

Southern Region Small Fruit Consortium

Progress Report Research

Title: Identification of Aromas Unique to Muscadine Grapes

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Public Abstract

In progress

Introduction

Muscadine grapes (*Vitis rotundifolia* Michx.) are a disease-resistant specialty crop native to the southeastern United States with potential for increased fresh-market expansion. Advances in U.S. muscadine breeding have resulted in unique traits emerging with commercial, fresh-market potential providing opportunity to strengthen the market presence for the muscadines. This research from the University of Arkansas System Division of Agriculture (UA System) will identify aroma attributes unique to muscadine grapes. This research will provide analytical and sensory data on the aroma attributes of muscadine grapes that can be used as a baseline for further breeding efforts.

Muscadines Aromas and Flavors

Muscadine grapes, and the juice and wine produced from muscadine grapes, have unique fruity and floral aromas and flavors. Flavor of grapes arises from perception of basic taste (sweet, sour, salty, bitter, and umami), volatile aroma compounds, and chemical sensitivity. **Volatile aroma compounds play a critical role in flavor perception.** Threlfall et al. (2007) found that commercial muscadine juices from Arkansas had cooked muscadine, apple, pear, cooked grape, green/unripe, and slightly musty aromas and flavors. Meullenet et al. (2008) found correlations between general muscadine flavor and musty flavor, general grape flavor and metallic flavor, green/unripe flavor and sourness/astringency, and sweetness and floral, apple, and pear flavors for Arkansas muscadine juice. Most research on muscadine flavor has been conducted with juice from processing cultivars, especially Carlos and Noble. However, Felts et al. (2018) developed a sensory lexicon for fresh-market muscadine grapes grown at the UA System Fruit Research

Station and found that panelists detected differences between genotypes in grape/overall, grape/muscadine, and fruity.

Important Muscadine Flavor Volatiles

Lamikanra (1987) determined that higher alcohols and fatty acid ethyl esters were numerically the largest classes of volatile aroma compounds in Noble muscadine wine. Lamikanra et al. (1996) found that 2-phenylethanol (rose and honey aroma) was predominantly synthesized during fermentation of muscadine wines but was also present in fresh muscadine grape skins. In an evaluation of Noble wine, Mayfield (2020) found that fruity esters were the largest class of volatile aroma compounds, followed by higher alcohols, notably 2-phenylethanol (rose and honey aroma). Baek et al. (1997) analyzed volatile aroma compounds in juice from Carlos grapes and found that furaneol and o-aminoacetophenone were likely responsible for characteristic candy and foxy-like aroma notes of muscadine grape juice. **It is important to note that few of these studies have paired GC/MS analysis of flavor volatiles with sensory assessments of aroma. Furthermore, no fresh-market muscadine cultivars have been analyzed for aroma volatiles.**

New Aromas and Flavors in UA System Breeding Selections

The UA System muscadine breeding program began in 2006 and since then, over 19,000 seedlings have been planted and 300 selections have been made. A group of advanced selections are currently being considered for cultivar release. These selections are notable for their interesting flavors. While some advanced selections have the intense grape flavor typically associated with muscadines, others have more tropical/pineapple or rosy/floral aromas. While the UA System breeding team enjoy these new flavors, it is not known whether a broad range of consumers would accept these uniquely flavored selections. It is also unknown which volatile compounds are responsible for the range of muscadine flavors in our breeding program. **This research will identify the important volatile compounds contributing to sensory perception of standard, ‘rosy’, and ‘tropical’ flavors in fresh-market muscadine grapes and assess consumer acceptance for these unique muscadine aromas.**

Objectives

This is a progress report on the research efforts expended. In February 2021, extreme freezing temperatures (-15 to -25 °F) in Arkansas destroyed muscadine plants to the ground or damaged the plants for many genotypes in the UA System breeding program. In addition, a late freeze in April caused additional damage to muscadines in Arkansas. Thus, muscadine fruit from Arkansas was limited.

Since muscadines were harvested in late September 2021, time was limited for both the volatile and sensory analysis for this project. The equipment for volatile analysis is shared with other researchers, and the equipment was being used for another project. Volatile analysis and volatile identification of the muscadines is planned for December 2021 and January 2022.

The Covid-19 pandemic impacted the objectives for this project in terms of implementing a sensory study which is planned for January 2022. If the sensory is not feasible, we will expand the volatile analysis portion of the project using muscadines from North Carolina.

Objectives:

1. Evaluate volatile aroma attributes of muscadine grapes

Measure volatile aroma attributes of muscadines grown in Arkansas

2. Evaluate sensory aroma attributes of muscadine grapes

Measure sensory aroma attributes of muscadines grown in Arkansas

Materials and Methods

The volatile and sensory attributes Arkansas-grown fresh-market muscadines will be evaluated. Fruit from genotypes (named cultivars and advanced breeding selections) were harvested from the UA System Fruit Research Station, Clarksville on September 20, 2021 (Table 1). The seven Arkansas muscadine genotypes evaluated were AM-26 (bronze), AM-70 (pink/red), AM-77 (dark/black), AM-135 (bronze), AM-148 (dark/black), AM-154 (dark/black), and AM-240 (dark/black). Approximately 1.8 kg of berries (four 1-quart clamshells) were harvested for each genotype. The volatile and sensory attributes of fresh-market muscadines will be evaluated at the UA System Department of Food Science, Fayetteville.

Berry weights and composition attribute analysis

The berry weights and composition (soluble solids, pH, and titratable acidity) attributes of each of the fresh-market muscadines grown in Arkansas were evaluated at the UA System. The experiment was organized as a completely randomized design with three replicates per genotype. The berry weights were measured at harvest and the berries for composition were placed in zip-type bags and stored at -10°C until analysis.

Berry size. Five berries per genotype and replication were evaluated for berry weight. Each berry was weighed (g) on a digital scale.

Composition. Five to twenty-five berries (depending on the size of the berries) per genotype and replication were evaluated for composition attributes. Berries were placed in cheesecloth to extract the juice from the berries. The juice from the berry samples was used to determine composition attributes.

Soluble solids. Soluble solids (expressed as percent) of the fruit was measured using an Abbe Mark II refractometer (Bausch and Lomb, Scientific Instrument, Keene, NH).

Titratable acidity and pH. Titratable acidity and pH were measured with an automated titrimeter and electrode standardized to pH 2.0, 4.0, 7.0, and 10.0 buffers. Titratable acidity was determined using 6 mL of juice diluted with 50 mL of deionized, degassed water by titration with 0.1 N sodium hydroxide (NaOH) to an endpoint of pH 8.2; results were expressed as % tartaric acid.

Aroma attribute analysis

The juice for the aroma attributes will be extracted from the grapes by squeezing the grapes in the bags, placing the grapes/juice into cheese cloth, and squeezing the grapes to extract the juice into containers. This process will simulate fresh-pressed juice production (grapes are crushed then pressed to extract juice).

Objective 1. Evaluate volatile aroma attributes of muscadine grapes

Measure volatile aroma attributes of muscadines grown in Arkansas

Volatile aroma. Volatile aroma of the juice from the muscadines will be identified and selectively quantified using solid-phase micro extraction (SPME)-GC-FID and SPME-GC-MS. The SPME fiber will then be manually injected into a Shimadzu GCMS-QP2012 SE standard gas chromatograph-mass spectrometer (Shimadzu Corp., Kyoto, Japan) with an Agilent HP-5 silica capillary column (30 m length, 0.25 mm i.d., 1 μ m film thickness) (Agilent Technologies, Santa Clara, CA, USA). Samples will be injected into the GC-FID injection port of the instrument, and compounds will be identified by a single sample injection into the GC-MS injection port. Compounds will be identified in scan mode with a mass scan range from 20 to 300 m/z . The Wiley7NIST0.5 MS library and relevant literature data will be used to identify volatile compounds. For further positive identification, the Kováts retention index will be calculated for each identified compound. Selected volatile compounds will be quantified using calibration curves with standard solutions.

Objective 2. Evaluate sensory aroma attributes of muscadine grapes

Measure sensory aroma attributes of muscadines grown in Arkansas

Sensory aroma. Consumer sensory analysis of the aroma of juice from fresh-market muscadine genotypes will be conducted at the UA System Food Science Department. Consumers (n=40-75) will be recruited to participate in a voluntary consumer sensory panel. Each consumer panelist will receive an incentive (candy or snack) for participation. The consumers will evaluate the aroma attributes of eight muscadine genotypes. The sample presentation order will be randomized and balanced so that the different genotypes appear the same number of times at each presentation position. Samples will be labeled with three-digit codes. Each consumer will be asked to evaluate aroma on the 9-point verbal hedonic scale (1 = dislike extremely; 9 = like extremely) or scales for the intensity of the aroma, as well as preference. Consumers will be asked to identify three key words that describe the aroma of the muscadines. Consumers will be asked demographic questions including: gender, age group, income and purchase habits. Data will be acquired using paper ballots.

Statistical analysis

Analysis of physical, composition, and postharvest attributes were conducted. Statistical analysis was conducted using JMP[®] Pro Statistical Software (version 16.0; SAS Institute Inc., Cary, NC). The study was analyzed as a completely randomized design with three replicates per genotype. A univariate analysis of variance (ANOVA) was used to determine the significance of the main factors. Tukey's Honest Significant Difference test or Student's T test were used to detect differences among means ($p < 0.05$).

Volatile aroma attributes. Relative peak areas (%) for each positively identified compound will be used for principal components analysis (PCA). Each compound will be assigned a chemical compound class and a general aroma category based on aroma descriptors reported in the databases. The relative peak areas of compounds within each compound class and aroma category will be summed to create general variables. PCAs will be conducted based on the compound class and aroma category variables and used to explore the relationship volatile aroma profiles.

Sensory aroma attributes. For the sensory panel, a univariate analysis of variance (ANOVA) will be used to detect the significance of the main effects for each hedonic-scaled

attribute or line-scaled attribute, and the Panelist main effect will be included to account for between-panelist variation. Nine-point hedonic scales will be converted to numerical values (dislike extremely = 1, dislike very much = 2, dislike moderately = 3, dislike slightly = 4, neither like nor dislike = 5, like slightly = 6, like moderately = 7, like very much = 8, like extremely = 9) for statistical analysis. For the preference-scaled data, an ordinal logistic model will be used to assess the likelihood of preferring the sprayed wine ($\chi^2 < 0.05$). For the aroma descriptor terms provided by panelists, the Text Explorer platform in JMP[®] will be used to determine the most frequently-used descriptors within and across the juice samples and generate a word cloud. Descriptors that were used less than five times overall were excluded from text analysis. The frequencies of occurrence of each descriptor will be determined. A PCA will be conducted, based on the descriptor frequencies, to explore the relationship between cultivars and aroma.

Results and Discussion

The berry size and composition of the muscadine grapes were significantly impacted by the genotype (Table 1). AM-135 had the highest berry weight (13.88 g) and AM-77 had the lowest (5.67 g). AM-135 had the highest soluble solids (19.47%). AM-240 had the highest pH (3.98) and AM-77 the lowest (3.04).

Conclusions

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Impact Statement

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Literature Cited

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Table 1. Muscadine grapes grown in Arkansas and evaluated at the University of Arkansas System Division of Agriculture (2021).

Genotype^z	Skin color	Berry weight (g)	Soluble solids (%)	pH	Titratable acidity (%)^y
AM-26	Bronze	11.08 bc	16.23 b	3.62 b	0.50 b
AM-70	Pink/red	13.50 ab	18.90 a	3.89 a	0.29 c
AM-77	Dark/black	<u>5.67 d</u>	<u>14.00 c</u>	<u>3.04 c</u>	0.88 a
AM-135	Bronze	13.88 a	19.47 a	3.89 a	0.28 c
AM-148	Dark/black	11.86 abc	16.30 b	3.67 b	0.54 b
AM-154	Dark/black	9.61 c	16.93 b	3.58 b	<u>0.25 c</u>
AM-240	Dark/black	13.49 ab	16.87 b	3.98 a	0.26 c
<i>P-value</i>		<i><0.0001</i>	<i><0.0001</i>	<i><0.0001</i>	<i><0.0001</i>

^z Genotypes were evaluated in triplicate. Means highlighted are highest value and means underlined are lowest. Means with different letters for each attribute within location are significantly different (p<0.05) using Tukey's Honestly Significant Difference test.