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Identification of Muscadine Wine Sulfur Volatiles: Pectinase versus Skin-Contact Maceration

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ABSTRACT: Muscadine grapes (*Vitis rotundifolia*) are widely grown in the southern United States, as the more common *Vitis vinifera* cannot be cultivated due to Pierce's disease. There is interest to determine if certain cultivars can be used for good-quality wine production. This study compared the effect of pectolytic enzyme pretreatment with conventional skin-contact fermentation on Muscadine (Noble, *Vitis rotundifolia*) wine major volatiles, aroma active volatiles, and volatile sulfur compounds (VSCs). Volatile composition, aroma activity, and VSCs in the initial juice and wine samples after 3 years were determined by gas chromatography in combination with mass spectrometry (GC-MS), olfactory detection (GC-O), and pulsed flame photometric detection (GC-PFPD). Forty-three nonethanol MS volatiles were common to all samples. Total ion chromatogram (TIC) MS peak area increased 91% in the skin-contact wines from the initial juice but only 24% in the enzyme-treated wine. Thirty-one VSCs were detected. Twenty-four sulfur volatiles were identified by matching their retention characteristics on polar and nonpolar columns with those of standards or MS spectrum matches. Six of these (sulfur dioxide, 1-propanethiol, 3-mercapto-2-pentanone, 3-mercapto-2-butanone, 2,8-epithio-*cis*-*p*-menthane, and 1-*p*-menthene-8-thiol) were reported for the first time in muscadine wine. Five additional VSCs were tentatively identified by matching standardized retention values with literature values, and two remain unidentified. Total sulfur peak areas increased 400% in the skin-contact wine and 560% in the enzyme-treated wine compared to the initial juice. There were 42 aroma-active volatiles in the initial juice, 48 in the skin-contact wine, and 66 in the enzyme-treated wine. Eleven aroma-active volatiles in the skin-contact wine and 16 aroma volatiles in the enzyme-treated wine appear to be due to sulfur volatiles. Pectolytic enzyme-treated wines contained less total volatiles but more sulfur and aroma-active volatiles than the traditional skin-contact wine.

KEYWORDS: *Muscadine wine, pectinase, sulfur volatiles, GC-PFPD, GC-olfactometry*

INTRODUCTION

Muscadine grapes (*Vitis rotundifolia*) are native to the southeastern United States, with current markets existing for juice, wine, and table grapes. They are particularly well suited to Florida's sandy soil since they grow best in soil which is well-drained. Their value is primarily due to their resistance to Pierce's disease (*Xylella fastidiosa*), to which *Vitis vinifera* is highly susceptible, and resistance to the insect phylloxera that can kill grapevine roots. This resistance makes them a valuable crop in areas where *V. vinifera* cannot be grown and currently offers good potential for expansion and further development.[1,2]

Wines have a complex mixture of components that are responsible for their aroma and taste. Wine volatiles can originate from the grapes, yeast, and bacterial metabolism, winemaking practices, and oak contact.[3] Among those volatiles are sulfur compounds, which may play an important role in wine aroma. Volatile sulfur compounds (VSCs) are formed by either biological or chemical mechanisms and often have extremely low sensory thresholds.[4] While some VSCs may have negative sensory attributes at the microgram per milliliter level, microgram per liter concentrations of specific compounds actually augment the favorable aroma of wine. For example, dimethyl sulfide at trace levels is often perceived as fruity,[5,6] whereas in higher concentrations, it is described as skunky or boiled cabbage.

Yeast is a major factor in wine development due to the correlation between yeast and volatile sulfur composition. For example, Howell et al.[7] reported that 4-mercapto-4-methyl-2-pentanone (4MMP) in grape juice is bound to cysteine as a nonvolatile compound and requires the action of yeast to release the aroma-active thiol during fermentation. Sulfur volatiles can also originate from the degradation of sulfur-containing amino acids such as cysteine and glutathione.[8] Additionally, VSCs can be generated from the reduction of sulfate in amino acid biosynthesis, from the catabolism of the sulfur containing amino acids, from the degradation of the tripeptide glutathione or its adduct, S-adenosyl methionine, and from the degradation of the S-containing vitamins biotin and thiamine.[9]

Analytical techniques such as high-pressure liquid chromatography (HPLC) and high-resolution capillary gas chromatography, alone (GC) or combined with sulfur chemiluminescence

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detection (GC-SCD) or mass spectrometry (GC-MS), can separate, identify, and quantify wine volatiles but cannot determine their flavor/aroma contribution. Through the use of gas chromatography−olfactometry (GC-O), human assessors are able to determine which eluted components possess aroma activity, and aroma assessors have been employed to examine wine volatiles for many years.[10]

Recent advances in winemaking practices have employed several types of enzymes. Macerating-type enzyme preparations (pectinase, cellulase, and hemicellulase) are commonly used in winemaking and fruit juice processing. In general, pectolytic enzymes are normally classified as pectin esterases (pectin methyl esterase) and pectin or pectate polymerases, which commonly consist of hydrolases and lyase.[11] Pectinase preparations are accepted as "generally recognized as safe" (GRAS) formulations by the U.S. Federal Bureau of Alcohol, Tobacco, and Firearms (ATF). Commercial pectolytic enzymes usually contain both groups of enzymes.[12] Pectolytic enzymes can have an important function in winemaking process due to their ability to increase free-run juice yield and improve extraction of color, aroma, and phenolic compounds and their use in the clarification and filtration process of must and wine.[12,13] Also, there is the advantage of impacting wine fermentation due to releasing of fermentable carbohydrates at earlier stages than traditional winemaking practices. Masino et al.[14] and Sánchez-Palomo et al.[15] also reported that enzymatic treatments improved perceived flavor.

One of the most crucial steps in any aroma analysis is the manner in which volatiles are collected for analysis.[8] Solvent extraction with immiscible organic solvents is one of the most frequently used methods for the isolation and concentration of volatiles.[20,21] Its primary advantage is that fairly high concentrations of volatiles can be collected if the extract is concentrated. The primary disadvantages are that some volatiles might be lost during concentration, highly volatile components are masked by the solvent peak, and nonvolatiles or semivolatiles are sometimes also extracted.

Lozano et al.[22] concluded that volatiles can also be adequately collected by either purge and trap or solid-phase microextraction (SPME) methods. SPME volatile collection is being increasingly employed for studies involving food, grapes, and wine[23−26] as it is a convenient, solventless technique that requires minimal sample manipulation. It was employed for the analysis of wine sulfur volatiles as early as 1998.[27] However, fiber type, extraction time, extraction temperature, and sample stirring are all factors that need to be optimized.[28] Torrens et al.[29] compared the extraction efficiencies of four fibers for the analysis of volatiles in red and white wines. Poly(dimethylsiloxane)−divinylbenzene−carboxen (PDMS−DVB−CAR) collected the widest volatile profile from these wines. Campillo et al.[30] found both the PDMS−DVB−CAR and carboxen−polydimethylsiloxane (CAR−PDMS) fibers provided essentially equivalent VSC results. Fang and Qian[16] found the CAR−PDMS fiber sufficiently sensitive to identify and quantify trace VSCs in commercial wines.[20] Fedrizzi et al.[31] reported that the PDMS−DVB−CAR fiber produced the highest sulfur chromatographic peaks.

In this study, muscadine (cv. Noble) juice was processed into wine by both pectolytic enzymatic pretreatment and classical skin-contact maceration techniques before yeast fermentation. The objectives of this study were to determine the effect of a pectolytic enzyme compared to skin-contact maceration on total wine volatiles, to identify VSCs in muscadine wine, and to determine which VSCs possess aroma activity.

To achieve these objectives, wine sulfur volatiles from both treatments were evaluated after 3 years of storage at 4 °C. GC with sulfur detection (by pulsed flame photometric detection, PFPD) was employed to specifically determine sulfur volatiles, and GC-MS was utilized to identify major volatiles. Time/intensity GC-O was employed to determine which of the complex collection of volatiles possessed aroma activity.

MATERIALS AND METHODS

Preparation of Must and Wine. Muscadine red grapes (*Vitis rotundifolia* cv. Noble) were collected at full maturity (15.5 °Bx) from a vineyard in Apopka, FL in 2004 and transported to the pilot-scale winery in Gainesville, FL, where they underwent destemming and crushing. The grapes were harvested by hand, stored under refrigerated conditions (4 °C), and processed into juice within 2 days of harvest. After the grapes were crushed, the macerated grapes, skin, and stems (grape mash) (∼68 kg) was divided into 2 portions and stored in large (30 L) glass demijohns. These were placed in a refrigerated room where skin-contact fermentation was allowed to occur.

The skin-contact maceration batch was immediately treated with potassium metabisulfide (50 mg of SO_2 kg) and left for 48 h under refrigeration. The other portion of the mash was treated with a pectolytic enzyme (0.03 g/L pectinase, Novozyme Corp., P2736 Sigma−Aldrich, St. Louis, MO) and allowed to react for 10 h under refrigeration before the addition of potassium metabisulfide (50 mg ofSO_2/kg). After each treatment, the musts were extracted from the mash by use of a vertical hydraulic basket press (Prospero's Equipment, Cort, NY) and then filtered through fine cheesecloth. The musts were ameliorated, total acidity of the juice was 3.15 g/L (as tartaric acid), and the water-soluble solids were 15.5 g/100 g. After chaptalization, the degree Brix was 23 g/100 g. The musts were stored for 3 weeks in a constant-temperature room (22 ± 2 °C) in 20 L glass fermentation vessels.

After fermentation, the wine was racked and filtered according to classical wine-processing production. The wine samples consisted of two treatments, those with skin-contact maceration and those with pectinase maceration.

Commercial active wine yeast 2% [6×10^8 colony-forming units (cfu)·mL^{-1}; *Saccharomyces bayanus*, Fermichamp no. 67 J, INRA, Narbonne, France] was inoculated into the musts in each separate sample until reducing sugars were exhausted. Wines were bottled following filtration and kept under cold conditions (4 °C) prior to analysis.

Reagents and Standards. All reagents and pure compounds used in this study were purchased: 3-methyl-3-furanthiol, dimethyl trisulfide, 2,8-epithio-cis-*p*-menthane, bis(2-methyl-3-furyl)disulfide, 3-mercaptohexanol, and 1-*p*-methene-8-thiol from Acros (Geel, Belgium); methanethiol, ethanethiol, and ethyl methyl sulfide from Fluka (Milwaukee, WI); 1-propanethiol, 2-butanethiol, thiazole, dimethyl disulfide, 3-mercapto-2-butanone, methional, diethyl disulfide, 3-mercapto-3-methylbutanol, dipropyl disulfide, 2-methylthiophene, 3-methylthiophene, and carbon disulfide from Sigma−Aldrich (St. Louis, MO); 3-mercapto-2-pentanone from Advocato Research Chemicals (Lancashire, U.K.); 4-mercapto-4-methyl-2-pentanone from Oxford Chemicals (Oxford, U.K.); *S*-methyl thioacetate from Alfa Aesar (Lancashire, U.K.); 3 mercaptohexyl acetate from Interchim (San Pedro, CA); and 4-methyl-5-thiazolylethyl acetate from Treatt (Lakeland, FL) .

Sample Preparation. Solid-phase microextraction (SPME) static headspace sampling was employed to collect and concentrate wine volatiles. SPME parameters (sample volume, magnetic stirring, temperature, and time of extraction) were optimized for extraction of VSCs. A CAR−PDMS fiber was utilized in the GC-S instrument, and a DVB−CAR−PDMS fiber was utilized with both GC-O and GC-MS instruments.

The wines were stored in dark glass containers at 4 °C until analyzed. Ten milliliters of wine was added to a 40 mL glass vial with a screw top and Teflon-lined septum. A 15 mm × 1.5 mm Teflon-coated micro stir bar (Fisher brand, catalog no. 791130-0029) was added to the wine. The sample vial was flushed with nitrogen to avoid oxidation.[16] It was then suspended in a 40 °C water bath placed on a stirring (200 rpm) hot plate (Isotemp, Fisher Scientific, Pittsburgh, PA), which was used to continuously heat and stir both the water and wine to maintain constant temperature. After equilibration with stirring for 20 min, volatiles from the wine headspace were extracted for 30 min via conditioned SPME [for GC-S, a 75 μm CAR−PDMS fiber (100 mm), and for GC-O and GC-MS, a PDMS−DVB−CAR fiber (100 mm); Supelco, Bellefonte, PA] and then desorbed in the injector port for 5 min. Prior to all analysis, the fiber was conditioned in a GC injector port (2 h at 300 °C). Before each extraction, the fiber was held at 260 °C for 5 min and then at room temperature for 2 min to allow the metal sheath to cool.

Gas Chromatography−Olfactometry. GC-O was carried out on an Agilent-6890N instrument with a sniffing port from DATU (Geneva, NY) plus a flame ionization detector (FID). The insulated stainless steel sniffing port tube was 70 cm long and 1 cm in diameter. The column effluent was split 3:1 in favor of the sniffing port, allowing simultaneous FID detection and sniffing of GC effluents. A deactivated SPME liner was used in the 200 °C injection port. Detector temperature was 250 °C. The columns used were a 30 × 0.32 × 0.5 μm DB-Wax column and a 30 × 0.32 × 0.5 μm DB-5 column, both from J&W Scientific (Folsom, CA). Helium was used as carrier gas at 1.5 mL/min. In addition to the helium, a heated (40 °C) and humidified air flow (1000 mL/min) was used. Oven temperature program was 40 °C, ramped at 7 °C/min to 265 °C, and then held for 5 min at the maximum temperature. Total run time was 37 min. All samples were analyzed by GC-O on both columns.

Purified air was obtained by passing it through activated charcoal, drierite, and a molecular sieve 5 Å (Alltech, Deerfield, IL) and then directed through a water-filled, round-bottom flask thermostated to 40 °C to provide warmed, humidified air. Assessors indicated the aroma intensity of each peak by use of a linear potentiometer with a 0−1 V signal. Time/intensity was continuously recorded via a separate chromatographic data channel and the aroma quality noted. The output of the FID was also recorded on a separate chromatographic software data channel. Chrom Perfect version 5.0.0 (Justice Innovations, Inc., Palo Alto, CA) was used for both data channels. Two screened and trained olfactometry panelists were employed. Each treatment was sniffed a minimum of five times by each panelist. Sensory descriptors for aroma-active peaks were transcribed into bound data files linking aroma descriptors with specific aroma peaks and were defined as aromagrams. Responses of each aroma-active compound were averaged. When a compound was not detected through GC-O, its value was treated as missing instead of zero, so as to reflect a more accurate average of the responses.

Average aromagram values were determined. A peak was considered aroma-active only if at least half the sniffs indicated similar times and descriptions. Peak areas were averaged, with zero values used if no peak was detected. Peaks were listed by linear alkane (LRI) and ethyl ester (EEI) retention index values, and calibration data from previously run series of alkane and ethyl ester standards were used for both columns.[30] Standards were sniffed under the same GC-O conditions as wine samples to accurately determine aroma character and retention characteristics for each column. Aroma and alkane LRI values of the standards were then compared with the wine sample aromagrams and data.

Gas Chromatography with Pulsed Flame Photometric Detection. Sulfur volatiles were separated and monitored on an Agilent 7890A gas chromatograph (Palo Alto, CA) with a pulsed flame photometric detector (O-I Analytical 5380 PFPD) using a DB-5 column (30 m × 0.32 mm i.d. × 0.25 μm) and DB-Wax column (30 m × 0.32 mm i.d. × 0.5 μm), both from J&W Scientific (Folsom, CA). The injector port was equipped with a deactivated SPME liner (1 mm) and was used in splitless mode. Initial oven temperature was 40 °C for the DB-Wax column, then was increased to 240 °C at 7 °C/min, and finally was held for 5 min. Initial oven temperature for the DB-5 column was 40 °C and then was increased to 265 °C at 7 °C/min. Helium was used as carrier gas at a flow rate of 1.5 mL/min. Nitrogen makeup gas was maintained at 19 mL/min, and air and hydrogen flame gases were maintained at 265 and 35 mL/min, respectively. Injector and detector temperatures were 220 and 265 °C, respectively. PFPD sulfur gate times were 6−25 ms. Chromatograms were recorded and integrated by use of Chrom Perfect (Justice Innovations, Inc., Mountain View, CA) with a data acquisition rate of 0.1 s/point. Samples were run in triplicate on both columns.

Gas Chromatography−Mass Spectrometry. GC-MS analyses were conducted on a Perkin/Elmer Clarus 500 quadruple GC-MS instrument equipped with Turbo Mass software (Perkin-Elmer, Shelton, CT). Conditions were as follows: Helium was used as the carrier gas with a constant flow mode of 2 mL·min^{-1}. The source was kept at 200 °C, and the transfer line and injector were kept at 220 °C. Compounds were separated on a 60 m × 0.25 mm i.d. × 0.5 μm Wax column and a 60 m × 0.25 mm i.d. × 0.5 μm Rtx-5 column (Restek, Bellefonte, PA). The mass spectrometer was operated in the total ion chromatogram (TIC) mode at 70 eV. Data were collected from 40 to 300 m/z. Mass spectral matches were made by comparison of NIST 2002 standard spectra (NIST, Gaithersburg, MD).

Identification. Identification of the wine aroma components was determined by comparison of their retention values and fragmentation patterns with authentic sulfur standards or literature values. Analyses for samples and standards were performed in triplicate.

Authentic standards were added to wine samples to confirm peak identification. Identification was also achieved by comparing GC-MS fragmentation patterns with the NIST library wherever possible, GC-O aroma descriptors, and retention characteristics from authentic compounds.[17−19]

Statistical Analysis. All data-evaluated statistical analyses were carried out with the SPSS software package (SPSS 11.5 SPSS Inc., Chicago, IL) and Minitab 14.0 (Minitab Inc., State College, PA).

RESULTS AND DISCUSSION

Gas Chromatography with Pulsed Flame Photometric Detection. A sample chromatogram of an enzyme-treated wine is shown in Figure 1. As seen in Table 1, the average PFPD chromatographic peak areas for all VSCs was 4−5 times greater in the fermented wines than in initial grapes, suggesting that some VSCs were bound in nonvolatile forms and released only during fermentation. There were two unidentified sulfur volatiles that were found in the initial grape as well as the wine.

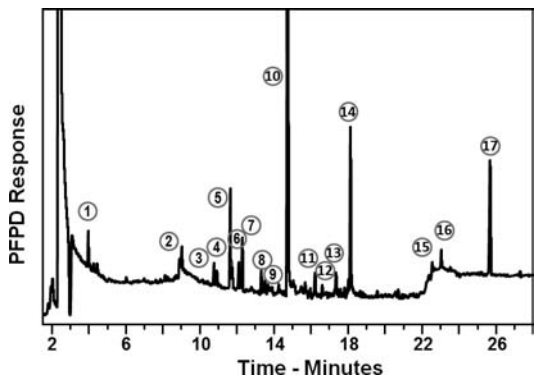

Figure 1. Sulfur volatiles of pectinase-treated wine on a polar column. Peak identifications are as follows: (1) *S*-methyl thioacetate, (2) 3-mercapto-2-pentanone, (3) dimethyl trisulfide, (4) 4 mercapto-4-methyl-2-pentanone, (5) 3-mercapto-3-methylbutanol, (6) not identified [NI], (7) NI, (8) dipropyl disulfide, (9) NI, (10) 3-mercaptohexyl acetate, (11) 3-mercaptohexanol, (12) NI, (13) NI, (14) NI, (15) bis(2-methyl-3-furyl)disulfide, (16) NI, (17) difuryl disulfide.

Table 1. Muscadine Wine Sulfur Compound Identification and Relative Composition[a]

GC-PFPD linear retention indices									
Rtx-5 column	std	Wax column	std	PLOT column	compd	identification[b]	grape, 2005	enzyme-treated, 2008	skin-contact, 2008
<500	490	528	600	<500	hydrogen sulfide	S[A,B]	30.4	9.3	10.3
<500	510	675	670	579	methanethiol	S[A,B]	146	81.7	67
540	546	722	700	<500	carbon disulfide	MS[A,B]		1040	768
508	560	722	729	636	ethanethiol	S[A,B]	45	192	91.9
519	574	736	743	721	dimethyl sulfide	MS/S[A,B,C,D]		17.8	26.2
<500		831		510	sulfur dioxide	S		63	98
623	610	861	861		ethyl methyl sulfide	S[A,D]		6.67	6.35
607	616	861	863		1-propanethiol	S		31.4	2.47
746	736	1071	1067	886	dimethyl disulfide	S[A,B,D,F]		8.9	10.5
726	725	1072	1075		*S*-methyl thioacetate	S[A,B,C,F]	4.13	8.05	8.7
773	774	1083			2-methylthiophene	tentative[A]		9.01	7.83
780	782	1098			3-methylthiophene	tentative		6.82	10.9
664		1143			2-butanethiol	S[A]		5.99	7.45
929	925	1211	1209		diethyl disulfide	S[A,B,C,F]		21.1	35
732	737	1213	1276		thiazole	S[H]		11.5	14
868	874	1284			2-methyl-3-furanthiol	S[G]	5.77	33.4	33.3
901	904	1352	1356		3-mercapto-2-pentanone	S	3.5	50.6	36.5
978	984	1355	1389	1330	dimethyl trisulfide	S[D]		5.74	5.88
955	947	1385	1389	597	4-mercapto-4-methyl-2-pentanone	S[A,E]	28	7.46	6.19
818	815	1455			3-mercapto-2-butanone	S		12.1	8.86
909	908	1460	1468	885	methional	S[A,F]		9.72	8.62
1264		1492			2,8-epithio-*cis*-*p*-menthane	S			1.02
1295	1266	1501			1-*p*-menthene-8-thiol	S	34.3	1.3	4.09
988		1676			3-mercapto-3-methylbutanol	S[A]	5.65	14.2	16.3
1249		1701			3-mercaptohexylacetate	S[A,E]		1.55	2.71
		1708			NI		3.64	3.03	6.73
		1727			NI		2.94	12.2	
1112	1126	1787			dipropyl disulfide	tentative	8.04	3.36	1.73
1123	1125	1982	1990		3-mercaptohexanol	S[A,E]		5.57	3.05
1394		2331			4-methyl-5-thiazolylethyl acetate	tentative	0		3.168
1694		2575			difurfuryl disulfide	tentative	0	13.2	16.4
						total S peak areas	317	1687	1319

[a]As average PFPD peak areas × 10^3. NI, not identified. [b]S indicates matches of LRI values with standards. Previous wine reports: A, ref 25; B, ref 4; C, ref 31; D, ref 30; E, ref 34; F, ref 47; G, ref 48; H, ref 49. Identification based on matching GC-MS spectra with those of standards.

Their contribution to the total PFPD peak area ranged from 2% in the grape samples to less than 1% in the wines. VSCs detected in the wine samples included thioesters, sulfides, polysulfides, thiosulfides, and heterocyclics. Concentration changes in VSCs during the aging of wines have been reported in a number of studies.[32−35] Kilmartin[36] indicated that wine oxidation can lead to the loss of existing aroma compounds, particularly those containing sulfur.

Fedrizzi et al.[35] found dimethyl sulfide and dimethyl disulfide increased with aging, and other studies also have reported changes in specific sulfur compounds over time.[31,34] The absence of methional in the original grape juice and presence of methional in the fermented samples is a clear indication that fermentation has liberated this VSCs due to degradation of the nonvolatile sulfur-containing amino acid methionine. Methional is produced by yeast from methionine, via deamination, followed by decarboxylation (Ehrlich reaction). The aldehyde thus formed, 3-(methylthio)-1-propanal (methional), is then reduced to the corresponding alcohol (methionol) or oxidized to the acid [3-(methylthio)propionic acid].[37]

Thirty-one sulfur volatiles were detected in this study (Table 1), of which 29 were identified or tentatively identified. Identifications were based on (a) sulfur-specific PFPD response indicating that the peaks detected contained sulfur and (b) chromatographic retention characteristics on 2−3 dissimilar column types compared to standards or literature values. As seen in Table 1, the retention values of the standards closely matched those observed in wine samples for the dissimilar chromatographic columns. Some VSCs such as MeSH, H_2S, SO_2, and dimethyl sulfide were difficult to separate on conventional column types (polar and nonpolar) due to their high vapor pressures and volatility. Therefore, a porous layer open tubular (PLOT), column, which contains a highly retentive porous lining, was employed as an additional source of validation (a third set of LRI values) for the early-eluting peaks.[38]

Of the VSCs present in all wine samples, carbon disulfide was the most prominent VSC (58−62%). This VSC was not observed in the grape juice. Carbon disulfide was first identified in wines by Leppänen et al.[39] and appears to be a common

volatile in both red and white wines.[4] Levels of this volatile were 26% lower in the skin-contact wine than in the pectolytic enzyme-treated wine. This compound may impact the ability to accurately quantify other sulfur compounds when CAR−PDMS fibers are used.[40] However, shorter extraction times can minimize this displacement.[41]

Almost half (46%) of the total sulfur volatiles in the grape juice samples were due to methanethiol (MeSH) alone. It also was identified in both wine types but accounted for only about 4−5% of total sulfur volatiles in wine. Methanethiol and ethanethiol were the second most notable sulfur compounds in both wines. Subileau et al.[43] reported that glutathione (GSH) derivatives might be the precursors of volatile thiols in wine aroma. GSH comprises almost 1% of the yeast *Saccharomyces cerevisiae* and represents more than 95% of the low molecular mass thiol pool.[42,44]

Adding to the actions of the yeast, enzymes and glutathione already present in the grape must may be responsible for the higher levels of thiols in the enzyme wines. Herbst-Johnstone et al.[37] determined 3-MHA reduction in wines over time was predominantly due to its hydrolysis to 3-MH. In that study, there was a reduction of 3-MHA over time, which was matched by an increase in 3-mercaptohexanol, 3-MH. In our study, relative levels of 3-MHA were higher in the skin-contact wines than in the enzyme-treated wines, suggesting that the commercial enzymes facilitated the hydrolysis of 3-MHA to 3-MH.

Six identified VSCs—hydrogen sulfide, ethyl methyl sulfide, *S*-methyl thioacetate, 2 methyl-3-furanthiol, dimethyl trisulfide, and methional—were present at similar levels in both wine types. Even though the enzyme-treated wines contained more total sulfur volatiles, skin-contact wines had higher levels of the following 14 compounds; dimethyl sulfide, dimethyl disulfide, sulfur dioxide, 3-methylthiophene, 2-butanethiol, diethyl disulfide, thiazole, 2,8-epithio-*cis*-*p*-menthane, 1-*p*-menthene-8-thiol, 3-mercapto-3-methylbutanol, 3-mercaptohexyl acetate, 4-methyl-5-thiazolylethyl acetate, difurfuryl disulfide, and one sulfur volatile that was not identified. Skin-contact wines generally contained the highest levels of sulfides. Dimethyl sulfide, sulfur dioxide, and diethyl disulfide exhibited levels ranging from 32% to 40% higher in the skin-contact wines. The remaining 11 VSCs followed the pattern of higher levels in enzyme samples compared to the skin-contact samples. Thiols were especially higher in enzyme-treated wines, particularly ethanethiol and 1-propanethiol.

Gas Chromatography−Olfactometry. As shown in Table 2, a total of 17 aroma-active sulfur volatiles were found in the two wine sample types. Aroma activity was attributed to sulfur volatiles if the aroma character and retention characteristics matched those of the sulfur-specific detector for both samples and standards on two columns of dissimilar polarity. This compares with the 32 sulfur volatiles detected by use of a sulfur-specific detector and listed in Table 1. Therefore, only about half of all the sulfur volatiles were aroma-active. However, the aroma-active sulfur peaks accounted for more than 90% of the total PFPD peak area. Identification, aroma descriptors, LRI values, occurrence, and intensity are presented in Table 2. Of the total GC-O aroma volatiles detected, sulfur compounds accounted for 37−39% of the total olfactory intensities. Enzyme-treated wines contained 16 sulfur aroma-active volatiles, as compared to 11 in the skin-contact samples. Both wines had 10 sulfur aroma components in common but with different intensities as shown in Table 2. These common

Table 2. GC-O Olfactory Descriptions of Sulfur Volatiles Found in Muscadine Wines and Average Aroma Intensities for Both Wine Treatments

LRI[a]	compd	odor description	enzyme-treated	skin-contact
612	hydrogen sulfide	rotten egg, pungent	2.4	3.9
675	methanethiol	pungent, rotten	4.9	ND
722	ethanethiol	onion, garlic	5.1	ND
831	sulfur dioxide	acrid	2.6	ND
950	dimethyl sulfide	herbaceous	ND	6.4
997	carbon disulfide	sour, paintlike	4.8	6.8
1182	*S*-methyl thioacetate	sulfurous, burnt	4.5	4.3
1213	thiazole	meaty	6.9	8.3
1295	dimethyl disulfide	sulfurlike	3.3	6.5
1369	2-methylthiophene	sulfur-like, burnt	2.5	ND
1410	3-methylthiophene	aromatic, nutty	3.3	4.6
1455	3-mercapto-2-butanone	meaty, greasy	4.0	3.9
1504	3-mercapto-2-pentanone	meaty	3.9	3.8
1568	diethyl disulfide	onion, viney	3.6	ND
1676	3-mercapto-3-methylbutanol	cooked leeks, green	6.4	4.6
1708	not identified	sour, moldy	3.5	ND
1727	not identified	green, slightly acidic	3.0	3.7
		total intensities	64.0	57.0

[a]Linear retention index on GC-PFPD Rtx−Wax column. ND, not detected.

aroma-active sulfur volatiles included hydrogen sulfide, carbon disulfide, dimethyl disulfide, *S*-methyl thioacetate, 3-methanethiophene, thiazole, 3-mercapto-2-pentanone, 3-mercapto-2-butanone, 3-mercapto-3-methylbutanol, and one unidentified compound. With the exception of *S*-methyl thioacetate, 3-methyl-2-pentanone, and 3-mercapto-3-methylbutanol, the seven remaining aroma volatiles were detected at levels slightly higher in skin-contact samples. While *S*-methyl thioacetate and 3-mercapto-2-pentanone levels in the enzyme were only slightly higher, the level of 3-mercapto-3-methylbutanol in the enzyme samples was almost 40% higher.

It is well-known that hydrogen sulfide, sulfur dioxide, and methanethiol have a negative impact on wine aroma. Schütz and Kunkee[45] found several factors that influence hydrogen sulfide release and determined that the yeast metabolism of sulfur precursors is responsible for the production of this volatile. Concentrations of *S*-methyl thioacetate ranging from 0 to 70 μg/L have been reported in wines, with concentrations up to 115 μg/L.[25,31] As listed in Table 2, this compound was found to possess an objectionable odor described as sulfurous or rotten/cooked vegetables. It was found at similar levels in both skin-contact and enzyme-treated wines. Dimethyl sulfide often contributes positively to wine aroma and in this study was detected only in the skin-contact wines.

Where the aroma-active sulfur volatiles were detected in both treatments, more often the intensities of enzyme-treated wines were lower. The only exceptions were *S*-methyl thioacetate, 3-mercapto-2-pentanone, and 3-mercapto-3-methylbutanol.

VSCs considered to add positively to aroma were identified as herbaceous, garlic, meaty, aromatic, nutty, viney, box tree, green, tropical, fruity, and spicy. It is interesting to note that the total aroma intensity of the enzyme-treated sulfur volatiles was

Table 3. GC-MS of Major Volatiles in Muscadine Wine (cv. Noble)[a]

	RT	LRI	compd	rel TIC peak area		
				grape juice	enzyme-treated wine	skin-contact wine
1	3.68	671	acetaldehyde[b]	0.48	5.92	1.47
2	6.20	882	ethyl acetate[b]	44.50	6.86	9.71
3	9.34	1027	ethyl butanoate[b]	1.70	1.49	0.39
4	9.69	1043	ethyl 2-methylbutanoate	6.18	1.14	0.06
5	10.15	1063	butyl acetate	1.31	0.15	0.79
6	11.28	1113	isobutyl acetate	0.44	0.12	0.44
7	11.34	1119	isoamyl acetate[b]	0.24	1.59	0.62
8	11.67	1130	*n*-butanol	0.31	0.03	0.03
9	12.09	1148	*β*-myrcene[b]	0.23	0.03	0.07
10	12.31	1158	ethyl 2-butanoate	8.75	1.96	1.11
11	13.07	1192	limonene[b]	0.53	10.29	2.86
12	13.13	1195	isoamyl alcohol	0.15	1.13	11.63
13	13.75	1223	ethyl hexanoate[b]	14.19	15.94	9.07
14	14.59	1263	cinnamol	0.45	0.43	0.97
15	14.77	1271	*p*-cymene[b]	0.36	0.21	0.01
16	15.86	1324	ethyl heptanoate[b]	1.24	0.07	0.43
17	16.15	1338	*n*-hexanol[b]	8.69	0.91	0.95
18	17.05	1382	butyl hexanoate	1.31	0.26	0.34
19	17.91	1426	ethyl octanoate	3.86	66.44	100.00
20	18.42	1452	acetic acid[b]	0.35	2.30	3.10
21	18.68	1466	octyl acetate[b]	0.28	0.96	0.18
22	19.19	1493	isoterpinolene	0.02	0.42	0.19
23	19.28	1497	*n*-decanal[b]	0.13	0.11	0.82
24	19.76	1523	*α*-terpinene[b]	0.15	0.18	0.62
25	19.80	1525	ethyl nonanoate[b]	0.10	0.10	0.86
26	19.88	1530	linalool[b]	0.01	0.49	0.56
27	20.11	1542	octanol[b]	6.07	1.46	1.36
28	21.64	1627	ethyl decanoate[b]	4.61	13.71	42.61
29	21.98	1647	isoamyl octanoate	0.39	0.22	2.03
30	22.38	1669	diethyl succinate	2.00	0.33	2.30
31	22.59	1681	ethyl 9-decenoate[b]	0.35	0.26	5.05
32	23.63	1744	decanol	0.24	0.13	0.20
33	24.17	1776	9-decynyl alcohol	0.24	0.01	0.12
34	24.28	1783	(*Z*)-4-decen-1-ol	4.49	1.34	6.39
35	25.02	1830	*β*-phenethyl acetate	0.39	0.80	0.15
36	25.19	1840	ethyl laurate[b]	0.46	0.21	2.30
37	25.34	1843	hexanoic acid[b]	0.19	0.48	1.17
38	25.63	1869	propanoic acid	0.27	0.17	0.69
39	26.49	1925	*β*-phenethyl alcohol	1.98	2.22	9.92
40	28.38	2054	octanoic acid	0.41	1.33	4.05
41	30.16	2180	eugenol	0.17	1.82	0.40
42	30.79	2225	ethyl palmitate	0.36	1.67	0.40
43	31.31	2261	decanoic acid[b]	0.36	2.26	1.58
			normalized totals	119	148	228

[a]Identification is based on GC-MS data along with pure compounds available in the lab. TIC peak areas are normalized to the largest peak on the basis of 100. [b]Confirmed with standards.

about 12% higher than the skin-contact wines. Over the 3 years of exposure time, enzyme-treated samples continued to exhibit more favorable aroma volatiles, which was also found in other studies.[14,15]

Mass Spectrometric Analysis. MS analysis is typically employed to identify food volatiles. However, sulfur volatiles are present at such low concentrations that they often cannot be detected in the presence of other volatiles found in much higher concentrations. MS could identify only a few muscadine wine VSCs with a reasonable level of assurance. Dimethyl sulfide and carbon disulfide could be identified by full spectral matches with standards. Over 100 total ion chromatogram (TIC) peaks were initially detected but only 44 are identified, as many of the smaller peaks did not produce a clean MS fragmentation spectrum even with background correction. Of the 43 non-ethanol volatiles determined by GC-MS to be common to all samples, there were 20 esters, 11 alcohols, five terpenes, two aldehydes, and five volatile acids. From the initial juice, total TIC MS peak area increased 91% in the skin-contact wines but only 24% in the enzyme-treated wine. Volatiles listed in Table 3 were common to both sample types. The major differences between the treatments were quantitative rather

than qualitative. Figure 2 is a MS-GC TIC chromatogram of the enzyme-treated wine. The numbered peaks refer to compound

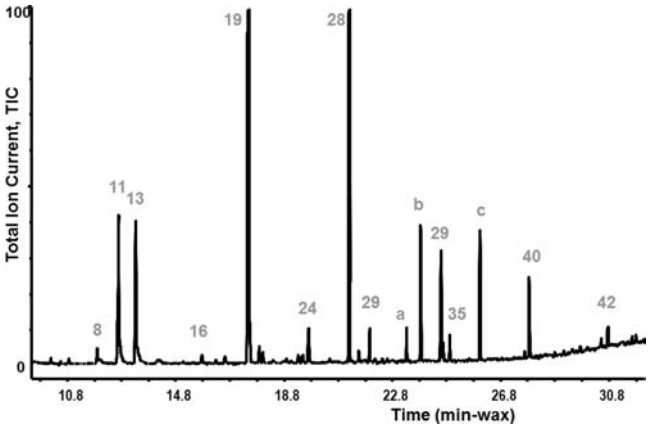

Figure 2. GC-MS separation and identification of pectinase-treated muscadine wine volatiles on a polar (Wax) column. Peak numbers correspond to those volatiles listed in Table 3. In addition, a = β-citronellol, b = *Z*-4-decen-1-ol, and c = 2-phenethyl alcohol.

numbers in Table 3. Three of the identified peaks (a, b, and c) were found solely in the enzyme samples and are identified in the footnotes. Wine total MS TIC peak areas were approximately 2−3 times greater than the grape values. There were 19 esters among the 45 volatiles identified. Esters were responsible for 73−76% of the total non-ethanol MS peak areas. Esters are the primary source of fruity aromas in wines, whose content can vary appreciably between cultivars.[18] Yeast is responsible for many of the ethyl esters as well as minor alcohols formed during fermentation.[46]

Minor alcohols are released from the slow acid hydrolysis of the corresponding esters, and their MS peak areas are considerably smaller than those of the parent esters.[18] The percentage of minor alcohols was almost double the amount in the skin-contact wines compared to the enzyme-treated wines. In this study, the total ester MS peak area was about 7 times greater than that of the corresponding minor alcohols. The terpenes and two aldehydes accounted for small percentages but were 2−3 times greater in the enzyme wines.

Nearly 50% of the total MS ion chromatogram peak area as produced from only seven compounds: ethyl acetate, limonene, isoamyl alcohol, ethyl hexanoate, ethyl octanoate, ethyl decanoate, and β-phenethyl alcohol. Limonene had a 28% greater MS peak area in the enzyme-treated samples than the skin-contact wines. The two alcohols, isoamyl alcohol and β-phenethyl alcohol, were 10−22% greater in the skin-contact wines. Furaneol and *o*-aminoacetophenone are semivolatiles that have been reported in wine studies employing solvent extraction. They are difficult to isolate and identify by SPME due to their low headspace concentrations and were not observed in this study.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

VSCs, volatile sulfur compounds; HPLC, high-pressure liquid chromatography; GC, gas chromatography; GC-SCD, gas chromatography with sulfur chemiluminescence detection; GC-MS, gas chromatography with mass spectrometry; GC-O, gas chromatography with an olfactory detector; SPME, solid-phase microextraction; PFPD, pulsed flame photometric detector; LRI, linear retention index; GC-S, gas chromatography with sulfur detector; PDMS−DVB−CAR, poly(dimethylsiloxane)−divinylbenzene−carboxen; CAR−PDMS, carboxen−poly(dimethylsiloxane); MeSH, methanethiol; GSH, glutathione; 3-MHA, 3-mercaptohexyl acetate; 3-MH, 3-mercaptohexanol

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