PROGRESS REPORT 201406

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

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III. Summary:

Volatile sulfur compounds (VSCs) are generally considered to be negative contribution to wine aroma. They usually give off very strong, offensive odors such as rotten eggs, rotten cabbage and garlic (1). The formation of VSCs is particularly challenging to winemakers because of their extreme low sensory threshold and the difficulty of removing them from wine.

Development of VSCs occur post-fermentation as wine aging on the wine lees, a well-known technique that is applied in almost all the winemaking process to improve wine quality such as increasing color stability and decreasing astringency and bitterness (2). It has been observed among Oregon wineries that sulfur off-flavor can develop during lees wine aging, and that the off-flavor problems are often more serious in cooler years such as 2007 and 2010. The formation of VSCs is thought to be associated with the yeast metabolism of nitrogen compounds particularly S-containing amino acids methionine and cysteine (3). Another potential source of VSCs could be the cysteine containing tripeptide glutathione which is accounts for about 1% of dry weight of *Saccharomyces cerevisiae* and is involved in cellular redox buffering (4).

It is our hypotheses that the amount of wine lees and lees contact time may affect the release of volatile sulfur precursors such as glutathione and sulfur-containing amino acids from lees to the wine, and cause sulfur off-flavor during barrel aging. In addition, grape nutrients, particularly amino acid composition, may affect the lees composition, resulting in the release of volatile sulfur compounds during aging.

In year 2013, initial experiments including the fermentation and lees treatment were conducted by Dr. James.P.Osborne as a part of a separate project "Formation of volatile sulfur compounds in Pinot noir post-fermentation. Part 1: Role of grape amino acid content and wine lees composition". Basically, initial experiments have been undertaken where Pinot noir wines were be produced 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 H_2S producing yeast strain *Saccharomyces cerevisiae* P1Y2 while a second set were 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 settling) which were named as heavy, medium and light treatments respectably in this progress report. Initial samples as well as samples that going through 2 weeks and 1, 2, 3, 6 and 9 months of lees-aging time were proposed to collect for analysis of volatile sulfur compounds.

Samples from collaborating wineries were collected to help determine the role grape composition plays in the formation of volatile sulfur compounds post-fermentation. Grape must samples were taken prior to fermentation while wine samples taken after pressing and settling, and 1, 3, and 6 months after wine has been placed in barrel. The wine/lees that showed signs of volatile sulfur compounds by sensory were taken prior to any remedial treatment being performed (racking, copper additions). Collaborating wineries have already provided juice and wine samples from the 2013 and continued every three months until the wines have been in barrel 6 months.

To analyze the volatile sulfur compounds in wine, we made a modification and validation based on a published method (5) using headspace-solid phase microextraction-Gas Chromatography-Pulsed Flame Photometric Detector (HS-SPME-GC-PFPD). The method enables the quantification of 10 volatile sulfur compounds in wine, with its utility demonstrated through application to studies relevant to wine fermentation and storage. By the time of reporting, the wines have gone through 8 months of lees aging. The initial samples and samples took after 2 weeks and 1, 2, 3 and 6 months of lees-aging time with three different lees treatments were performed analysis for volatile sulfur compounds.

V. Summary of Major Research Accomplishments and Results:

1. Method development to analyze the VSCs in wine by HS-SPME-GC-PFPD

Chemicals and reagents

Dimethyl sulfide (DMS), ethyl methyl sulfide (EMS), diethyl disulfide (DEDS) were obtained from TCI America (Portland, OR, USA); CS_2 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 methanthiol (MeSH) and hydrogen sulfide (H_2S) 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 eight milliliters of Milli-Q water. An aliquot 100μL of the internal standard solution (500ppb EMS and 2ppb Isopropyl DS) and 50μL 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 85μm 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 500 rpm of agitation, and then extracted with the SPME fiber at headspace for 20 min with 250 rpm agitation at the same temperature.

The samples will be prepared one more set for quantification of mehionol. The only difference was to set up the equilibration and extraction temperature at 50 °C instead of 30°C while other conditions including sample preparation and chromatographic conditions were all 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 by using a DB-FFAP capillary column (30m×0.32 mm I.D., 1.0μm 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 injection of the 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 (H_2S) standard stock solution was generated by bubbling pure H_2S gas into 0.5M NaOH solution and the concentration was calculated by weight. Individual standard stock solutions of CS_2, DMS, DES, DEDS, MeSOAc, EtSOAc, DMDS, DMTS and Methionol were prepared by weighing a certain amount of standard into specific volume of pure ethanol. All the standard stock solutions were stored at −18°C until use.

Individual standard stock solution of EMS and IsopropylDS was made by dissolving certain amount of standard in pure ethanol. An internal standard solution was prepared by diluting and mixing to the proper concentrations containing 500 μg/L of EMS and 2μg/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 20mL autosampler vials and then diluted with eight milliliters of Milli-Q water. An aliquot 100μL of the internal standard solution (500ppb EMS and 2ppb Isopropyl DS) and 50μL 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. As the sample preparation, a calibration curve for methionol will be 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.

Result and Discussion

1. Results on wines from OSU winery trail

Table 1 Concentraion of hydrogen sulfide in wines with 3 different lees level during 6 months of aging time(μg/L)

H_2S	0 week	2 weeks	1 month	2 months	3 months	6 months
	Saccharomyces cerevisiae P1Y1					
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
P1Y 2Medium	2.47±0.10	2.38±0.20	3.52±0.72	2.17±0.33	2.01±0.19	1.47±0.24
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
	Saccharomyces cerevisiae 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
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
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

Table 2 Concentraion of methanthiol in wines with 3 different lees level during 6 months of aging time(μg/L)

Methanthiol	0 week	2 weeks	1 month	2 months	3 months	6 months
	Saccharomyces cerevisiae P1Y1					
P1Y2 Light	1.00±0.01	1.38±0.19	1.33±0.22	2.45±0.45	2.04±0.33	0.78±0.11
P1Y 2Medium	1.00±0.14	1.33±0.13	1.28±0.10	1.68±0.30	2.15±0.18	0.84±0.05
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
	Saccharomyces cerevisiae RC212					
RC212 Light	1.49±0.10	1.63±0.05	1.54±0.06	1.45±0.12	1.93±0.14	0.69±0.18
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
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

Table 3 Concentraion of Carbon disulfide in wines with 3 different lees level during 6 months of aging time(μg/L)

CS_2	0 week	2 weeks	1 month	2 months	3 months	6 months
	Saccharomyces cerevisiae P1Y1					
P1Y2 Light	0.040±0.007	0.035±0.004	0.032±0.003	0.048±0.005	0.048±0.002	0.023±0.000
P1Y 2Medium	0.038±0.010	0.049±0.006	0.038±0.003	0.051±0.001	0.051±0.004	0.030±0.001
P1Y2 Heavy	0.034±0.004	0.047±0.002	0.044±0.009	0.057±0.006	0.054±0.003	0.030±0.004
	Saccharomyces cerevisiae RC212					
RC212 Light	0.050±0.002	0.049±0.002	0.042±0.001	0.065±0.004	0.066±0.003	0.029±0.001
RC212 Medium	0.053±0.003	0.055±0.002	0.041±0.004	0.068±0.004	0.062±0.007	0.029±0.004
RC212 Heavy	0.043±0.002	0.056±0.004	0.047±0.001	0.078±0.002	0.076±0.002	0.051±0.002

Table 4 Concentraion of Dimethyl sulfide in wines with 3 different lees level during 6 months of aging time(μg/L)

DMS	0 week	2 weeks	1 month	2 months	3 months	6 months
	Saccharomyces cerevisiae P1Y1					
P1Y2 Light	1.59±0.20	1.94±0.22	1.91±0.16	3.75±0.34	3.25±0.11	2.76±0.07
P1Y 2Medium	1.10±0.04	2.02±0.15	1.81±0.14	3.69±0.22	3.26±0.19	2.94±0.25
P1Y2 Heavy	1.97±0.05	2.95±0.08	2.60±0.05	4.77±0.10	4.65±0.21	3.69±0.02
	Saccharomyces cerevisiae RC212					
RC212 Light	1.89±0.08	2.07±0.10	1.79±0.08	3.17±0.37	3.20±0.28	2.66±0.12
RC212 Medium	1.88±0.27	2.01±0.13	1.79±0.13	2.95±0.59	2.96±0.37	2.49±0.20
RC212 Heavy	1.82±0.29	2.70±0.14	2.71±0.18	4.62±0.12	4.51±0.18	3.77±0.24

Table 5 Concentraion of Methyl Thiolacetate in wines with 3 different lees level during 6 months of aging time(μg/L)

MeSoAc	0 week	2 weeks	1 month	2 months	3 months	6 months
	Saccharomyces cerevisiae P1Y1					
P1Y2 Light	N.D.	0.67±0.01	0.58±0.03	0.66±0.04	0.94±0.10	0.66±0.05
P1Y 2Medium	N.D.	0.44±0.15	0.58±0.09	0.55±0.09	0.52±0.05	0.74±0.07
P1Y2 Heavy	0.40±0.02	0.83±0.05	0.56±0.04	0.56±0.10	0.49±0.08	0.64±0.08
	Saccharomyces cerevisiae RC212					
RC212 Light	3.61±0.01	4.09±0.10	3.23±0.07	4.12±0.17	4.06±0.17	3.66±0.03
RC212 Medium	3.35±0.14	3.75±0.66	3.36±0.13	4.69±0.07	3.97±0.07	3.66±0.09
RC212 Heavy	3.25±0.51	4.54±0.09	3.10±0.08	3.73±0.11	3.48±0.11	2.98±0.06

Table 6 Concentraion of Dimethyl disulfide in wines with 3 different lees level during 6 months of aging time(μg/L)

DMDS	0 week	2 weeks	1 month	2 months	3 months	6 months
	Saccharomyces cerevisiae P1Y1					
P1Y2 Light	N.D.	0.14±0.01	0.17±0.02	0.23±0.03	0.16±0.02	0.095±0.001
P1Y 2Medium	N.D.	0.11±0.02	0.20±0.04	0.25±0.02	0.21±0.03	0.091±0.01
P1Y2 Heavy	0.12±0.02	0.16±0.02	0.25±0.02	0.22±0.03	0.24±0.04	0.11±0.02
	Saccharomyces cerevisiae RC212					
RC212 Light	0.16±0.00	0.16±0.03	0.13±0.01	0.11±0.01	0.12±0.01	0.075±0.01
RC212 Medium	0.09±0.03	0.12±0.01	0.19±0.03	0.11±0.02	0.11±0.02	0.093±0.01
RC212 Heavy	0.22±0.04	0.087±0.015	0.17±0.03	0.15±0.02	0.17±0.02	0.096±0.01

Table 7 Concentraion of Dimethyl trisulfide in wines with 3 different lees level during 6 months of aging time(μg/L)

DMTS	0 week	2 weeks	1 month	2 months	3 months	6 months
	Saccharomyces cerevisiae P1Y1					
P1Y2 Light	N.D.	0.040±0.002	0.12±0.02	0.096±0.01	0.021±0.003	N.D.
P1Y 2Medium	N.D.	0.13±0.01	0.20±0.01	0.071±0.02	0.022±0.001	N.D.
P1Y2 Heavy	N.D.	0.18±0.02	0.15±0.01	0.086±0.01	0.053±0.005	N.D.
	Saccharomyces cerevisiae RC212					
RC212 Light	N.D.	0.10±0.01	0.15±0.03	N.D.	N.D.	N.D.
RC212 Medium	N.D.	0.054±0.01	0.18±0.03	0.020±0.02	0.024±0.002	N.D.
RC212 Heavy	N.D.	0.045±0.02	0.21±0.01	0.13±0.02	0.053±0.011	N.D.

Table 8 Concentraion of Methionol in wines with 3 different lees level during 6 months of aging time(mg/L)

Methionol	0 week	2 weeks	1 month	2 months	3 months	6 months
	Saccharomyces cerevisiae P1Y1					
P1Y2 Light	1.74±0.01	1.94±0.16	1.64±0.05	1.59±0.11	1.49±0.08	1.36±0.00
P1Y 2Medium	1.85±0.10	1.67±0.05	1.76±0.19	1.58±0.07	1.45±0.05	1.31±0.08
P1Y2 Heavy	2.54±0.07	1.73±0.04	1.71±0.29	1.65±0.12	1.56±0.05	1.31±0.07
	Saccharomyces cerevisiae RC212					
RC212 Light	3.30±0.10	3.06±0.21	2.84±0.26	2.80±0.08	2.69±0.04	2.26±0.16
RC212 Medium	3.11±0.13	2.93±0.12	2.80±0.12	2.79±0.10	2.76±0.09	2.35±0.14
RC212 Heavy	3.58±0.33	2.99±0.07	2.93±0.14	2.91±0.14	2.87±0.11	2.37±0.04

Eight volatile sulfur compounds were detected in the Pinot Noir wine produced in OSU winery by 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) since no sulfur compounds were beyond the sensory threshold (see table 12). For the 3 levels of lees load, there are no obvious differences were observed through aging despite of yeasts used in fermentation. Unexpectedly, there is no sulfur off-flavor detected neither by sensory trail nor by instrumentation.

During 6 months of lees-aging time, we can see that some sulfur compounds continually decreased through aging time such as methionol either through adsorption by wines lees or by degradation. Some sulfur compounds kept almost consistent through lees aging like methyl thioacetate. Other sulfur compounds like hydrogen sulfide, carbon disulfide, dimethyl sulfide, dimethyl disulfide and dimethyl disulfide followed a general trend that increased to a certain level at first several months then started to decrease at some point. This might be the combined effect of yeasts activity to generate sulfur compounds from precursors and the adsorption effect by lees. As to the two different fermentation yeasts, *Saccharomyces cerevisiae* P1Y2 produced lower levels of methyl thioacetate and methionol than *Saccharomyces cerevisiae* RC212 while other sulfur compounds had similar levels. Interestingly, after fermentation and press, the wines fermented with *Saccharomyces cerevisiae* P1Y2 had lower hydrogen sulfide than *Saccharomyces cerevisiae* RC212. However, after 6 months of lees aging time, all the wines have very similar levels of hydrogen sulfide despite of lees levels and yeasts used in fermentation. This results indicated that the lees might not cause the problem of the hydrogen sulfide off-flavor in wines during aging, in contrary, the lees could help reduce the hydrogen sulfide during aging, and the final level of hydrogen sulfide after aging were mainly determined by grape composition related to the YAN level as well as the amino acid compositions.

2. Results on wines from the collabaraing wineries

Table 9 Concentration of volatile sulfur compounds in wines from Adelsheim Vineyards after settling (μg/L)

Wine Code	H_2S	MeSH	CS_2	DMS	MeSOAc	DMDS	EtSOAc	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	0.82±0.03	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	0.55±0.03	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	0.58±0.10	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.	0.93±0.02	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	0.49±0.04	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	0.43±0.06	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	1.29±0.09	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	1.17±0.08	0.27±0.03	4.08±0.01

Table 10 Concentration of volatile sulfur compounds in wines from Adelsheim Vineyards and Lemelson Vineyards after 1 months of barrel aging (μg/L)

Wine Code	H_2S	MeSH	CS_2	DMS	MeSOAc	DMDS	EtSOAc	DMTS	Methionol[a]
CHPOM 8 days settling	4.46±0.63	2.88±0.37	0.063±0.005	4.54±0.37	12.8±0.1	0.27±0.01	3.60±0.01	0.10±0.02	1.57±0.08
MY 6D/3A 4DAYS settling reduction noted	3.21±0.13	2.90±0.04	0.038±0.004	3.50±0.38	14.7±0.4	0.27±0.01	3.03±0.05	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	0.050±0.001	2.70±0.02	13.9±0.2	N.D.	2.78±0.03	0.072±0.002	1.05±0.11
Lemelson ST4	2.71±0.06	1.86±0.27	0.058±0.004	3.82±0.12	10.7±0.4	0.16±0.03	1.03±0.04	0.097±0.017	1.37±0.15
JN9 post settling	2.38±0.26	2.13±0.30	0.040±0.01	4.30±0.12	9.24±0.42	0.12±0.01	0.69±0.04	0.057±0.010	1.29±0.20
JN9 after copper addition 0.3ppm	2.80±0.33	1.73±0.09	0.074±0.004	1.97±0.21	9.18±0.01	0.081±0.010	0.66±0.02	0.040±0.002	1.26±0.03
RM 16	3.53±0.10	2.03±0.18	0.13±0.02	3.97±0.19	4.57±0.17	0.33±0.01	N.D.	0.15±0.01	1.65±0.19
CL15 11/13 1 month barrel	2.39±0.59	1.24±0.10	0.18±0.01	2.63±0.22	8.39±0.22	0.14±0.03	0.44±0.05	0.025±0.002	1.81±0.04
BBO3-1month barrel	8.55±0.52	3.49±0.15	0.19±0.01	3.58±0.04	16.9±0.6	0.37±0.03	0.95±0.05	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 (µg/L)

Wine code	H_2S	MeSH	CS_2	DMS	DES	MeSOAc	DMDS	EtSOAc	DEDS	Methionol[a]
BC08-affected	1.68±0.21	3.37±0.12	0.21±0.01	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	0.074±0.003	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	0.19±0.00	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	0.20±0.01	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 Aroma descriptors and sensory thresholds for Volatile Sulfur Compounds reported in Wine(6-9)

	Synonyms	Descriptor	Sensory threshold (µg/L)
Hydrogen sulfide	H2S	Rotten eggs	40-100
Methanethiol	MeSH	Rotten vegetables, putrefaction	1.8-3.1
Dimethyl sulfide	DMS	Black currant, cooked cabbage, canned corn, asparagus	25-60
Carbon disulfide	CS2	Sweet, ethereal, rubber	>38
Diethyl sulfide	DES	Garlic, rubbery	0.92-18
Methyl thioacetate	MeSOAc	Cooked cabbage	50
Dimethyl disulfide	DMDS	Rotten vegetables, onion	11.2-23.6
Ethyl thioacetate	EtSOAc	Sulfurous, garlic, onion	10
Diethyl disulfide	DEDS	Onion	1.4-4.3
Dimethyl trisulfide	DMTS	Vegetal, cabbage, intense onion-like	
3-methylthio-1-propanol	Methionol	Potato, soup or meat like,	1200-4500

For the winery samples, 2 of the 8 samples from Adelsheim Vineyards after settling can be detected sulfur off-flavor sensorially which were coded as SUB2A and BBO3. The quantification results on sulfur compounds showed that the concentration of H_2S were much higher than H_2S in other wines and also higher than sensory threshold (table 12). After 1 month barrel aging, the H_2S in BBO3 were greatly reduced either due to copper addition and racking treatment by wine makers or just due to lees contact.

However, both of the samples MY 6D/3A after 4DAYS settling with reduction noted and MY 6D/3A with 0.3ppm copper addition as well as JN9 post settling and MY 6D/3A with 0.3ppm copper addition had very similar sulfur compounds levels. We did not detect any sulfur issues on those wines.

As to the wines from Adelsheim Vineyards after 6 months of barrel aging, only BC08-affected among the 4 samples were detected sulfur off-flavor by sensory. The differences between BC08-affected and BC08- unaffected were MeSOAc, EtSOAc, DEDS and DES, although these 4 sulfur compounds in affected BC08 were all lower than the sensory threshold reported by literature. The probable explanations for the remarkable sulfur off-flavor notes were: firstly, the sensory threshold of those sulfur compounds should be revalued since there is no agreement in different literatures; Secondly, the off-flavor could be the combination of several sulfur compounds due to synergy effect.

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