm 0.07

Table 6 Concentraion of dimethyl disulfide in wines with 3 different lees level during 9 months of aging time($\mu\text{g/L}$)

DMDS	0 week	2 weeks	1 month	2 months	3 months	6 months	9 months
<i>Saccharomyces cerevisiae</i> PIY1							
PIY2 Light	N.D.	0.14 \pm 0.01	0.17 \pm 0.02	0.23 \pm 0.03	0.16 \pm 0.02	0.095 \pm 0.001	N.D.
PIY2 Medium	N.D.	0.11 \pm 0.02	0.20 \pm 0.04	0.25 \pm 0.02	0.21 \pm 0.03	0.091 \pm 0.01	N.D.
PIY2 Heavy	0.12 \pm 0.02	0.16 \pm 0.02	0.25 \pm 0.02	0.22 \pm 0.03	0.24 \pm 0.04	0.11 \pm 0.02	N.D.
<i>Saccharomyces cerevisiae</i> RC212							
RC212 Light	0.16 \pm 0.00	0.16 \pm 0.03	0.13 \pm 0.01	0.11 \pm 0.01	0.12 \pm 0.01	0.075 \pm 0.01	N.D.
RC212 Medium	0.09 \pm 0.03	0.12 \pm 0.01	0.19 \pm 0.03	0.11 \pm 0.02	0.11 \pm 0.02	0.093 \pm 0.01	N.D.
RC212 Heavy	0.22 \pm 0.04	0.087 \pm 0.015	0.17 \pm 0.03	0.15 \pm 0.02	0.17 \pm 0.02	0.096 \pm 0.01	N.D.

Table 7 Concentration of dimethyl trisulfide in wines with 3 different lees level during 9 months of aging time($\mu\text{g/L}$)

DMTS	0 week	2 weeks	1 month	2 months	3 months	6 months	9 months
<i>Saccharomyces cerevisiae</i> PIY1							
PIY2 Light	N.D.	0.040 \pm 0.002	0.12 \pm 0.02	0.096 \pm 0.01	0.021 \pm 0.003	N.D.	N.D.
PIY2 Medium	N.D.	0.13 \pm 0.01	0.20 \pm 0.01	0.071 \pm 0.02	0.022 \pm 0.001	N.D.	N.D.
PIY2 Heavy	N.D.	0.18 \pm 0.02	0.15 \pm 0.01	0.086 \pm 0.01	0.053 \pm 0.005	N.D.	N.D.
<i>Saccharomyces cerevisiae</i> RC212							
RC212 Light	N.D.	0.10 \pm 0.01	0.15 \pm 0.03	N.D.	N.D.	N.D.	N.D.
RC212 Medium	N.D.	0.054 \pm 0.01	0.18 \pm 0.03	0.020 \pm 0.02	0.024 \pm 0.002	N.D.	N.D.
RC212 Heavy	N.D.	0.045 \pm 0.02	0.21 \pm 0.01	0.13 \pm 0.02	0.053 \pm 0.011	N.D.	N.D.

Table 8 Concentration of Methionol in wines with 3 different lees level during 9 months of aging time(mg/L)

Methionol	0 week	2 weeks	1 month	2 months	3 months	6 months	9 months
<i>Saccharomyces cerevisiae</i> PIY1							
PIY2 Light	1.74 \pm 0.01	1.94 \pm 0.16	1.64 \pm 0.05	1.59 \pm 0.11	1.49 \pm 0.08	1.36 \pm 0.00	1.34 \pm 0.01
PIY2 Medium	1.85 \pm 0.10	1.67 \pm 0.05	1.76 \pm 0.19	1.58 \pm 0.07	1.45 \pm 0.05	1.31 \pm 0.08	1.35 \pm 0.00
PIY2 Heavy	2.54 \pm 0.07	1.73 \pm 0.04	1.71 \pm 0.29	1.65 \pm 0.12	1.56 \pm 0.05	1.31 \pm 0.07	1.32 \pm 0.09
<i>Saccharomyces cerevisiae</i> RC212							
RC212 Light	3.30 \pm 0.10	3.06 \pm 0.21	2.84 \pm 0.26	2.80 \pm 0.08	2.69 \pm 0.04	2.26 \pm 0.16	2.26 \pm 0.20
RC212 Medium	3.11 \pm 0.13	2.93 \pm 0.12	2.80 \pm 0.12	2.79 \pm 0.10	2.76 \pm 0.09	2.35 \pm 0.14	2.06 \pm 0.07
RC212 Heavy	3.58 \pm 0.33	2.99 \pm 0.07	2.93 \pm 0.14	2.91 \pm 0.14	2.87 \pm 0.11	2.37 \pm 0.04	2.11 \pm 0.08

2. Results on wines from the collaborating wineries-2013 harvest

Table 9 Concentration of volatile sulfur compounds in wines from Adelsheim Vineyards after settling ($\mu\text{g/L}$)

Wine Code	H ₂ S	MeSH	CS ₂	DMS	MeSOAc	DMS	DMS	MeSOAc	DMS	DMS	MeSOAc	DMS	EtSOAc	DMS	DMTS	Methionol ^a
bkpn#1	27.0±0.06	2.62±0.04	0.038±0.001	2.49±0.36	13.8±0.09	0.11±0.01	0.11±0.01	0.82±0.03	0.044±0.013	0.044±0.013	0.82±0.03	0.044±0.013	0.82±0.03	0.044±0.013	0.044±0.013	7.66±0.66
dfpn#4	24.1±2.70	1.94±0.05	0.043±0.005	1.45±0.22	12.3±0.2	0.085±0.020	0.085±0.020	0.55±0.03	0.080±0.013	0.080±0.013	0.55±0.03	0.080±0.013	0.55±0.03	0.080±0.013	0.080±0.013	4.19±0.03
dfpn#0	18.6±3.07	2.30±0.22	0.036±0.005	2.07±0.16	8.85±0.40	0.087±0.010	0.087±0.010	0.58±0.10	0.010±0.007	0.010±0.007	0.58±0.10	0.010±0.007	0.58±0.10	0.010±0.007	0.010±0.007	3.33±0.25
dfpn#4	26.8±1.6	1.90±0.29	0.041±0.001	2.34±0.28	17.39±1.16	N.D.	N.D.	0.93±0.02	0.057±0.017	0.057±0.017	0.93±0.02	0.057±0.017	0.93±0.02	0.057±0.017	0.057±0.017	4.05±0.25
CUS	23.2±3.9	2.49±0.01	0.27±0.00	2.49±0.40	10.4±0.1	0.19±0.01	0.19±0.01	0.49±0.04	0.14±0.003	0.14±0.003	0.49±0.04	0.14±0.003	0.49±0.04	0.14±0.003	0.14±0.003	4.53±0.36
RC3	9.45±1.05	1.91±0.23	0.22±0.01	4.17±0.40	8.84±0.10	0.14±0.02	0.14±0.02	0.43±0.06	0.035±0.05	0.035±0.05	0.43±0.06	0.035±0.05	0.43±0.06	0.035±0.05	0.035±0.05	3.25±0.21
SUB2A	57.4±8.9	3.26±0.27	0.28±0.01	3.94±0.29	15.8±0.24	0.11±0.01	0.11±0.01	1.29±0.09	0.11±0.03	0.11±0.03	1.29±0.09	0.11±0.03	1.29±0.09	0.11±0.03	0.11±0.03	3.55±0.17
BBO3	96.2±4.8	4.03±0.48	0.31±0.04	4.21±0.46	20.3±2.3	0.18±0.17	0.18±0.17	1.17±0.08	0.27±0.03	0.27±0.03	1.17±0.08	0.27±0.03	1.17±0.08	0.27±0.03	0.27±0.03	4.08±0.01

Table 10 Volatile sulfur compounds in wines from Adelsheim Vineyards and Lemelson Vineyards after 1 months of barrel aging ($\mu\text{g/L}$)

Wine Code	H ₂ S	MeSH	DMS	MeSOAc	DMS	DMS	MeSOAc	DMS	DMS	MeSOAc	DMS	DMS	EtSOAc	DMS	DMTS	Methionol ^a
CHPOM 8 days settling	4.46±0.63	2.88±0.37	4.54±0.37	12.8±0.1	0.27±0.01	0.27±0.01	12.8±0.1	4.54±0.37	12.8±0.1	0.27±0.01	0.27±0.01	12.8±0.1	3.60±0.01	0.10±0.02	0.10±0.02	1.57±0.08
MY 6D/3A 4DAYS settling reduction noted	3.21±0.13	2.90±0.04	3.50±0.38	14.7±0.4	0.27±0.01	0.27±0.01	14.7±0.4	3.50±0.38	14.7±0.4	0.27±0.01	0.27±0.01	14.7±0.4	3.03±0.05	0.15±0.03	0.15±0.03	1.17±0.01
MY 6D/3A 11/2/13 0.3ppm copper addition	2.75±0.04	1.93±0.29	2.70±0.02	13.9±0.2	N.D.	N.D.	13.9±0.2	2.70±0.02	13.9±0.2	N.D.	N.D.	13.9±0.2	2.78±0.03	0.072±0.002	0.072±0.002	1.05±0.11
Lemelson ST4	2.71±0.06	1.86±0.27	3.82±0.12	10.7±0.4	0.16±0.03	0.16±0.03	10.7±0.4	3.82±0.12	10.7±0.4	0.16±0.03	0.16±0.03	10.7±0.4	1.03±0.04	0.097±0.017	0.097±0.017	1.37±0.15
JN9 post settling	2.38±0.26	2.13±0.30	4.30±0.12	9.24±0.42	0.12±0.01	0.12±0.01	9.24±0.42	4.30±0.12	9.24±0.42	0.12±0.01	0.12±0.01	9.24±0.42	0.69±0.04	0.057±0.010	0.057±0.010	1.29±0.20
JN9 after copper addition 0.3ppm	2.80±0.33	1.73±0.09	1.97±0.21	9.18±0.01	0.081±0.010	0.081±0.010	9.18±0.01	1.97±0.21	9.18±0.01	0.081±0.010	0.081±0.010	9.18±0.01	0.66±0.02	0.040±0.002	0.040±0.002	1.26±0.03
RM 16	3.53±0.10	2.03±0.18	3.97±0.19	4.57±0.17	0.33±0.01	0.33±0.01	4.57±0.17	3.97±0.19	4.57±0.17	0.33±0.01	0.33±0.01	4.57±0.17	N.D.	0.15±0.01	0.15±0.01	1.65±0.19
CL15 11/13 1 month barrel	2.39±0.59	1.24±0.10	2.63±0.22	8.39±0.22	0.14±0.03	0.14±0.03	8.39±0.22	2.63±0.22	8.39±0.22	0.14±0.03	0.14±0.03	8.39±0.22	0.44±0.05	0.025±0.002	0.025±0.002	1.81±0.04
BBO3-1month barrel	8.55±0.52	3.49±0.15	3.58±0.04	16.9±0.6	0.37±0.03	0.37±0.03	16.9±0.6	3.58±0.04	16.9±0.6	0.37±0.03	0.37±0.03	16.9±0.6	0.95±0.05	0.19±0.03	0.19±0.03	1.65±0.12

Table 11 Concentration of volatile sulfur compounds in wines from Adelsheim Vineyards after 6 months of barrel aging ($\mu\text{g/L}$)

Wine code	H ₂ S	MeSH	DMS	DES	MeSOAc	DMS	EtSOAc	DEDS	Methionol ^a
BC08-affected	1.68±0.21	3.37±0.12	2.36±0.10	0.17±0.00	20.6±0.10	0.11±0.00	6.69±0.41	0.58±0.05	5.10±0.33
BC08-Unaffected	1.49±0.13	1.37±0.09	2.19±0.11	N.D.	10.9±0.1	0.097±0.025	0.57±0.05	N.D.	5.76±0.38
Cl1S	1.43±0.31	0.90±0.10	7.50±0.28	0.30±0.00	8.10±0.01	0.10±0.02	0.48±0.02	N.D.	3.22±0.35
Cl08	1.99±0.26	1.34±0.28	6.46±0.36	N.D.	13.3±0.2	0.14±0.00	1.48±0.07	N.D.	3.23±0.13

* the concentration unit for this compounds is mg/L

Table 12 Concentration of volatile sulfur compounds in wines from Vineyards after 9 months of barrel aging $\mu\text{g/L}$)

Wine code	H ₂ S	MeSH	DMS	MeSOAc	DMS	EtSOAc	DMTS	DEDS	Methionol ^a
1bk	N.D.	3.32±0.56	3.96±0.02	10.96±0.58	N.D.	N.D.	N.D.	N.D.	5.05±0.16
4bk	N.D.	1.17±0.06	2.16±0.25	8.95±0.14	N.D.	N.D.	N.D.	N.D.	3.95±0.17
4df	N.D.	N.D.	5.11±0.20	7.33±0.03	N.D.	N.D.	N.D.	N.D.	2.23±0.05
bb03140324	N.D.	3.07±0.48	6.49±0.19	12.47±0.61	N.D.	N.D.	N.D.	N.D.	3.70±0.20
bb08lees	6.16±0.31	13.45±1.12	2.19±0.03	12.16±0.63	1.84±0.23	4.52±0.31	0.15±0.03	0.25±0.01	6.50±0.37
bkpn13	7.10±0.35	5.80±0.75	8.75±0.17	25.62±0.36	N.D.	6.48±0.04	N.D.	0.09±0.01	2.79±0.12
bkpn15	5.56±0.44	4.94±0.04	6.87±0.11	21.2±0.29	N.D.	5.27±0.05	N.D.	N.D.	2.56±0.06
bkpn17	2.96±0.19	4.69±0.25	7.67±0.03	17.85±0.79	N.D.	5.10±0.38	N.D.	N.D.	3.09±0.11
odf	N.D.	1.25±0.17	5.72±0.33	7.19±0.45	N.D.	N.D.	N.D.	N.D.	2.65±0.07

* the concentration unit for this compounds is mg/L

3. Results on wines from the collaborating wineries –year 2014 harvest

Table 13 Concentration of volatile sulfur compounds in wines from collaborating wineries ($\mu\text{g/L}$)

Wine code	H ₂ S	MeSH	CS ₂	DMS	DES	MeSOAc	DMDS	EtSOAc	DEDS	DMTS
1	33.5±10.8	ND	ND	ND	ND	16.9±0.5	ND	4.4±0.1	ND	ND
2	ND	ND	0.10±0.02	ND	ND	9.6±0.2	ND	1.6±0.0	ND	ND
3	ND	ND	0.13±0.01	7.2±0.8	ND	9.3±0.2	ND	1.7±0.0	ND	ND
4	ND	ND	0.13±0.00	5.5±0.5	ND	9.2±0.1	ND	2.2±0.2	ND	ND
5	29.1±5.2	ND	0.095±0.009	5.7±0.3	ND	9.3±0.0	ND	2.2±0.2	ND	ND
6	ND	ND	0.13±0.01	5.5±0.4	ND	9.2±0.0	ND	2.2±0.2	ND	ND
7	ND	ND	0.14±0.01	6.1±0.1	ND	9.2±0.0	0.46±0.07	2.3±0.1	ND	ND
8	11.8±1.0	ND	0.075±0.00	4.4±0.3	ND	17.1±0.3	ND	4.8±0.1	ND	ND
9	10.4±0.8	ND	0.11±0.00	8.7±0.9	ND	16.7±0.5	ND	4.6±0.1	ND	ND
10	ND	ND	0.098±0.02	9.7±0.1	ND	20.4±0.5	ND	6.0±0.1	ND	ND
11	ND	ND	0.091±0.00	4.9±0.1	0.97±0.1	9.9±0.1	0.57±0.09	2.0±0.1	ND	ND
12	9.1±2.0	ND	0.11±0.01	5.9±0.3	ND	11.4±0.1	ND	1.8±0.0	ND	ND
13	ND	ND	0.13±0.00	9.1±0.6	ND	14.6±0.2	0.54±0.04	3.0±0.0	ND	ND
14	ND	ND	0.16±0.01	9.8±0.2	ND	15.6±0.3	ND	2.9±0.1	ND	ND
15	6.8±0.7	ND	0.10±0.01	10.0±0.0	ND	14.8±0.3	ND	3.1±0.1	ND	ND
16	ND	ND	ND	15.7±0.4	ND	11.9±0.2	0.47±0.07	ND	ND	ND
17	ND	ND	0.20±0.00	23.9±0.3	ND	13.4±0.1	0.46±0.02	1.6±0.1	ND	ND
18	ND	ND	0.18±0.02	17.5±0.2	ND	7.4±0.4	0.49±0.07	0	0	ND
19	ND	ND	0.18±0.00	10.3±1.2	0.95±0.13	14.5±0.1	0.32±0.01	1.8±0.1	0.058±0.010	ND
20	ND	ND	0.23±0.00	10.8±0.4	ND	18.2±0.5	0.47±0.02	2.6±0.0	0.061±0.011	ND
21	ND	ND	0.20±0.01	41.5±0.9	1.5±0.0	16.0±0.7	0.38±0.00	2.9±0.3	0.15±0.02	ND
22	ND	ND	ND	37.7±1.9	1.2±0.1	15.5±0.4	0.43±0.01	2.8±0.2	ND	ND
23	ND	ND	0.24±0.02	19.6±0.2	ND	16.3±0.4	0.34±0.03	2.4±0.1	ND	ND
24	ND	ND	ND	14.0±1.1	ND	13.0±0.2	ND	1.8±0.1	ND	ND
25	ND	ND	0.22±0.03	11.9±0.0	1.6±0.2	14.0±0.0	0.38±0.02	3.2±0.2	0.11±0.01	ND
26	ND	ND	ND	14.4±1.1	ND	8.0±0.1	0.38±0.01	ND	ND	ND
27	ND	ND	0.21±0.04	13.7±0.5	ND	14.9±0.2	0.41±0.00	2.1±0.1	ND	ND

28	ND	ND	ND	12.4±0.4	ND	12.8±0.7	ND	3.3±0.2	ND	ND
29	ND	0.12±0.01	ND	22.0±0.6	ND	13.3±0.5	0.62±0.00	3.2±0.3	ND	ND
30	ND	0.10±0.00	ND	17.3±0.9	ND	12.0±0.2	ND	1.8±0.0	ND	ND
31	ND	0.13±0.00	ND	15.9±0.5	ND	13.1±0.2	ND	2.5±0.0	0.12±0.02	ND
32	ND	0.086±0.004	ND	27.0±1.3	ND	13.7±0.2	ND	2.8±0.1	ND	ND
33	ND	ND	ND	11.3±0.1	ND	6.9±0.0	ND	ND	ND	ND
34	ND	ND	ND	10.3±0.2	ND	10.7±0.2	ND	1.7±0.0	ND	ND
35	ND	0.13±0.00	ND	8.7±0.4	ND	14.6±0.1	ND	2.6±0.1	ND	ND
36	ND	0.13±0.00	ND	17.7±0.1	ND	11.4±0.0	ND	1.7±0.2	ND	ND
37	ND	0.15±0.01	ND	ND	ND	13.0±0.2	ND	4.3±0.2	ND	ND
38	ND	ND	ND	90.1±1.3	ND	10.3±0.1	ND	1.9±0.1	ND	ND
39	ND	ND	ND	ND	ND	6.3±0.2	ND	ND	ND	ND
40	ND	ND	ND	ND	ND	21.7±1.3	ND	8.4±0.5	0.18±0.00	ND
41	ND	ND	ND	ND	ND	15.6±0.3	ND	5.9±0.1	ND	ND
42	12.4±3.9	ND	0.092±0.000	ND	ND	17.8±0.5	ND	6.4±0.2	ND	ND

Table 14 Sample information from collaborating wineries

Code	Sample information									
1	9/28/14	PNPom-grafted-F5	Post Press Sample	CHEHALEM-Stoller Vinerards						
2	9/30/14	14PNSTWAD-grafted	Pressing Sample	CHEHALEM-Stoller Vinerards						
3	10/2	14PNSTWAD-grafted	Barrel Sample	CHEHALEM-Stoller Vinerards						
4	10/18/14	14PNRCBabiesWAD-F6	Press Sample	CHEHALEM						
5	10/21/14	14PNRCBabiesWAD-F6	Barrel-down sample	CHEHALEM						
6	11/05/2014	14PNRCBabiesWAD-F6	Barrel 2 WEEKS	CHEHALEM						
7	11/19/2014	14PNRCBabiesWAD-F6	Barrel Sample 4 weeks	CHEHALEM						
8	10/2	14PNSTPom-grafted	Barrel Sample	CHEHALEM-Stoller Vinerards						
9	10/15/2014	14PNSTPom-grafted	2 week in barrel	CHEHALEM						
10	10/30/2014	14PNSTPom-grafted	4 Weeks in barrel	CHEHALEM						
11	10/15/2014	14PNSTWAD-grafted	2 week in barrel	CHEHALEM						
12	10/30/2014	14PNSTWAD-grafted	4 Weeks in barrel	CHEHALEM						
13	11/30/14	14PNJH4W40%	2of2	CHEHALEM						
14	11/30/14	14PNJH4W40%	1of2	CHEHALEM						

CHEHALEM

15	11/30/14	14PNJH4ETS250%	1 of1	
16	9/26/14	14PNBB05B	Pressed on 9/22	
17	9/26/14	14PNCL07	Barrel initial	
18	9/26/14	14PNBB05A	Pressed 9/25	
19	9/26/14	14PNBB02	Pressed on 9/26	
20	9/26/14	14PNBB04	Barrel initial	
21	9/26/14	14PNBB03B	Pressed on 9/23	
22	9/27/14	14PNBB03B	Initial barrel	
23	9/26/14	14PNBB03A	Barrel initial	
24	9/27/14	14PNBB05B	Initial barrel	
25	9/29	14PNLS07	Barrel initial	
26	10/2	14PNBB05A	Barrel initial	
27	10/4	14PNBB02	Barrel initial	
28	9/24	BB03A		
29	9/24	BB03B		
30	9/24	CL07		
31	10/10/14	BB3A	2 WK BBL	Adelsheim
32	10/10/14	BB3B	2 WK BBL	Adelsheim
33	10/10/14	BB5A	2 WK BBL	Adelsheim
34	10/10/14	BB5B	2 WK BBL	Adelsheim
35	10/10/14	BB4	2 WK BBL	Adelsheim
36	10/10/14	CL7	2 WK BBL	Adelsheim
37	10/23	CH 6B	Post press	pressed on 10/23
38	10/29	JN 1A	Post press	drained+pressed on 10-28
39	10/20	WV 1A	Post press	pressed on 10/17
40	10/28	ST 7C	Post press	drained+pressed on 10-27
41	10/28	ST 8C	Post press	drained+pressed on 10-27
42	10/20	ST3#2	Post press	pressed on 10/16

Table 15 Aroma descriptors and sensory thresholds for Volatile Sulfur Compounds reported in Wine(ref. 6-9)

	Synonyms	Descriptor	Sensory threshold (µg/L)	Detect limit (µg/L)
Hydrogen sulfide	H ₂ S	Rotten eggs	40-100	3
Methanethiol	MeSH	Rotten vegetables, cooked cabbage	1.8-3.1	0.1
Dimethyl sulfide	DMS	Canned corn, asparagus	25-60	2
Carbon disulfide	CS ₂	Sweet, ethereal, rubber	>38	0.01
Diethyl sulfide	DES	Garlic, rubbery	0.92-18	0.3
Methyl thioacetate	MeSOAc	Cooked cabbage	50	1
Dimethyl disulfide	DMDS	Rotten vegetables, Onion	11.2-23.6	0.1
Ethyl thioacetate	EtSOAc	Sulfurous, garlic, onion	10	0.5
Diethyl disulfide	DEDS	Onion	1.4-4.3	3
Dimethyl trisulfide	DMTS	Vegetal, cabbage, intense onion-like		0.05
3-methylthio-1-propanol	Methionol	Potato, soup or meat like,	1200-4500	200

The samples from collaborating wineries were complicated due to various treatments and remedies performed at winery. As showed in Table 9, H₂S was the major volatile sulfur compound in those wine samples. Two of the wine samples Adelsheim Vineyards (Table 9) had very high concentration of H₂S (57 and 96 ppb). High levels (up to 20 ppb) of methyl thioacetate were also present in many of these wine samples. This observation is important because the sulfur-containing esters can be hydrolyzed to methanethiol during storage, and methanethiol has a very low sensory threshold of 1.8 ppb. The levels of methanethiol were all above the sensory threshold. After one month of barrel aging, the H₂S in BBO3 as well as from other wineries was greatly reduced either due to copper addition or racking treatment by wine makers. Although the level of H₂S was dropped to 2-8 ppb in these wines, high levels of methyl thioacetate as well as methanethiol were still present in these wines (Table 10). All other samples had very similar volatile sulfur levels (Table 10).

At 6 months aging, the BC08-affected wine from Adelsheim Vineyards showed high level of methyl thioacetate, ethyl thioacetate and methanethiol (Table 11). At 9 months of barrel aging, high levels of methyl thioacetate were detected in many samples, and methanethiol in bb08lees sample reached as high as 13 ppb (Table 12).

More winery samples were studied for 2014 harvest and the results were listed in Table 13 and 14. High levels of hydrogen sulfide, DMS and methyl thioacetate were detected in most of those wines. Unfortunately, due to heavy cut of funding last year, we were unable to follow through these any further.

We collaborated with Dr. James Osborne further studied the effect of YAN on volatile sulfur formation (Table 16). Detailed results were submitted as a single report separately. The results showed that the levels of YAN did not affect the generation of MeSH, CS₂, DMDS and DMDS. *Saccharomyces cerevisiae* P1Y2 did not generate thioacetates regardless the level of the YAN or the type of YAN. However, the amount of YAN and the type of YAN did affect the formation of thioacetates by *Saccharomyces cerevisiae* UCD522. *Saccharomyces cerevisiae* UCD522 generated more volatile sulfur compounds including methyl thioacetate. DAP treatment generated higher thioacetates.

Table 16. Concentraion of volatile sulfur compounds in synthetic medium with different levels of YAN ($\mu\text{g/L}$)

Sulfur compounds	H ₂ S	MeSH	CS ₂	MeSOAc	DMDS	EtSOAc	DMTS
<i>Saccharomyces cerevisiae</i> P1Y2							
P1Y2 Low	ND	5.2±0.0	2.7±1.3	ND	0.4±0.1	ND	0.06±0.02
P1Y 2 High	ND	ND	3.2±0.7	ND	1.3±0.2	ND	0.08±0.01
P1Y2 DAP	ND	2.5±0.0	3.4±0.8	ND	0.7±0.3	ND	0.09±0.00
<i>Saccharomyces cerevisiae</i> UCD522							
UCD522 Low	ND	4.6±0.8	5.2±1.2	9.8±1.7	2.8±0.3	4.6±0.4	0.6±0.3
UCD522 High	3.1	4.4±0.7	5.6±1.3	8.2±0.7	1.5±0.5	3.2±0.1	0.3±0.1
UCD522 DAP	12.1	6.4±0.6	3.3±1.0	11.4±0.3	2.1±1.3	5.3±1.0	0.4±0.3

Low: amino acids at 100 mg/L YAN, High: amino acids at 350 mg/L YAN, DAP: supplemented with DAP (diammonium phosphate) to add up at 350 mg/L YAN, ND: Blow the detect limit

We also studied the effect of elemental sulfur on volatile sulfur composition, and the detailed report was submitted separately. Although the residual sulfur affected hydrogen sulfide production during fermentation (see the separate report), sulfur addition did not affect the volatile sulfur compounds in the final wine except for methyl thioacetate. With higher sulfur addition, more MeSOAc was generated, which was observed in both *Saccharomyces cerevisiae* P1Y2 and UCD522 (Fig.1). The *Saccharomyces cerevisiae* P1Y2 even generated higher MeSOAc than *Saccharomyces cerevisiae* UCD522 although P1Y2 is a non-H₂S producing *Saccharomyces cerevisiae*.

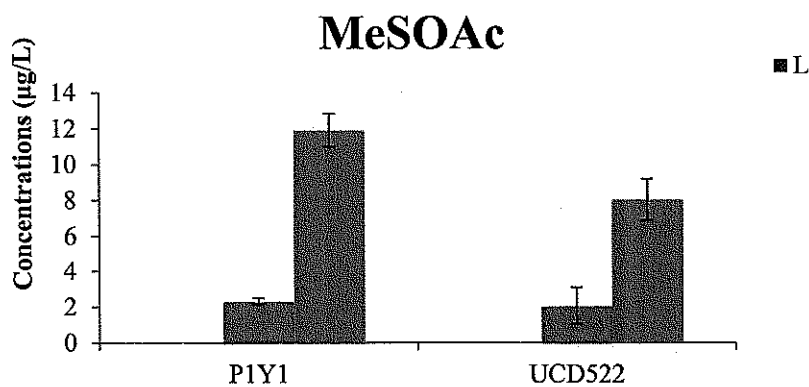


Fig.1 Concentrations of MeSOAc in wine with different levels of colloidal sulfur additon($\mu\text{g/L}$)

VI. OUTSIDE PRESENTATIONS OF RESEARCH

A poster presentation was given at the 2014 “OWRI Grape Days” (April 1st). Results to date from the study have also been shared and discussed with the Willamette Valley Enology Technical group during two meetings in 2014. A oral presentation has been scheduled for the grape day of 2015. Two abstracts for the annual American Society of Enology and Viticulture national meeting will be submitted.

VII. RESEARCH SUCCESS STATEMENTS:

The development of volatile sulfur off-flavor during winemaking is an ongoing issue in the wine industry. The exact nature of the off-flavor is seldom known, and treatment is often difficult due to lack of information on the off-flavor. The results from this study identified that hydrogen sulfide (H₂S), methylmercaptan (methanethiol) and methyl thioacetate (sulfur containing ester) were the major sulfur compounds of the concern in the wines. Lee contact has impact on the accumulation of these sulfur compounds. However, the impacts are dependent on the types of lee, lee level and the specific compound.

Collaboration with wine industry successfully identified hydrogen sulfide is the major cause of off-flavor at beginning of barrel aging. However, it appeared that methanethiol and methyl thioacetate were the major sulfur off-flavor compounds at later stage of in barrel aging. More winery samples were studied for 2014 harvest and high levels of hydrogen sulfide, DMS and methyl thioacetate were detected in most of those wines.

We further studied the effect of YAN on volatile sulfur formation. The study showed the amount of YAN and the type of YAN affected the formation of thioacetates by *Saccharomyces cerevisiae* UCD522. DAP addition generated higher thioacetates. We also studied the effect of elemental sulfur on volatile sulfur composition and found sulfur addition generated more methyl thioacetate by both *Saccharomyces cerevisiae* P1Y2 and UCD522.

High levels of methyl thioacetate could be an important issue for winery. Methyl thioacetate impart sulfur off-flavor in wine, it can also be converted to methanethiol that has a very low sensory threshold. The results suggested the methyl thioacetate and methanethiol could be the major culprits for sulfur off-flavor development during barrel aging. This new finding is significant and needs to have further investigation in future.

VIII. FUND STATUS:

The project was only funded for \$15,000 which cannot cover a graduate student. The student worked on this project has to work on other projects to cover the living expenses. All of the fund has been spent.

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