MARSHALL PLAN RESEARCH PROJECT REPORT

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TITLE OF PROJECT:

"Aroma Characterization of Wines Produced from Grapes Grown in the State of Arkansas"

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RESEARCH PROBLEM

The genus *Vitis* contains over 60 species[1], with most of the commercially important wine grape varieties belonging to the *vinifera* species. While *vinifera* grapes have traditionally preferred flavor characteristics, they are highly vulnerable to pests, diseases, and extreme temperatures[2] and are therefore incredibly difficult to grow in the U.S. state of Arkansas. Species native to the U.S., such as the muscadine grape (*V. rotundifolia*), are generally better-adapted to surviving such stressors[3]. However, these native species often have a low crop yield and produce wines with "unfavorable" characteristics, such as high acidity, low astringency, and excessive herbaceous aromas[2].

Muscadine grapes are approximately 2.5-4.0 centimeters in diameter and have thick, tough skins that protect them from heat, UV radiation, humidity, insects, and fungi. They are the most widely-grown grape in the southeastern United States because they are well-accustomed to warm, humid climates[4]. Muscadines can be either light- or dark-skinned[5] and are marketed in fresh and processed forms such as juice, wine, and jam. However, a majority of the commercial crop is used to produce wine[6]. Research has shown that muscadines contain a variety of antioxidant phenolic compounds[5], which have been linked to many positive human health benefits[7]. As consumers have become more aware of these health benefits, the demand for both fresh and processed muscadine products has increased. In fact, the muscadine grape industry is currently experiencing its greatest growth in decades[8].

Although a majority of the research on muscadine juice/wine has focused on antioxidant properties, there have been several studies examining volatile aroma profiles. For example, Baek et al.[9] identified the predominant aroma compounds in muscadine grape juice, and it was proposed that furaneol (burnt sugar, cotton candy aroma) and *o*-aminoacetophenone (foxy "artificial grape" aroma) were responsible for the characteristic candy and foxy aroma of the juice. Relative to muscadine juice, work on the volatile compounds of muscadine wine is even more limited. Lamikanra et al.[10] analyzed flavor development in muscadine wine during fermentation and aging. The complexity of the aroma profile increased with time, and it was determined that 2-phenylethanol and fatty acid esters were significant aroma compounds. As a majority of the commercial muscadine harvest is used to produce wine[6], it would be of interest to conduct further research on how winemaking techniques affect aroma profile.

In September 2017, muscadine grapes (cultivar "Noble") were harvested from a commercial vineyard in Arkansas and used to produce wine with three different durations of fermentation on the skins: 0 days, 3 days, and 7 days. In addition, each of these wines was split into two batches at bottling (May 2018), and β-glucosidase enzyme, which releases glucose-bound volatile compounds, was added to one of the batches, while the other was treated as a control (no enzyme). The basic composition, nutraceutical content, and color of the wines are being evaluated at the University of Arkansas Department of Food Science (Fayetteville, AR, USA). The volatile aroma profile, including gas chromatography-olfactometry (GC-O), was analyzed at Graz University of Technology in the spring of 2019.

In order to combat the warm, humid climate in the southeastern U.S., commercial grape-growers often plant species native to the area. However, these species can have undesirable characteristics that are not seen in *vinifera* grapes. Another alternative to *vinifera* grapes, in addition to muscadines, are the so-called "French-American hybrids", which have been created by grape breeders to reap advantageous traits from both parents, such as heat-tolerance from wild

species and desirable yield and flavor from *vinifera* species[3]. "Chambourcin", a red wine grape, is a popular French-American hybrid cultivar that is commercially grown in much of the U.S., including the state of Arkansas.

Inactive dry yeasts (IDYs) are *Saccharomyces cerevisiae* byproducts that are often used during winemaking to enhance or preserve wine aroma and improve mouthfeel[11]. IDYs are typically added to juice/wine before, during, or after fermentation[12] and are used as fermentation enhancers to promote yeast resistance to osmotic stress, improve nitrogen compound assimilation, and enhance sensory profiles of wine[13].

Although IDYs are commonly used in the wine industry, they are typically added to juice/wine during the vinification process. LalVigne® is an organic foliar fertilizer spray developed by Lallemand, Inc. (Montreal, Canada) to be used on grapevines in the vineyard at the point of veraison. It is said to quicken fruit ripening, promote even ripeness, increase phenolic maturity, concentrate and increase aroma precursors, and improve overall mouthfeel and quality of resulting wine. Despite recent use of LalVigne® in the wine industry, there are very few published studies examining its use[11,14]. Villangó et al.[14] evaluated the use of LalVigne® on Syrah (*V. vinifera*) grapevines grown in a cool climate (Eger, Hungary). It was determined that grapes from treated vines had thicker skins and greater phenolic potential than grapes from untreated vines. Šuklje et al.[11] evaluated the use of LalVigne® on Sauvignon Blanc (*V. vinifera*) grapes grown in South Africa. Wine produced from treated grapes had increased glutathione (GSH) antioxidant concentrations, differences in individual higher alcohol acetate (HAA) and fatty acid ethyl ester (FAEE) concentrations at the end of fermentation, and slower degradation of HAAs and FAEEs relative to the control wine. In addition, sensory analysis showed that wines produced from treated grapes had greater perceived fruitiness, whereas control wines were more commonly described as green/unripe. Although the previously mentioned studies demonstrate the ability of LalVigne® to improve the quality of *vinifera* grapes and the resulting wine, there has been no published research on the application of LalVigne® to non-*vinifera* wine grape cultivars, such as French-American hybrids. This would of particular interest for areas with climates unsuitable to *vinifera* grapes, like the state of Arkansas.

During the summer of 2018, LalVigne® MATURE spray was applied to four rows of Chambourcin hybrid wine grapes at a commercial vineyard (Hindsville, AR) as part of a project funded by Lallemand, Inc. A separate four rows were designated as the "control" (unsprayed) plot. Berries from both treatments were sampled during ripening and evaluated for basic composition, physical properties, and nutraceutical content. Grapes were hand-harvested from sprayed and control plots for wine production at the University of Arkansas Department of Food Science in late August 2018. Wines were bottled in January 2019 and the basic composition, nutraceutical content, and color of the wines are being evaluated at the University of Arkansas Department of Food Science. Descriptive and industry consumer sensory testing will be conducted through the University of Arkansas Department of Food Science during the summer of 2019. The volatile aroma profile, including GC-O, were analyzed at Graz University of Technology in the spring of 2019.

OBJECTIVES

The objectives of this research were to:

1. Determine how skin contact time and application of a glycosidic enzyme affect the aroma profile of Noble muscadine wine

2. Evaluate effects of inactive dry yeast application at veraison on the aroma profile of Chambourcin wines

MATERIALS AND METHODS

Muscadine Wine

Wine Production:

Approximately 110 kg of Noble muscadine grapes were hand-harvested from a commercial vineyard in Ozark, Arkansas, USA in September 2017. Grapes were transported to the University of Arkansas Department of Food Science (Fayetteville, AR, USA) and split randomly into six-18 kg batches. Two batches were designated "0 days skin contact", two batches were designated "3 days skin contact", and two batches were designated "7 days skin contact". Therefore, there were initially six samples: three skin contact times x two replicates each. Wines were produced according to the traditional red wine style. Each batch of grapes was passed twice through a crusher/destemmer and 30 ppm SO_2 was added at crush. Must sugars/acids were adjusted to appropriate levels. The "0 days skin contact" batches were pressed prior to inoculation with a 70 L Enoagricola Rossi Hydropress at a pressure of 30 psi. The "0 days" juice batches and the "3 days" and "7 days" must were inoculated with Lalvin QA23® wine yeast according to manufacturer's recommendations (1 g/gal estimated juice). The "3 days" and "7 days" batches were pressed after three and seven days of fermentation, respectively.

When fermentation was complete, SO_2 was adjusted to 60 ppm and wine was cold settled at 4°C. After cold settling, each of the six treatments was split into two treatments each: "enzyme" and "no enzyme". Scottzyme® BG glycosidic enzyme (Scott Laboratories, Petaluma, CA, USA) was added to the "enzyme" treatments according to manufacturer's recommendations (5 g/hL). Therefore, there were a total of 12 treatments at this point: three skin contact time levels x two replicates each x two enzyme levels. Samples were coded as 0 days no BG (2 replicates), 0 days BG (2 replicates), 3 days no BG (2 replicates), 3 days BG (2 replicates), 7 days no BG (2 replicates), and 7 days BG (2 replicates). In May 2018, wines were bottled into 375 mL glass bottles and stored at 15°C. In January 2019, samples were re-bottled into 25 mL vials and shipped to Graz University of Technology for analysis of aroma profiles.

Solid-Phase Microextraction (SPME)-GC-Mass Spectrometry (GC-MS) Analysis of Aroma Profiles:

Volatile aroma profiles of muscadine wines were analyzed at Graz University of Technology (Graz, Austria) Institute of Analytical Chemistry and Food Chemistry in March 2019. Volatile compounds were extracted from 1 mL of sample in a 10-mL glass vial using SPME with a 2-cm DVB/CAR/PDMS fiber for 30 minutes at 40°C. GC (Shimadzu GC 2010) in combination with MS (Shimadzu QP 2010) was used to identify volatile compounds. An autosampler (PAL HTX) was used for all extractions and injections. Samples were extracted/injected in analytical triplicate. Analysis was done on two separate columns: a nonpolar column and a wax (polar) column. For the nonpolar column, volatiles were separated on a Restek Rxi 5MS column (30 m x 0.25 mm x 1 μm) with a temperature gradient program: 30°C (hold 1 min) to 230°C at 5°C/min then to 280°C (hold 1 min) at 20°C/min with a constant helium flow of 35 cm/min. Data was recorded in the scan mode (*m/z* 35-350) with a 9.8 min solvent cut time and a detector voltage relative to the tuning result. For the polar (wax) column, volatiles were separated on a Phenomenex ZB-Wax column (20 m x 0.18 mm x 0.18 μm) with a temperature gradient program: 40°C (hold 1 min) to 240°C (hold 2 min) at 7.5°C/min with a constant helium flow of 35 cm/min. Data was

recorded in the scan mode (*m/z* 46-350) with a 2.3 min solvent cut time and a detector voltage relative to the tuning result.

For both the nonpolar and wax column analysis, data was recorded with the Shimadzu GCMS Postrun Analysis software. Compounds were identified using comparison of mass spectra with NIST14, FFNSC3, and Adam's Essential Oils libraries and comparison of calculated Kovats retention indices (RI) with previously reported values. A matching library result and a RI within ±40 of previously reported values was considered a positive identification. Total ion chromatogram (TIC) peak areas were obtained for each compound peak and used as a semi-quantitative measure for multivariate analysis.

Identification of Aroma-Active Compounds with SPME-GC-O:

In order to determine which volatile compounds were aroma-active, GC-O was performed on a Hewlett Packard HP5890 gas chromatograph equipped with a flame ionization detector (FID) and an olfactory detection port (ODP). Volatiles were extracted using SPME with a DVB/CAR/PDMS fiber. Varying amounts of sample were placed in a 10-mL glass vial and equilibrated for 5 min at 40°C, followed by extraction for 30 min at 40°C. Separation of volatile compounds was performed on a nonpolar column (Agilent HP5, 30 m length, 0.32 mm id, 0.25 μm film thickness) using a temperature gradient: 35°C to 280°C at 10°C/min. At the end of the column, a splitter was used to divide the effluent with a 1:1 ratio between the FID and olfactory port. Panelists used the ODP to sniff the effluents. GC effluents were combined with humidified air in order to avoid nasal dehydration.

Five trained, internal panelists were used to evaluate the wines. Results of previous GC-MS analyses showed that all wines, regardless of treatment, contained the same aroma compounds (but possibly in varying amounts). Because GC-O does not provide any quantitation of compounds, only the 7 days BG sample was evaluated. Varying amounts of sample ("sample levels") were extracted with SPME prior to injection on the GC (500 μL, 100 μL, 50μL, and 10 μL) in order to determine which compounds were the most aroma-active even at lower concentrations. Panelists evaluated each sample level one time, and order was randomized among panelists. Panelists were instructed to sniff for 15 minutes, indicating (through the press of a button) when they perceived an odor, and describing that odor if possible. Data was collected using the Agilent GC ChemStation software. FID and ODP chromatograms were generated, and panelists' voice comments were overlaid with the ODP chromatograms. Nasal impact factors (NIF) were calculated as a percentage of panelists that perceived a particular odorant. A NIF of 60% (3 out of 5 panelists) was considered a "positive identification". An alkane standard mixture was also run on the GC-FID to calculate Kovats RI values for each compound, and thus identify compounds by comparison with GC-MS data.

Statistical Analysis:

JMP Pro 14.0.0 statistical software (SAS Institute) was used to conduct all statistical tests.

GC-MS Data: Areas under TIC curve peaks were used as a semi-quantitative comparison of aroma compound concentrations among sample treatments. A hierarchical clustering analysis was conducted in order to determine if treatments could be distinguished/grouped based on the areas of the positively identified peaks that were seen on both the nonpolar and wax columns. A principle components analysis (PCA) was also conducted with peak areas, but the results were inconclusive.

The Functional Data Explorer platform in JMP, which can compare samples based on their entire TIC, rather than just the areas under selected peaks, was used to build a functional PCA model.

GC-O Data: NIF values were calculated for each sample level as a percentage of the panelists that detected a particular odor. A NIF>60 was determined to represent a positive identification of that substance as an aroma-active compound.

Chambourcin Wine

Grape Treatment:

Four rows (approximately 200 m long) of Chambourcin grapevines at a commercial vineyard in Hindsville, AR, USA were sprayed with LalVigne® MATURE (Lallemand, Inc.) at 5% veraison (5% red color development) on July 20, 2018 and again 10 days after veraison. This was the sprayed treatment. An additional four rows were left unsprayed and designated the control treatment. There was a 10 row buffer between the sprayed and control rows.

Wine Production:

Approximately 100 kg of Chambourcin grapes were hand-harvested from each treatment (200 kg total) on August 27, 2018. The grapes were transported to the University of Arkansas Department of Food Science and randomized into two 50 kg batches for each treatment. Therefore, there were four wines total: two spray treatments (sprayed and control) with two replicates each. Wines were produced according to the traditional red wine style. Each batch of grapes was passed twice through a crusher/destemmer and 30 ppm SO_2 was added at crush. After 6-8 hours, 20 mL/ton PEC5L enzyme was added to all treatments to increase juice yield at pressing. Must sugar was adjusted to 22°Brix and pH was adjusted to 3.6 with tartaric acid. Lalvin ICV D254® wine yeast (20 g/hL) was rehydrated with 3 lb/1000 gal Go-Ferm Protect Evolution® and must was inoculated. At the onset of fermentation, 20 g/hL Fermaid® O yeast nutrient was added to the wines, and another 20 g/hL was added when the °Brix had decreased by 1/3. Must was fermented on the skins until °Brix ≤ 0 and wines were pressed with a 70 L Enoagricola Rossi Hydropress at a pressure of 30 psi. After pressing, all four wines were inoculated with Lalvin MBR VP41® malolactic fermentation culture (1 g/hL) to induce malolactic fermentation. When the malic acid level had decreased to < 10 ppm, the SO_2 level was adjusted to 0.8 ppm molecular SO_2 based on the pH and wine was racked off the lees.

In January 2019, wines were bottled into 375 mL glass bottles and stored at 15°C. In February 2019, samples were re-bottled into 25 mL vials and shipped to Graz University of Technology for analysis of aroma profiles.

Solid-Phase Microextraction (SPME)-GC-Mass Spectrometry (GC-MS) Analysis of Aroma Profiles:

Volatile aroma profiles of Chambourcin wines were analyzed at Graz University of Technology (Graz, Austria) Institute of Analytical Chemistry and Food Chemistry in April 2019 using SPME-GC-MS. Volatiles were extracted, analyzed, and identified in triplicate according to the same SPME-GC-MS procedures described above for muscadine wines. Analysis was done on both a nonpolar and polar (wax) column.

Identification of Aroma-Active Compounds with SPME-GC-O:

In order to determine which volatile compounds were aroma-active, GC-O was performed using the same SPME-GC-O procedure described for muscadine wines. Five trained, internal panelists were used to evaluate the wines. Results of previous GC-MS analyses showed that all wines, regardless of spray treatment, contained the same aroma compounds. Therefore, only the sprayed sample was evaluated. Varying amounts of sample ("sample levels") were extracted with SPME prior to injection on the GC (500 µL, 100 µL, 50µL, and 10 µL). Panelists evaluated each sample level one time, and order was randomized among panelists.

Quantification of Ethyl Esters using SPME-GC-MS:

To quantify ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate in Chambourcin wine samples, a standard additions procedure was used with the same nonpolar column SPME-GC-MS procedure described above for identification of all aroma compounds. Four standard solutions were prepared with 10 ng/µL internal standard (hexyl butanoate) and either 0, 5, 10, or 25 ng/L of each ethyl ester in methanol. 100 µL of each wine sample and 10 µL of standard solution (corresponding to 0, 0.5, 1, and 2.5 additional mg/L of each ethyl ester in the wine) were added to 890 µL of an artificial wine matrix (12% v/v ethanol in water with 350 mg/L tartaric acid) in triplicate. Ester compounds were quantified using the integrated peak areas, corrected for the internal standard.

Statistical Analysis:

JMP Pro 14.0.0 statistical software was used to conduct all statistical tests.

GC-MS Data: Areas under TIC curve peaks were used as a semi-quantitative comparison of aroma compound concentrations among sample treatments. A hierarchical clustering analysis and a PCA were conducted with peak areas, but the results were inconclusive.

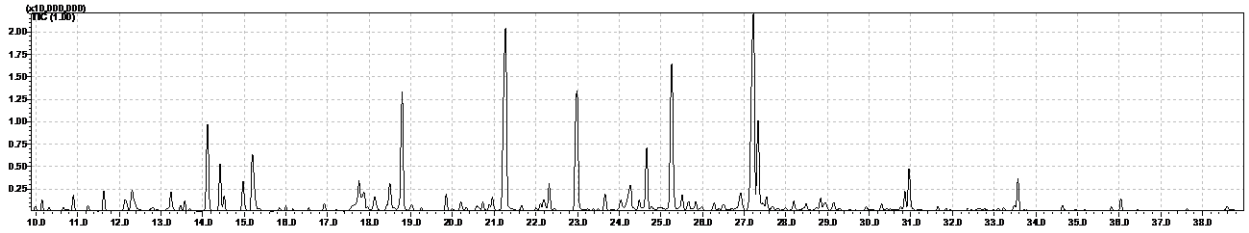
GC-O Data: NIF values were calculated for each sample level as a percentage of the panelists that detected a particular odor. A NIF>60 was determined to represent a positive identification of that substance as an aroma-active compound. A PCA analysis was done using the nonpolar column GC-MS area values of those compounds that were identified as aroma active from GC-O.

Ester Quantitation: An ordinary least squares analysis with a student's t-test ($p<0.05$) was conducted to determine if there were significant differences in wine ethyl ester concentrations among spray treatments.

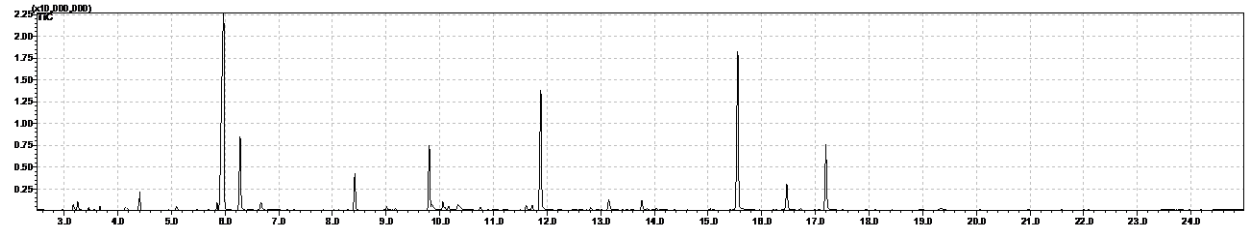
RESULTS

Muscadine Wine SPME-GC-MS Analysis:

On both the non-polar and wax column GC-MS analyses, the same compounds were seen in all samples, regardless of winemaking treatment. There were 59 compounds positively identified in all samples using the nonpolar column and 48 compounds positively identified using the wax column. TIC plots for the 7 days BG sample on both columns are shown in Figure 1.



(a)



(b)

Figure 1. Total ion chromatogram (TIC) plot obtained from SPME-GC-MS analysis of a Noble muscadine wine (2017) sample with 7 days of fermentation on the skins and with β-glucosidase enzyme added at bottling using a nonpolar GC column (a) and a wax GC column (b).

In order to narrow data down for statistical analysis, only compounds that were positively identified on both columns were further considered. There were 27 such compounds, and they could be divided into five "aroma categories"- floral, fruity, green, herbal, and rancid/unpleasant (Table 1).

Table 1- Aroma compounds positively identified in muscadine wines (2017) on both the nonpolar and wax columns. Wines were produced with varying times of fermentation on the skins (0, 3, and 7 days) and with different levels of β-glucosidase (BG) glycosidic enzyme (no BG and BG).

Compound	Compound class	Odor description	Aroma category
2-Phenylethanol	Aromatic alcohol	Honey, spice, rose, lilac, yeast	Floral
Benzyl alcohol	Aromatic alcohol	Floral, fruity	Floral
Acetophenone	Aromatic ketone	Must, flower, almond, cheese, sweat	Floral
Citronellol	Monoterpene	Rose, clove, citrus, floral	Floral
Linalool	Monoterpene	Floral, lavender	Floral
2-Ethylhexanol	Primary alcohol	Rose, green, citrus, floral	Floral
(Z)-Ethyl cinnamate	Ester	Honey, cinnamom	Fruity
Ethyl 2-methylbutyrate	Ester	Apple, strawberry, blackberry, green apple	Fruity
Ethyl 3-hydroxybutyrate	Ester	Grape, nutty, coconut, marshmallow	Fruity
Ethyl butyrate	Ester	Apple, strawberry, bubblegum, pineapple	Fruity
Ethyl decanoate	Ester	Grape	Fruity
Ethyl dodecanoate	Ester	Leaf, mango	Fruity
Ethyl hexanoate	Ester	Apple peel, fruit, strawberry, anise	Fruity
Ethyl isovalerate	Ester	Fruit, cashew, anise, apple, blackcurrant	Fruity
Ethyl nonanoate	Ester	Fruit, rose, wax, tropical	Fruity
Ethyl octanoate	Ester	Fruit, fat, floral, green, leafy	Fruity
Isoamyl acetate	Ester	Banana, pear	Fruity
Isobutyl acetate	Ester	Fruit, apple, banana, pear, floral	Fruity
Nonanal	Aldehyde	Fat, citrus, green, waxy	Green
1-Hexanol	Primary alcohol	Resin, flower, green, herbal, woody, leafy	Green
1-Nonanol	Primary alcohol	Fat, green	Green
Eucalyptol	Monoterpene	Mint, camphor, licorice, pine	Herbal
α-Terpineol	Monoterpene	Oil, anise, mint, peach, floral, toothpaste	Herbal
Hexanoic acid	Carboxylic acid	Sweat, pungent, cheese, rancid	Unpleasant/rancid
Octanoic acid	Carboxylic acid	Sweat, cheese, fat	Unpleasant/rancid
1-Decanol	Primary alcohol	Fat	Unpleasant/rancid
Octanol	Primary alcohol	Chemical, metal, burnt, sulfurous	Unpleasant/rancid

A hierarchical clustering analysis was conducted using the TIC peak area values of the 27 compounds positively identified on both columns. Only area values from the nonpolar column analysis were used, because nonpolar columns are generally better-suited for analysis of samples containing alcohol. Figure 2 shows the clustering constellation plot. Samples appeared to divide into four clusters: “cluster 1” containing 0 days BG, 3 days BG, and 7 days BG samples, “cluster 2” containing 7 days no BG sample, “cluster 3” containing 0 days no BG and 3 days no BG samples, and “cluster 4” containing three samples that appear to be outliers. Thus, only the first three clusters will be considered further.

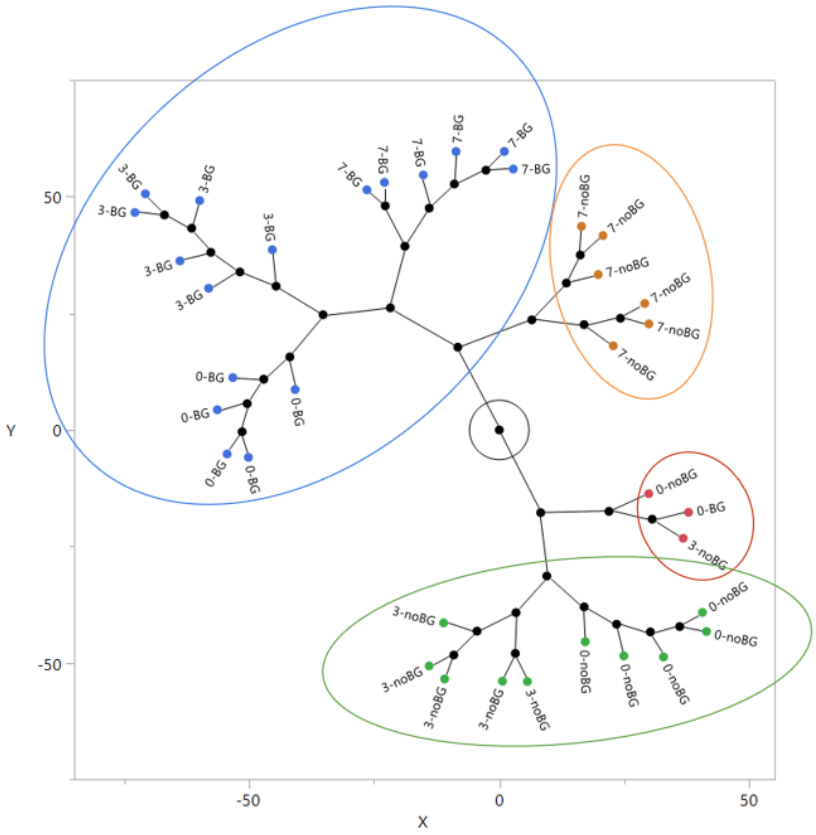


Figure 2. Hierarchical clustering constellation plot based on TIC peak areas for muscadine wine (2017) aroma compounds analyzed by GC-MS with a nonpolar column. Wines were produced with varying times of fermentation on the skins (0, 3, and 7 days) and with different levels of β-glucosidase (BG) glycosidic enzyme (no BG and BG).

All samples with the BG enzyme could be grouped together, whereas the 0 and 3 days no BG samples were grouped and the 7 days no BG sample was in its own group. Therefore, it is likely that use of the β-glucosidase glycosidic enzyme lead to wines with similar aroma profiles, and wines with longer fermentations on the skins were also different in terms of aroma. In order to investigate relationships between the clusters and aroma compounds, parallel coordinate plots were generated (Figure 3). The plot for cluster 1 (all BG samples) appeared to show high levels of fruity compounds (left portion of graph) and floral/herbal terpenes and fruity fatty acid esters (right portion of graph). For cluster 2 (7 days no BG), there appeared to be high levels of fruity compounds (left side of left), similar to the cluster 1 samples, but lower amounts of the fruity fatty acid esters on the right side of the graph. The plot for cluster 3 (0 and 3 days no BG) showed lower amounts of fruity compounds on both the left and right sides of the graph, relative to the other two clusters.

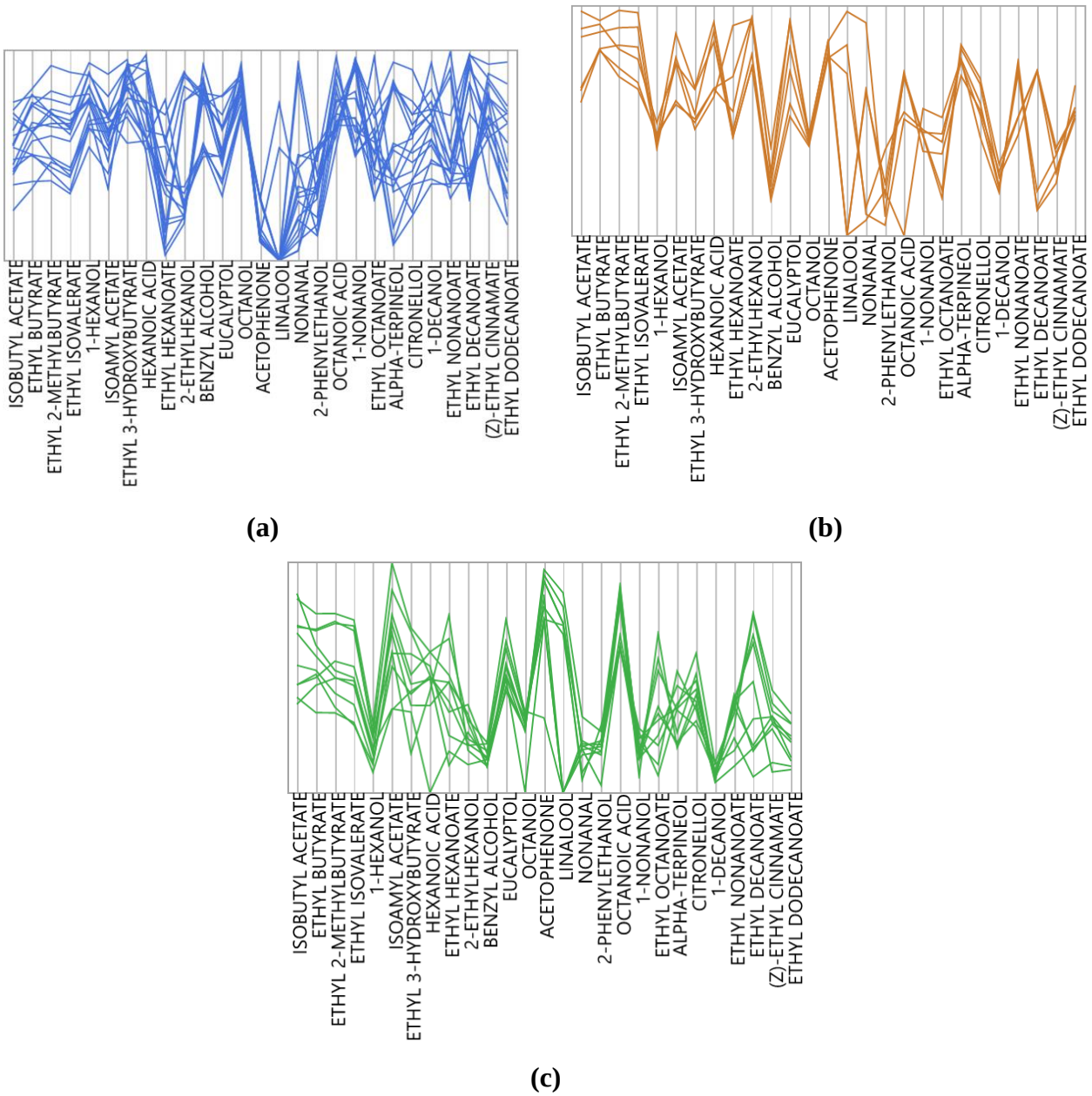


Figure 3. Hierarchical clustering parallel coordinate plots for (a) cluster 1 (all BG samples), (b) cluster 2 (7 days no BG), and (c) cluster 3 (0 and 3 days no BG) based on nonpolar column GC-MS area values of Noble muscadine wine (2017) samples. Wines were produced with varying times of fermentation on the skins (0, 3, and 7 days) and with different levels of β-glucosidase (BG) glycosidic enzyme (no BG and BG).

Therefore, it is likely that wines produced with the BG enzyme will be perceived as fruitier, and that increasing skin fermentation time could also lead to increased fruitiness. However, the clustering analysis only took into account the 27 compounds that were positively identified on both the nonpolar and wax columns. It is possible that compounds seen only on the nonpolar column, or compounds that could not be positively identified, were aroma-active and could provide

further distinction among samples. Thus, the Functional Data Explorer platform in JMP was used to compare the full chromatogram curves for each sample.

As GC-MS data were being collected, a retention time (x-axis) and intensity (y-axis) were recorded for each data point, with one data point recorded every 0.005 minutes. There were a total of 5820 data points recorded for each sample run. The Functional Data Explorer takes all of these data points into account for each sample. Initial data plots were built for each treatment (Figure 4).

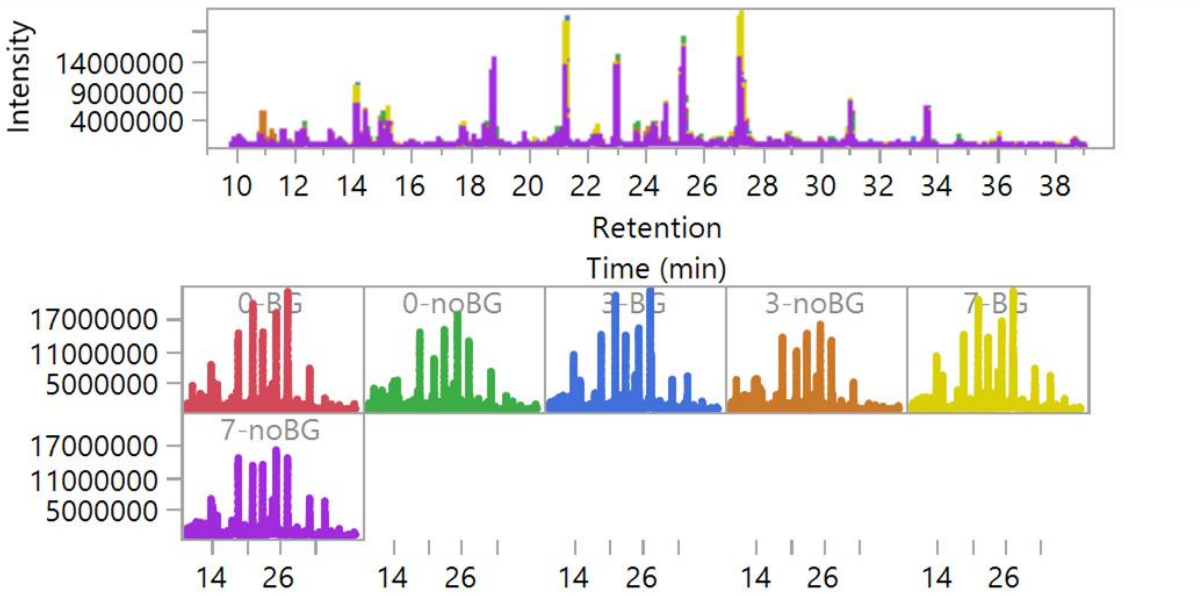


Figure 4. Initial data plots (chromatogram retention time vs. intensity) for each muscadine winemaking treatment, built using the Functional Data Explorer platform in JMP Pro 14.0.0. Noble muscadine wines (2017) were produced with varying times of fermentation on the skins (0, 3, and 7 days) and with different levels of β-glucosidase (BG) glycosidic enzyme (no BG and BG).

These plots appeared to show that the BG samples have greater intensities across the chromatogram than their respective no BG samples. The 7 days BG sample appeared to stand out above the others in the overlaid chromatogram. A B-spline model was fit to the dataset with 10 knots (AIC = 6562263.9 and BIC=6562540.7). With this model, a functional PCA was conducted, in which 97.3% of the variation in the data was accounted for by one component (Figure 5).

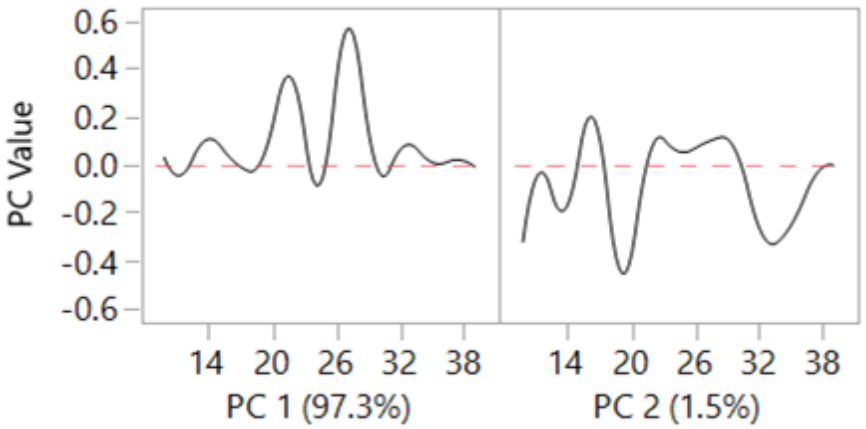


Figure 5. Function PCA plots for PC1 and PC2 built using the Functional Data Explorer platform in JMP Pro 14.0.0. Noble muscadine wines (2017) were produced with varying times of fermentation on the skins (0, 3, and 7 days) and with different levels of β-glucosidase (BG) glycosidic enzyme (no BG and BG).

The first component represented high levels of compounds that eluted from 20-25 minutes (benzyl alcohol, eucalyptol, linalool, 2-phenylethanol) and 26-31 minutes (citronellol, ethyl nonanoate, ethyl decanoate). PC2 represented low levels of compounds that eluted from 16-19 minutes (1-heptanol, hexanoic acid, 1-octen-3-ol, octanal) and 31-33 minutes (dodecanal, octanoic acid, 1-dodecanol). Therefore, PC1 was thought to represent high levels of fruity/floral compounds and PC2 was thought to represent low levels of fatty, waxy, rancid, vegetal, and green compounds. The functional PCA score plot (Figure 6) showed clear separation among BG and no BG treatments and among the 0 and 3 days skin contact and the 7 days skin contact treatments. These groupings were similar to those seen with the hierarchical clustering analysis. The BG treatments had higher loadings on PC1, and therefore it was concluded that these treatments had greater amounts of fruity/floral compounds. The 0 and 3 days skin contact time treatments had higher loadings on PC2, so it was determined that these treatments represented lower amounts of green/unripe compounds.

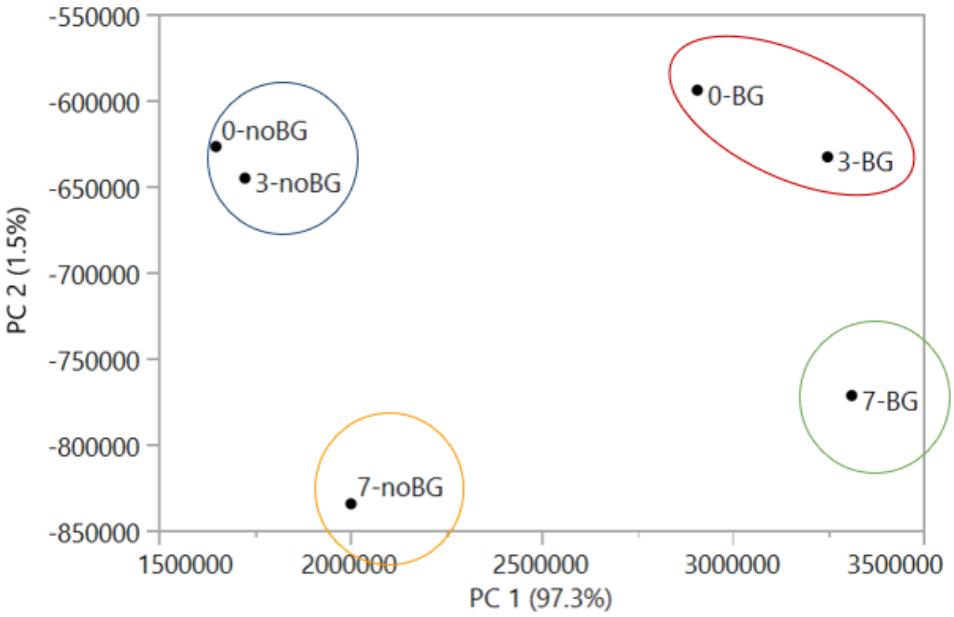


Figure 6. Functional PCA score plot for muscadine wine (2017) samples built with the Functional Data Explorer platform in JMP Pro 14.0.0. Wines were produced with varying times of fermentation on the skins (0, 3, and 7 days) and with different levels of β-glucosidase (BG) glycosidic enzyme (no BG and BG).

From the results of the Functional Data Explorer analysis, it was determined that the winemaking treatments could be clearly distinguished based on their entire chromatograms. The samples treated with the β-glucosidase enzyme could be characterized by higher amounts of fruity/floral compounds, whereas samples with longer durations of fermentation on the skins displayed higher levels of green/unripe compounds. However, not all of these compounds were necessarily odor-active in the wine. Therefore, GC-O analysis was conducted to determine which compounds were odor-active.

Muscadine Wine SPME-GC-O Analysis:

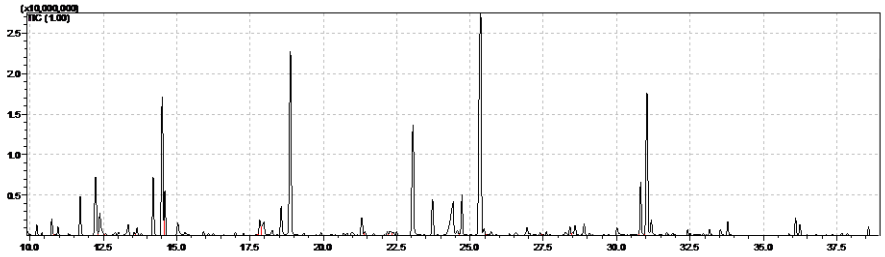
There were eight compounds identified as odor-active (NIF>60 in at least one sample) in the muscadine wine (Table 2). Compounds included the fruity/floral ethyl esters ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate, the roasty/sweet/malty methyl ester methyl hexanoate, the cheesy/sweaty carboxylic acid 2-methylbutyric acid, the floral/sweet aromatic alcohol 2-phenylethanol, and the mushroom-smelling alkenyl alcohol 1-octen-3-ol. It was of particular interest that the ethyl esters were aroma active at almost all sample levels, as these compounds were seen to provide separation among BG and no BG samples from the GC-MS results. Therefore, it is likely that, when tasted, wines produced with the addition of the β-glucosidase enzyme at bottling would be perceived as fruitier.

Table 2- Odor-active compounds, nasal impact factors (NIF) and odor descriptors for the muscadine wine (2017) 7 days BG sample, which was assumed (from GC-MS results) to be representative of all samples in this study. Wines were produced with varying times of fermentation on the skins (0, 3, and 7 days) and with different levels of β-glucosidase (BG) glycosidic enzyme (no BG and BG).

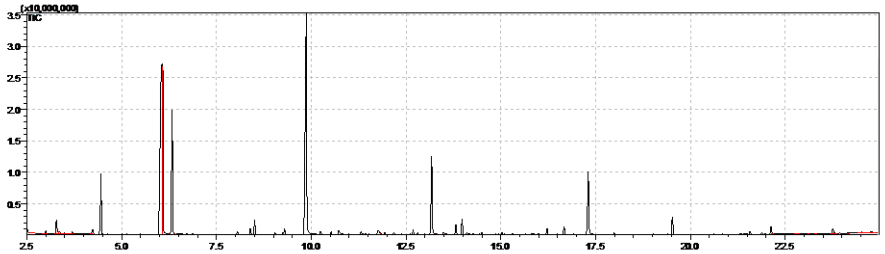
	NIF				
Compound	**500 uL**	**100 uL**	**50 uL**	**10 uL**	**Odor descriptors**
Ethyl butanoate	100	100	80	100	Red fruit, artificial, strawberry, apple
Ethyl hexanoate	100	80	80	40	Green apple, grape, artificial, candy
Methyl hexanoate	100	80	80	100	Bread, roasty, fruity, malty, sweet
2-Methylbutyric acid	80	80	40	20	Cheese, sweat, feet, stinky
2-Phenylethanol	80	80	60	80	Rose, floral, honey, wine
Ethyl octanoate	80	40	80	80	Grape, artificial, wine, slightly waxy
1-Octen-3-ol	60	60	60	60	Mushroom, forest
Ethyl decanoate	60	0	40	0	Fruity, floral, wine, grape, rum

Chambourcin Wine SPME-GC-MS Analysis:

On both the non-polar and wax column GC-MS analyses, the same compounds were positively identified in all samples, regardless of winemaking treatment. There were 56 compounds positively identified in all samples using the nonpolar column and 58 compounds positively identified using the wax column. TIC plots for the sprayed sample on both columns are shown in Figure 7.



(a)



(b)

Figure 7. Total ion chromatogram (TIC) plot obtained from SPME-GC-MS analysis of a Chambourcin wine (2018) produced from grapes sprayed with LalVigne® MATURE at veraison using a nonpolar GC column (a) and a wax GC column (b).

Like with the muscadine wine data, compounds were narrowed down to only those seen on both the nonpolar and wax column (Table 3). There were 34 compounds such compounds, and they could be divided into five aroma categories: floral, fruity, fusel/alkane, green/vegetal, and unpleasant/rancid.

Table 3- Aroma compounds positively identified in Chambourcin wines (2018) on both the nonpolar and wax columns. Wines were produced from grapes treated with LalVigne® at veraison (sprayed) and from untreated grapes (control).

Compound	Compound class	Odor description	Aroma category
2-Phenylethanol	Aromatic alcohol	Honey, spice, rose, lilac, yeast	Floral
Benzyl alcohol	Aromatic alcohol	Floral, fruity	Floral
2-Phenylethyl acetate	Ester	Honey, floral, rose	Floral
Citronellol	Monoterpene	Rose, clove, citrus, floral	Floral
2-Ethylhexanol	Primary alcohol	Rose, green, citrus, floral	Floral
2,3-Butanediol	Diol	Fruit, onion	Fruity
Diethyl succinate	Ester	Wine, fruit, watermelon	Fruity
Ethyl 2-methylbutyrate	Ester	Apple, strawberry, blackberry, green apple	Fruity
Ethyl butyrate	Ester	Apple, strawberry, bubblegum, pineapple	Fruity
Ethyl decanoate	Ester	Grape	Fruity
Ethyl dodecanoate	Ester	Leaf, mango	Fruity
Ethyl hexanoate	Ester	Apple peel, fruit, strawberry, anise	Fruity
Ethyl isovalerate	Ester	Fruit, cashew, anise, apple, blackcurrant	Fruity
Ethyl octanoate	Ester	Fruit, fat, floral, green, leafy	Fruity
Hexyl acetate	Ester	Fruit, herb, spicy, sweet wine	Fruity
Isoamyl acetate	Ester	Banana, pear	Fruity
Isoamyl butyrate	Ester	Sweet, apricot, banana	Fruity
Isobutyl acetate	Ester	Fruit, apple, banana, pear, floral	Fruity
Methyl decanoate	Ester	Wine, fruity	Fruity
Methyl hexanoate	Ester	Fruit, fresh, sweet, paint thinner, acetone	Fruity
3-Methyl-1-pentanol	Primary alcohol	Wine, cognac, whiskey, fruity, green	Fruity
Nonanal	Aldehyde	Fat, citrus, green, waxy	Fusel/alkane
Heptadecane	Alkane	Alkane, fusel	Fusel/alkane
Hexadecane	Alkane	Alkane, fruit, fusel	Fusel/alkane
Pentadecane	Alkane	Alkane, green, fusel	Fusel/alkane
Tridecane	Alkane	Alkane, citrus, fruity, fusel	Fusel/alkane
Methionol	Alkyl sulfide	Potato, garlic, cooked vegetable	Green/vegetal
1-Heptanol	Primary alcohol	Chemical, green, fresh	Green/vegetal
1-Hexanol	Primary alcohol	Resin, flower, green, herbal, woody, leafy	Green/vegetal
Decanoic acid	Carboxylic acid	Rancid, fat, soap	Unpleasant/rancid
Hexanoic acid	Carboxylic acid	Sweat, pungent, cheese, rancid	Unpleasant/rancid
Octanoic acid	Carboxylic acid	Sweat, cheese, fat	Unpleasant/rancid
1-Decanol	Primary alcohol	Fat	Unpleasant/rancid
1-Dodecanol	Primary alcohol	Fat, wax	Unpleasant/rancid

A hierarchical clustering analysis and PCA analysis were attempted using the nonpolar area values of the 34 compounds in Table 3. However, these analyses were inconclusive.

Chambourcin Wine SPME-GC-O Analysis:

In order to determine which compounds were odor-active in the wines, SPME-GC-O analysis was conducted. There were 10 compounds identified as odor-active (NIF>60 for at least one sample level). Compounds included the fruity/floral esters ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, isoamyl acetate, and diethyl succinate, the floral/sweet aromatic alcohol 2-phenylethanol, the vegetal/green ester methyl hexanoate, the cheesy/sweaty methylbutyric acid isovaleric acid, and the vegetal alkyl sulfide methionol (Table 4).

Table 4- Odor-active compounds, nasal impact factors (NIF) and odor descriptors for the sprayed Chambourcin wine (2018) sample treated with LalVigne® MATURE at veraison.

	NIF				
Compound	**500 uL**	**100 uL**	**50 uL**	**10 uL**	**Odor descriptors**
Methyl hexanoate	100	100	100	100	Vegetal, bread dough, leaves, green
Ethyl decanoate	100	100	80	0	Grape juice, wine, red fruit, cherries
Ethyl hexanoate	100	80	60	80	Apple, fresh, artificial, red fruit
Ethyl butanoate	100	60	80	40	Red fruit, strawberry, artificial, bubble gum
Isovaleric acid	80	100	100	100	Cheese, sweat, vomit
2-Phenylethanol	80	80	100	100	Rose, honey, fermented, wine
Methionol	80	60	80	40	Slight mushrooms, fatty, musty
Isoamyl acetate	60	80	60	80	Banana, pear, apple, artificial, ripe
Ethyl octanoate	60	60	40	20	Wine, fermented fruit, grape, caramel
Diethyl succinate	60	20	20	20	Fruity, flowery, spicy, roasted

Similar to the muscadine wine analysis, the four major wine ethyl esters (ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate) were odor active at almost all sample levels. In order to determine whether wines from each spray treatment could be distinguished based on only the odor-active compounds, a PCA was conducted using the nonpolar GC-MS area values of the compounds in Table 4.

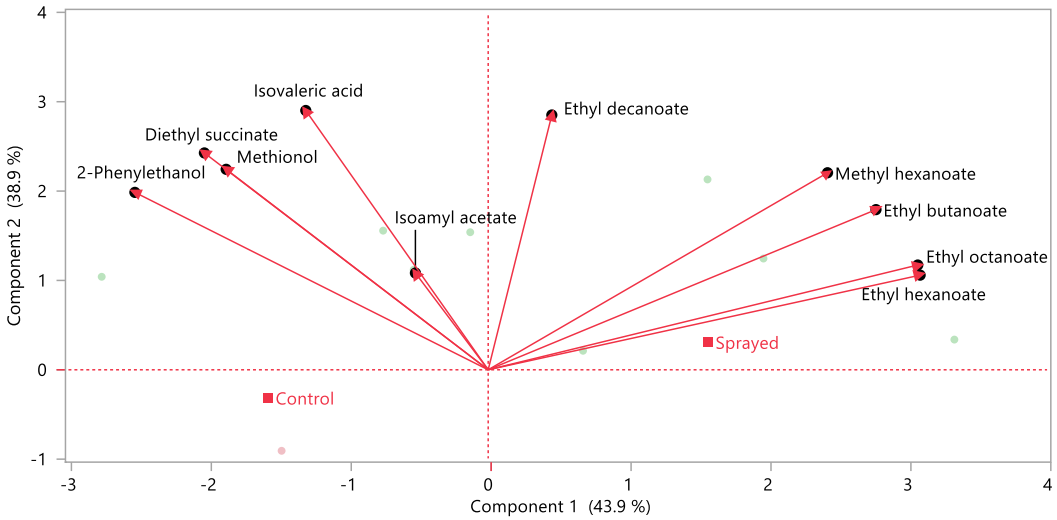


Figure 8. PCA biplot for the aroma-active compounds in sprayed and control Chambourcin wines (2018). Wines were produced from grapes treated with LalVigne® at veraison (sprayed) and from untreated grapes (control).

The first component appeared to represent high levels of the fruity ester compounds ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate, and the vegetal/green ester methyl hexanoate (Figure 8). The second component appeared to represent high levels of all compounds. The sprayed treatment had a higher loading than the control treatment on PC1, which indicated that it likely had higher amounts of the fruity esters. The sprayed treatment also had a higher loading than the control treatment on PC2, although this difference was less pronounced.

Chambourcin Wine Ester Quantitation:

The results of GC-O and PCA analysis showed that the four major wine ethyl esters (ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate) were aroma-active in the wines and that the wines produced from grapes treated with LalVigne® likely had higher levels of these compounds. Therefore, these four compounds were quantified using a standard additions SPME-GC-MS procedure. This was done to obtain an absolute quantitative measure, since the area values obtained from standard GC-MS analysis were only pseudo-quantitative at best and were not corrected with an internal standard.

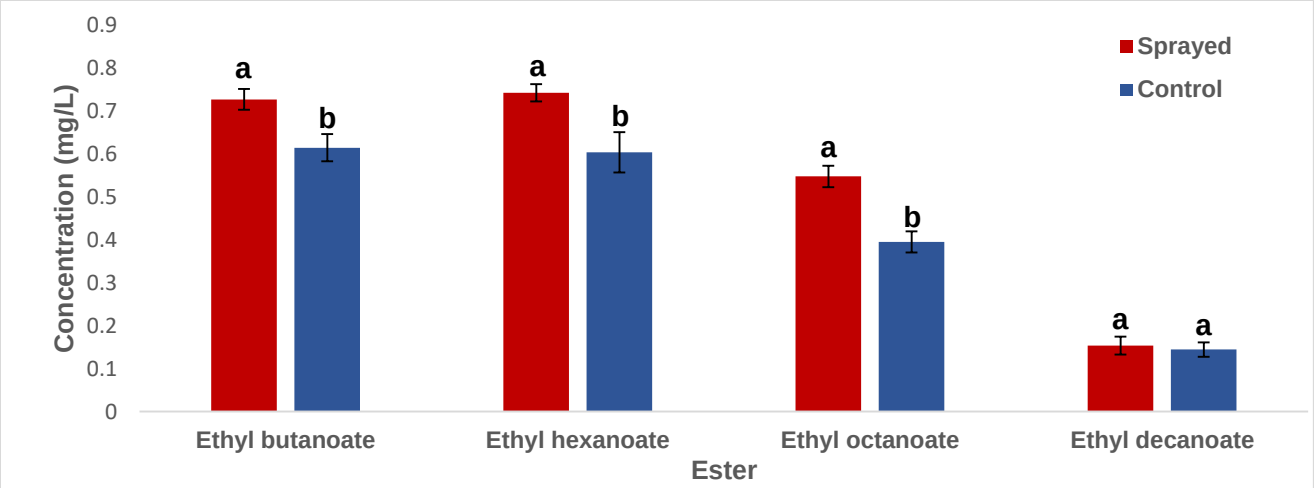


Figure 9. Concentrations of ethyl esters ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate in Chambourcin wines produced from grapes treated with LalVigne® at veraison (sprayed) and from untreated grapes (control) determined from SPME-GC-MS analysis. Error bars indicate standard deviation. Means not connected by the same letter within the same compound are significantly different according to student's t-test ($p<0.05$).

Figure 9 shows that the concentration of ethyl butanoate (0.73 mg/L vs. 0.61 mg/L), ethyl hexanoate (0.74 mg/L vs. 0.60 mg/L), and ethyl octanoate (0.55 mg/L vs. 0.39 mg/L) were present in significantly greater concentrations in the Chambourcin wine produced from sprayed grapes than in the wine produced from control grapes. There was no difference in the concentration of ethyl decanoate among wine treatments (0.15 mg/L for sprayed and 0.14 mg/L for control). Ethyl butanoate and ethyl hexanoate were present in the greatest concentration, followed by ethyl octanoate. Therefore, it is likely that Chambourcin wines produced from grapes treated with LalVigne® MATURE at veraison will be perceived as fruitier during consumption.

DISCUSSION

In the muscadine wines, a majority of the compounds identified could be classified as fruity or floral. This result was logical since muscadine wines are known for their excessive, almost candy-like fruitiness. In previous research on the aroma profile of Noble muscadine wines, Lamikanra et al.[10] analyzed flavor development during fermentation and aging. It was determined that 2-phenylethanol and the fatty acid esters, which form from condensation of fatty acids with ethanol, were significant aroma compounds. Both 2-phenylethanol and the fatty acid ethyl esters ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate were observed to be odor-active in the Noble muscadine wines in this study. Furaneol (burnt sugar, cotton candy aroma) and *o*-aminoacetophenone (artificial grape, foxy aroma) are widely known to be major contributors to the aroma of fresh muscadine berries and juice[9]. However, these compounds were not seen in the wine samples in this study, and were not determined to be predominant contributors to aroma in the study of Lamikanra et al.[10]. Authors proposed that the intense, almost artificial, candy-like fruitiness of the wines in this study was due to excessive amounts of esters, which were perceived during GC-O analysis as having strawberry, apple, bubble gum, artificial, and candy aromas.

Muscadine wine samples with different skin contact times and enzyme levels could be readily distinguished based on their GC-MS profiles. Increasing the duration of fermentation on the skins of wine is known to lead to increased extraction of phenolic compounds from the skins and seeds[2], thus deepening the color and yielding more astringent/tannic wines. However, authors wanted to determine if the volatile aroma profile could be also be impacted by variation in the skin contact time. Many volatile compounds are present in wine as their glycosidic (sugar-bound) forms, which leads to decreased (if any) volatility. The addition of a glycosidic enzyme, which cleaves the sugar and restores volatility, is a common winemaking practice to increase fruitiness and overall aroma impact. In this study, it was determined from the clustering analysis that all samples with the BG enzyme had similar aroma profiles. For samples without the addition of BG, the 0 and 3 days skin contact samples were similar, and the 7 days sample stood on its own. The functional data explorer output showed similar groupings, except that the 7 days BG sample was in its own group. Samples with the addition of the BG enzyme were associated with high levels of fruity/floral compounds, such as esters and monoterpenes, and the 7 days skin contact samples were associated with high levels of vegetal/green compounds, such as alcohols and aldehydes. These results were in line with what would be expected from the winemaking treatments tested: adding a glycosidic enzyme at bottling could potentially increase wine fruitiness, and leaving must to ferment on skins/seeds for a longer amount of time could lead to wines that are perceived as more green/unripe.

In the Chambourcin wines, a majority of the compounds identified were classified as fruity and floral, similar to the muscadine wines. However, the aroma and flavor of Chambourcin wines are considered closer to that of a traditional *vinifera* wine than a muscadine. Therefore, it is likely that other components, such as those with more spicy, peppery, and herbal notes, also play a role in the aroma of Chambourcin wines but were not seen in this study. This was confirmed from the results of the GC-O analysis. While the fruity esters and 2-phenylethanol appeared to dominate the data, compounds overall were described as having more roasted, fermented, vegetal, spicy, caramel, and "generic wine" aromas. This could be because the fruity esters were not present in as excessive of amounts and thus did not linger and mask the aromas of other compounds, or it could be due to compounds that co-eluted with the identified compounds and contributed to the aroma but could not be identified. In order to determine the overall aroma impact of these Chambourcin wines, rather than just the perceptions of separated individual compounds, a descriptive sensory analysis would need to be conducted. In fact, such a study is being done in the sensory lab at the University of Arkansas Department of Food Science. The analytical data from the work at Graz Technical University will be combined with the sensory data in order to build a more complete picture of how foliar treatment with LalVigne® affects the overall aroma/flavor quality of Chambourcin wines.

When only the odor-active compounds in the Chambourcin wine were considered for multivariate analysis, the wines produced from grapes sprayed with LalVigne® at veraison could clearly be distinguished from those produced with unsprayed grapes. Wines from sprayed grapes were more strongly associated with the fruity fatty acid ethyl esters ethyl butanoate (red fruit, strawberry aroma), ethyl hexanoate (apple, fresh fruit aroma), ethyl octanoate (wine, fermented fruit, grape aroma), and ethyl decanoate (grape juice, red fruit, cherry aroma). In addition, the sprayed wine was associated with higher levels of all odor-active compounds, although the differentiation between the sprayed and control wines in terms of the second principle component was less apparent. Therefore, it is likely that Chambourcin wines produced from grapes treated

with LalVigne® will be perceived as fruitier and have a greater aroma impact overall. Descriptive sensory analysis and industry consumer panels will be conducted on the Chambourcin wines through the University of Arkansas Department of Food Science during the summer of 2019. The results from these studies will be paired with the analytical data obtained from the work at Graz Technical University.

Because the fatty acid ethyl esters appeared to provide the most distinction between the sprayed and control Chambourcin wines, these four compounds were selected for quantitation using a standard additions procedure with GC-MS. The peak area values obtained from the initial GC-MS scan allowed for comparison among samples with multivariate statistical techniques, but these area values did not provide true quantitative measures and were not corrected relative to an internal standard. It was shown that the wines produced from Chambourcin grapes treated with LalVigne® at veraison had significantly greater concentrations of ethyl butanoate (0.73 mg/L vs. 0.61 mg/L), ethyl hexanoate (0.74 mg/L vs. 0.60 mg/L), and ethyl octanoate (0.55 mg/L vs. 0.39 mg/L). All three of these compounds were odor-active at all sample levels tested. Šuklje et al.[11] evaluated the application of LalVigne® to Sauvignon Blanc grapes grown in South Africa and evaluated the volatile aroma compound profiles and sensory attributes of the wines produced from sprayed and control grapes. While wines from sprayed grapes were generally perceived as fruitier by the sensory panel, only minor differences were observed in the concentrations of fatty acid ethyl esters. For example, the concentrations of ethyl decanoate and ethyl dodecanoate were greater in the wines from sprayed grapes after 2 months storage, but the concentrations of these compounds were minor relative to those of the other fatty acid ethyl esters, despite having much higher perception thresholds. Therefore, the results of the Chambourcin fatty acid ethyl ester quantitation were particularly encouraging, as it could potentially explain why wines produced from grapes treated with LalVigne® are often perceived as fruitier and as having improved overall aroma quality.

CONCLUSIONS

Muscadine grapes, juice, and wine are considered a regional specialty in the southern U.S. Their unique flavor is what sets them apart from the other varieties of grapes that can be grown in the area. However, if careful consideration is not taken during winemaking, muscadine wines can turn out less than optimal. There are a variety of winemaking techniques that can be altered to improve wine quality, and this study tested the effect of skin contact time and addition of a glycosidic enzyme at bottling. Authors concluded that the use of β-glucosidase glycosidic enzyme could lead to wine with enhanced fruitiness, and thus improved quality. In terms of skin contact time, selecting the optimal level would likely be a careful balance between a desire to deepen the color and preventing excessive green/unripe aromas and excessive phenolic bitterness/astringency.

Chambourcin is a French-American hybrid red wine grape that is well-suited for the difficult Arkansas climate, and is thus widely grown in much of the Midwestern/Eastern U.S. The goal of this study was to determine if the application of LalVigne® could improve the aroma quality of wines produced from Chambourcin grapevines. Although Chambourcin is considered one of the better, more *vinifera*-like hybrids, wines produced from hybrid grapes are not viewed as highly as those produced from *vinifera* grapes in most markets. The results of this study demonstrated that the application of LalVigne® at veraison could potential increase the fruitiness and overall

aroma impact of Chambourcin wines. Evaluation of the aroma profile of these wines will continue during storage, and results will be paired with sensory analyses.

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