5-2020

Techniques to Enhance the Attributes of Wines Produced from Grapes Grown in Arkansas

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Techniques to Enhance the Attributes of Wines Produced from Grapes Grown in Arkansas

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Food Science

by

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May 2020
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ABSTRACT

Grapevines (*Vitis* spp.) are one of the most widely-planted horticultural crops, and the United States plays a major role in grape and wine production. Arkansas has a long history of grape and wine production with grapes grown in Arkansas including mostly native species, such as muscadines, and hybrids (crosses of *Vitis* spp.), such as Chambourcin. In addition, the University of Arkansas System Division of Agriculture (UA System) grape breeding program has cultivars and selections that have shown potential for wine production. The objectives of this research were to: evaluate effects of specific inactivated yeast application to Chambourcin grapevines on attributes of grapes and wine; determine impacts of winemaking methods on Noble muscadine wine attributes; evaluate impacts of winemaking methods on Enchantment wine attributes; and explore attributes of wines from UA System white wine genotypes (Opportunity, A-2359, and A-2574). In 2018 and 2019 at a commercial Arkansas vineyard, four rows of Chambourcin grapevines were sprayed with inactivated yeast (spray treatment) and four rows were unsprayed (control treatment). Berries were sampled from each treatment during ripening and at harvest and wines were produced from each treatment. Sprayed Chambourcin berries had higher skin elasticity, lower pH, and higher anthocyanins than control berries. Wines from sprayed grapevines had higher red color than control wines over 12-months storage, higher concentrations of fruity ester aroma compounds in analytical studies, and higher red color and better mouthfeel in sensory studies. This is the first data on inactivated yeast application to Chambourcin, but it shows potential for grapes with better winemaking attributes and wines with deeper red colors and improved sensory attributes. In 2018, Noble muscadine grapes were used to produce wines with different skin contact times and with and without the addition of a glycosidic enzyme. Noble wines with increased skin contact had higher anthocyanins and red

color and spicy, dark-fruit aromas. Wines with 0-days skin contact had strawberry and candy aromas characteristic of muscadine juice. Noble wines without glycosidic enzyme had fruitier, more pleasant aromas. Therefore, skin contact time and glycosidic enzyme addition impacted the color and sensory attributes of Noble muscadine wine. Wines were produced from Enchantment grapes in 2017 and 2018 with and without the addition of tannin and oak. Enchantment wines had *V. vinifera*-like anthocyanins and deep red color. Enchantment wines with oak were associated with oaky, roasted, and caramelized aromas, and wines with tannin had lower overall aromas. These results suggested the potential of Enchantment grapes for producing high-quality, deeply red-colored wines with aging potential. Wines were produced from Opportunity, A-2359, and A-2574 in 2015, 2017, and 2018. The aroma/flavor of Opportunity wines was described as spicy, green apple, and peach, A-2359 wine was described as floral, grapefruit, and Muscat, and A-2574 wine was described as spicy, rose, and peach. This demonstrated that UA System white wine grapes produced wines with unique/pleasant sensory characteristics and could provide new opportunities for the Arkansas grape and wine industry. Therefore, viticultural and enological techniques enhanced the attributes of wines produced from grapes grown in Arkansas.

ACKNOWLEDGEMENTS

I would first and foremost like to acknowledge my advisor, Dr. Renee Threlfall, for her guidance and support throughout this entire process. She provided me with opportunities to work on research projects that I was truly interested in and passionate about and exposed me to the fields of viticulture and enology. I would also like to thank my committee, Dr. Luke Howard, Dr. Han-Seok Seo, Dr. Andy Mauromoustakos, and Dr. Jack Lay, for their insights and critical evaluations over these last few years. In addition, I would like to thank my labmates, Molly Felts, Virginia Beasley, Morgan Gramlich, and Herbie Evans, for their support, especially when they had to wake up at 5:00 AM to harvest and sample grapes with me. Finally, I would like to acknowledge Cindi Brownmiller and Luke Holcombe. Cindi provided valuable assistance with instrument operation and troubleshooting throughout this project, as well as insights into method development and sample preparation. Luke donated much of his time and knowledge as an industry collaborator, working with us in both vineyard and winery settings. With the help of everyone mentioned, I was able to conduct research on a topic that I am truly passionate about and was able to grow as both a flavor/aroma chemist and food scientist.

DEDICATION

This dissertation is dedicated to my grandmother, Mary Mayfield (1931-2018). Growing up, she taught me that food could provide more than basic sustenance- it can be a source of joy, it can bring people together, and, most importantly, it can be something to build a career upon. She earned her M.S. in Food and Nutrition from Colorado A & M College in 1955, where she conducted research on lysine fortification in baked goods. After graduate school, she went on to serve as an Assistant Professor in the College of Home Economics at the University of Washington in Seattle. At UW, she taught classes on foods and nutrition, food preparation, and experimental food preparation and appeared on a local cooking show. She also worked as an Assistant Professor of Nutrition in the College of Home Economics at the University of Arizona, where she taught classes and conducted research on the effect of caffeine on the human body.

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OVERALL INTRODUCTION

Grapevines (*Vitis* spp.) are one of the most widely-planted horticultural crops in the world and are cultivated for fresh fruit consumption (table grapes) and production of juice, wine, and other products. Grapevines are in the family *Vitaceae*, which includes *Vitis*, the genus of the grapevine (Creasy and Creasy 2009). Although there are over 24,000 cultivars of grapevines, the International Organization of Vine and Wine (OIV) lists 250 cultivars significant to the wine industry, with *V. vinifera* as the most widely-planted grape species (OIV 2000). There were approximately 77.8 million tonnes of grapes harvested worldwide in 2018, and 57% were used for wine and juice production (OIV 2019). While European growers have traditionally produced a majority of the world's wine grapes, other countries, such as Australia, New Zealand, South Africa, Argentina, Chile, and the United States, have expanded production. Almost seven million tonnes of grapes were harvested in the United States in 2017, and 63% of these grapes were used for wine production (USDA NASS 2019). There were 292 million hectoliters of wine produced worldwide in 2018, and since 2014, just 10 countries were responsible for over 80% of production: Italy, France, Spain, the United States, Argentina, Chile, Australia, Germany, South Africa, and China (OIV 2019).

The United States is the world's fourth-largest wine producer by volume, with five states (California, Washington, New York, Pennsylvania, and Oregon) responsible for 95% of grape and wine production (TTB 2015, USDA NASS 2019). This is because *V. vinifera* grapes are highly vulnerable to pests, diseases, and extreme temperatures (Waterhouse et al. 2016) and are difficult to grow in much of the United States, including Arkansas. The high cost of maintaining *V. vinifera* grapevines in non-ideal climates typically offsets the profit from producing these wines. Native species, such as *V. rotundifolia* (muscadine) and *V. aestivalis*, and hybrids are

better-adapted to surviving stressors that devastate *V. vinifera* grapes (Reisch et al. 2012). Hybrid grapes are created by grape breeders to reap advantageous traits from both parents, such as the cold-hardiness of native species and the desirable yield and flavor of *V. vinifera*. However, hybrid and native species can have low crop yields and produce wines with unfavorable characteristics, such as high acidity, low astringency, and excessive herbaceous aromas (Waterhouse et al. 2016).

Despite the challenges, grape and wine production contribute significantly to the Arkansas economy. Arkansas was ranked twenty-first among U.S. states for grapevine area in 2017, with 322 hectares (USDA NASS 2019), and about 14 wineries (Arkansas Department of Parks, Heritage 2019). In a 2010 study on the economic impact of Arkansas grapes and wine, it was reported that the Arkansas grape and wine industry was responsible for 1,700 jobs and over $42 million in wages. Wine-related tourism generated $21 million (Frank 2010). Therefore, it would be of interest to explore methods to improve the quality of grapes and wine produced in Arkansas.

Grapes grown in Arkansas include mostly native species and hybrids. Chambourcin (Seyve-Villard 12-417 x Chancellor) is an interspecific French-American hybrid red wine grape grown throughout the midwestern and eastern United States, including Arkansas (Homich et al. 2016, Prajitna et al. 2007). Chambourcin has higher disease and winter resistance than *V. vinifera* grapevines and is considered one of the best red-wine hybrid cultivars for producing quality wine (Dami et al. 2006).

Chambourcin grapevines experience issues with delayed/uneven ripening (Dami et al. 2006, Ferree et al. 2004) and are subjected to the typical disease pressures of Arkansas (Creasy and Creasy 2009, Urbez-Torres et al. 2012), which can affect the quality of grapes for wine

production. While Chambourcin wines have good compositions and deeper red color than other hybrid red wines (Zhu et al. 2012), they can have high acid retention and sourness (Homich et al. 2016) and lower tannin concentrations and therefore less complex mouthfeel than traditional *V. vinifera* wines (Norton et al. 2020). Chambourcin is one of the most economically-important hybrid wine grapes in the United States and Canada (Robinson et al. 2012), but research is still lacking on the effects of vineyard and/or winemaking treatments on the ripening and harvest parameters of Chambourcin grapes and the quality and sensory attributes of Chambourcin wine.

LalVigne® (Lallemand, Inc., Montreal, Canada) is a specific inactivated dry yeast that is rehydrated and applied foliarly to grapevines in the vineyard. This product has been shown to enhance physical properties and composition and increase red-colored anthocyanin compounds in *V. vinifera* wine grapes (Giacosa et al. 2019, Villangó et al. 2015) and improve the sensory attributes of *V. vinifera* wine (Šuklje et al. 2016). Therefore, it would be of interest to evaluate the effect of LalVigne® application of the ripening and harvest parameters of Chambourcin grapes and on the quality and sensory attributes of Chambourcin wine.

Muscadine grapes (*V. rotundifolia*) are a species of grapes native to Arkansas and the southeastern United States that produce wines with unique fruity characteristics (Creasy and Creasy 2009, Sims and Bates 1994). Muscadine grapevines can withstand disease pressures and hot, humid environments that are unfavorable for *V. vinifera* grapevines (Gürbüz et al. 2013, Talcott and Lee 2002, Zhang et al. 2017). Consumption of muscadine grapes and related products has grown in recent years due to their reported human health benefits (Banini et al. 2006, Manach et al. 2005, Marshall et al. 2012). A majority of the commercial muscadine crop is used to produce wine (Sims and Morris 1985), and muscadines are one of the most commonly-grown grape species in Arkansas (Alman 2016). Striegler and Morris (1984) determined that

Noble (black-skinned) muscadine grapes grown in Arkansas were excellent for wine production. Wines produced from muscadine grapes have unique fruity, candy, and floral aromas (Lamikanra et al. 1996, Threlfall et al. 2007).

Despite their unique and appealing aromas and flavors, muscadine wines can have high bitterness and astringency, poor color and color stability, and cloudiness caused by ellagic acid precipitation during storage (Sims et al. 1995). Muscadine wines contain only diglucoside anthocyanins, which are unable to form stable polymeric pigment complexes that protect them from color degradation (Sims and Morris 1985). Sims and Bates (1994) observed an increase in anthocyanin content and therefore red color with increasing skin contact time (duration of fermentation with skins, seeds, pulp, and juice) for Noble muscadine wines. However, increasing skin contact resulted in higher astringency and lower fruity and floral aromas. Muscadine grapes and wine contain significant amounts of non-volatile glycoside aroma compounds, consisting of a non-sugar component (aglycone) attached to one or more sugar moieties. Glycosidic enzymes can release the aglycone from the sugar, converting it to a free volatile form. Glycosidic enzyme addition has been shown to increase the fruitiness of muscadine grape juice (Baek and Cadwallader 1999), but studies on enzyme addition to wines have shown mixed results (Cabaroglu et al. 2003, Rodríguez-Bencomo et al. 2013, Segurel et al. 2009). Therefore, it would be of interest to determine how variations in skin contact time and glycosidic enzyme addition affect the color and aroma attributes of Noble muscadine wine.

The University of Arkansas System Division of Agriculture (UA System) has a Fruit Breeding Program established in 1964 located at the Fruit Research Station in Clarksville, AR. This program has released many cultivars of blackberries, peaches and nectarines, table and juice grapes, and blueberries, and began breeding wine grapes over 40 years ago. The goal of the wine

grape breeding program was to develop new hybrid wine grape cultivars that grow well in Arkansas, have desirable flavor attributes, and are suitable for winemaking. In 2016, the first wine grape cultivars, Enchantment (red-wine cultivar) and Opportunity (white-wine cultivar) were released from the UA System. Two other white-wine advanced breeding selections, A-2359 and A-2574, are being evaluated for potential release. These cultivars and advanced selections show potential for regions that have limited productivity of wine grape cultivars.

The Enchantment grapevine produces teinturier (red-fleshed) berries with a dark purple color in the flesh and juice in the grape. In preliminary evaluations, Enchantment wines were noted to have acceptable compositions, intense color, and Syrah-like aroma. Because of the promise Enchantment has shown for grape growers and wine makers in the mid-South United States, it would be of interest to explore techniques to improve the quality of Enchantment wines. Oak addition is known to give red wines smoky, spicy, and vanilla aromas (Schahinger 2005, Singleton 1995), and exogenous tannin addition can help prevent oxidation in wines and has been correlated with improved mouthfeel (Mercurio and Smith 2008, Robichaud and Noble 1990). Tannin addition is especially helpful for wines produced from hybrid grapes, such as Enchantment, as these wines typically have lower tannins than those from *V. vinifera* grapes (Harbertson et al. 2012, Norton et al. 2020).

Opportunity, A-2359, and A-2574 grapevines produce wines with acceptable compositions. Opportunity wines have spicy, Semillon-like characteristics, while A-2359 and A-2574 wines have Muscat and Gewürztraminer characteristics, respectively. As these white wines have shown promise for grape growers and wine makers in the mid-South United States, further exploration of winemaking potential and the unique flavors and aromas of these wines would be

of interest. Since the Fruit Breeding Program is no longer breeding wine grapes, these would be the last wine grapes released by the U of A System.

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OBJECTIVES

The objectives of this research were to:

1. Evaluate the effects of specific inactivated yeast application to Chambourcin grapevines on the physical, composition, and phenolic attributes of grapes.
2. Evaluate the effects of specific inactivated yeast application to Chambourcin grapevines on the composition, anthocyanin, color, and sensory attributes of wines.
3. Evaluate the effects of skin contact time and glycosidic enzyme addition on the composition, anthocyanin, color, aroma, and sensory attributes of Noble muscadine wines.
4. Evaluate the effects of tannin and oak addition on the composition, anthocyanin, color, and aroma attributes of Enchantment wines during one year of storage.
5. Evaluate the composition, color, aroma, and sensory attributes of wines produced from the UA System white wine grape cultivars and breeding selections.

LITERATURE REVIEW

Grapevines and Grapes

History of grapevine cultivation

Grapevines (*Vitis* spp.) are cultivated worldwide and can be used for a wide range of purposes, including fresh fruit consumption (table grapes) and the production of preserves, wine, juice, and raisins. Evidence has shown that *V. vinifera* grapes, the most widely grown species, originated in southern Caucasia (modern-day northwest Turkey, northern Iraq, Azerbaijan, and Georgia) (Mullins et al. 1992). From Caucasia, grapevines were taken on trading routes to Palestine, Syria, Egypt, Mesopotamia, and the Mediterranean. Once the Greeks and Romans realized the value of the grape, particularly for wine production, the grapevine, and methods for its cultivation and processing, were spread throughout Europe. Interest in worldwide exploration meant that grapevines were eventually brought to North America, Peru, and Chile by traders and explorers. Thus, grapevines evolved in many different environments, leading to diversification and the development of many species (Creasy and Creasy 2009).

Grapevine cultivation statistics

Worldwide grapevine cultivation. According to the International Organization of Vine and Wine (OIV), there were approximately 7.4 million hectares of grapevines cultivated worldwide in 2018. Just five countries (Spain, China, France, Italy, and Turkey) are responsible for approximately 50% of the grapes grown in the world. There were 77.8 million tonnes of grapes harvested in 2018, and 57% were used for wine and juice production, 36% were sold as table grapes, and 7% were used for dried fruit (raisins) (OIV 2019).

While European growers have traditionally produced an overwhelming majority of the world's wine grapes (and therefore wine), there has been a shift in recent years as other

countries, such as the United States, Australia, New Zealand, South Africa, Argentina, and Chile, have rapidly expanded their wine grape production. The trend has been to produce better-quality grapes and wine more inexpensively (Creasy and Creasy 2009).

United States grapevine cultivation. According to the Unites States Department of Agriculture (USDA) National Agricultural Statistics Service (NASS), over 400,000 hectares of grapevines were grown in the United States in 2017. This produced almost seven million tonnes of grapes, and 63% of these grapes were used for wine production. Just five states (California, Washington, New York, Oregon, and Pennsylvania) were responsible for over 95% of United States grapevine cultivation, with California representing 83% of the total (USDA NASS 2019).

Within the United States, American Viticultural Areas (AVAs) are federally-recognized grape growing regions, established by the Alcohol and Tobacco Tax and Trade Bureau (TTB). A particular AVA has "specific geographic or climatic features that distinguish it from the surrounding regions and affect how grapes are grown" (TTB 2019). There are currently 242 AVAs in the United States, and California has the most AVAs of any state, with 139. It is common for wineries to label their wine with an 'appellation of origin'. If this origin is a particular AVA, then at least 85% of the grapes used to produce a particular wine must have come from that AVA and the wine must have been fully finished within the state (or one of the states) that contains the AVA (The Wine Institute 2005, TTB 2019).

Arkansas grapevine cultivation. In 2017, Arkansas was ranked twenty-first among U.S. states for grapevine area, with 322 hectares. From 2008-2015, the amount of grapes harvested and the price per tonne in Arkansas fluctuated. Grape production peaked in 2010 at over 2,300 tonnes, and the price peaked at about $1,290/tonne in 2012 (USDA NASS 2019).

Unfortunately, the USDA does not report detailed information on grape production, such as the distribution of grapes designated for fresh market or processing, for Arkansas. In a 2016 Arkansas grape industry assessment survey conducted by the University of Arkansas Department of Horticulture, 18 grape growers from across the state provided information about their operations (Alman 2016). It was reported that 80% of the grapes grown in Arkansas were used for wine production, whereas 16% were used for juice production, 3% were designated as table grapes, and the remaining 1% were used to produce other value-added products (jams, jellies, raisins, etc.). Muscadines grapes (*V. rotundifolia*) were the most common cultivar, with 11 out of 18 growers indicating they cultivated muscadines for a variety of purposes. After muscadines, Cynthiana (native cultivar), Chambourcin (French-American hybrid), Vignoles (French-American hybrid), and Traminette (hybrid from Illinois) were the most commonly-grown cultivars. In terms of cultivars designated for wine production, Cynthiana was the most common, followed by Chambourcin, Vignoles, and Traminette (Alman 2016).

There are currently three AVAs in Arkansas: the Altus AVA, the Arkansas Mountain AVA, and the Ozark Mountain AVA. The Altus AVA is located in northwestern Arkansas, near the town of Altus in Franklin County. This region is a plateau above the Arkansas River to the south and below the Boston Mountains to the north. A majority of wine grapes in Arkansas come from the Altus AVA. The Arkansas Mountain AVA is located in the Ozark Mountains of northwestern Arkansas and surrounds the Altus AVA. The Ozark Mountain AVA is located in northwestern Arkansas, southern Missouri, and northeast Oklahoma, and is the sixth-largest AVA in the United States by area, covering almost 1.5 million hectares (TTB 2019).

Grapevine cultivars and taxonomy

Grapevines are one of the most widely-planted horticultural crops in the world, with a range of existing cultivars that vary depending on where they are grown and their typical end use (wine, table grapes, juice, etc.). Creasy and Creasy (2009) wrote a comprehensive book on the taxonomy, anatomy, growth, and composition of grapevines and grapes, which will be referenced throughout this review. The entire genome of *V. vinifera* has now been sequenced (Velasco et al. 2007), making the grape the second food plant to achieve this milestone after rice. This genetic information is useful in the development of new cultivars, especially when breeding resistance to diseases and pests.

There are approximately 24,000 named cultivars of grapevines (Viala and Vermorel 1909), and the OIV lists 250 cultivars as significant to the wine industry (OIV 2000). Although there is often pressure on growers to plant only the most recognizable, 'popular' varieties in lieu of more traditional regional specialties, individual regions continue to grow local cultivars to support small but thriving markets.

Grapevines are in the family Vitaceae, which is made up of mostly woody, tree-climbing vines characterized by the presence of tendrils and inflorescences opposite the leaves. Within Vitaceae, there are 12 genera, including *Vitis*, the genus of the grapevine. The *Vitis* species contains two subgenera: *Muscadinia* and *Euvitis. Muscadinia* grapes have different seed shapes than *Euvitis,* and they have simple rather than branched tendrils, smooth bark, fewer berries per cluster, and berries that easily fall off the stem (Bailey 1934, Einset and Pratt 1975, Williams 1923). There are three named species within the *Muscadinia* subgenus, the most important of which is *rotundifolia*, the muscadine grape. Muscadines are native to the southeastern United States and today are primarily grown in that region. They support a small but persistent wine,

table grape, juice, and preserves market. Noble, Scuppernong, Carlos, Magnolia, and Fry are well-known muscadine cultivars.

There are many species within the *Euvitis* subgenus, including *V. vinifera*, the most widely-planted grape species worldwide. *V. vinifera* is used for wine, table grape, juice, and raisin production. All well-known European grapevine species are *V. vinifera*, including Cabernet Sauvignon, Chardonnay, and Pinot noir. Another example of a *Euvitis* species is *V. labrusca*, which is native to North America. Cultivars such as Concord, Catawba, Delaware, and Niagara belong to the *labrusca* species. Although *labrusca* grapes are more disease/pest resistant than *vinifera*, they typically contain 'undesirable' flavor attributes, including foxy (intense artificial grape) aroma.

Although they can possess undesirable flavor attributes, non-*vinifera* cultivars native to North America have been incredibly important for the commercial development of *vinifera* cultivars. The crossing of *vinifera* and non-*vinifera* cultivars became widespread in the 19th century as a means to combat the Phylloxera grapevine epidemic spreading throughout Europe. Phylloxera are sap-sucking insects that feed on and deform the roots and leaves of grapevines. Phylloxera killed a majority of the grapevines in Europe, including 90% of French vines. There is no chemical control for Phylloxera, but species native to the United States have natural resistance, exuding a repellant sap and forming protective wound-repairing tissue. It was discovered that if *vinifera* vines were grafted onto resistant rootstock, resistance would be conferred. Cultivars used for their rootstock included *V. rupestris, V. ruparia,* and *V. berlandieri*. (Wine and Spirit Education Trust (Great Britain) 2012).

Growth cycle of the grapevine

The grapevine is botanically a liana, a woody climbing vine. It can be classified as a woody perennial, meaning that it has an active growing season every year and retreats into dormancy during the winter months, then re-emerges again the following season when environmental conditions become favorable. Unlike herbaceous perennials, grapevine shoots eventually lignify, becoming 'canes' and forming a hard outer periderm layer. Thus, the grapevine trunk is built upon year after year, ceasing growth only during the dormant season (Srivastava 2001). The shoots of the grapevine experience indeterminate growth, meaning that they have no set endpoint for growth. Therefore, grapevines must be pruned every winter to keep vines manageable and to produce a commercial crop (Creasy and Creasy 2009).

Grapevines experience a period of dormancy during the fall/winter seasons and re-emerge from dormancy in the spring, to be harvested in late summer/early fall. Winter dormancy allows the vine to grow and survive in areas where the temperature drops well below freezing in the winter. Some species can survive temperatures as low as -40°C (Pierquet et al. 1977). However, *V. vinifera* grapevines generally cannot withstand winter temperatures less than -15°C without sustaining damage (Clore et al. 1974). In general, grapevines will stop growing and move into dormancy when temperatures fall below 10°C.

Budburst, shoot, and leaf development. As soil temperatures rise at the beginning of the growing season, the shoot primordia begin to grow, pushing out of the buds. This process, known as budbreak, typically occurs around 10°C (Williams et al. 1985, Winkler et al. 1974). After budbreak, pre-formed leaves and internodes (spaces on the canes between nodes) expand, and the first shoots are initiated by energy derived from stored carbohydrates (May 1986,

Winkler et al. 1974). Temperature is the main influencer of shoot growth, and the rate of shoot development increases with increasing temperatures throughout the season.

Because the grapevine is a perennial plant, the rate of photosynthesis one season affects the amount of carbohydrates stored for the dormant season and emergence from dormancy the following season. These carbohydrates are stored in the roots and other woody, lignified tissues, and they supply energy to the growing parts of the vine when photosynthesis is slow or non-existent. Photosynthesis will occur as soon as green tissues develop.

Inflorescence development and flower formation. Grapevine flowers are in highly branched clusters called panicle inflorescences (Pratt 1971). Flowers can be perfect (have both male and female anatomy), male, or female. On cultivated vines, the flowers are typically perfect, which allows self-pollination. Differentiation of individual flowers on the inflorescence begins near the time of budburst (Srinvasan and Mullins 1981). However, the shoot must develop leaves capable of generating the necessary carbohydrates for the rest of the vine before flowering can occur.

Duration of flowering is defined as the time from initial floral development to bloom and pollination and is very dependent on the environment. Cool, cloudy weather and rainfall during flowering will increase the duration of flowering, and warm, sunny weather will quicken it. Flowering can last from a few days to over a month. Temperature requirements for flowering vary by cultivar, but the optimal range for most vines is 30-35°C (Buttrose 1969). After shoots have developed approximately 15-17 nodes, flowers will begin to open, calyptra will fall from the flower, and the process of pollination and berry formation begins (Pratt and Coombe 1978).

Berry growth, veraison, and ripening. Grapes are a true berry, as they contain seeds on the inside of ovarian tissue (the flesh). The berry skin is a layer of epidermal cells protecting the berry from physical damage and pests and containing flavor and color compound. The seeds of

the berry encase the embryo that can develop into a new grapevine. Grapes from the *Euvitis* subgenus can have up to four seeds (Winkler and Williams 1935), whereas those from the *Muscadinia* subgenus can have up to six (Olien 1990). Although some table grape cultivars have been bred to be seedless, most wine grapes contain seeds. Grape berries develop from fertilized flowers, and fertilization typically occurs two to three days after pollination. Fertilization is highly temperature dependent, and even brief periods of cool temperature will cause embryos to degenerate and decrease the chance of fruit set (Ebadi et al. 1996).

After fertilization, cell division and expansion cause berries to grow rapidly. Increases in berry weight, diameter, and volume fit a double sigmoid curve pattern. There are three periods of growth. Phase I represents initial rapid growth following fertilization. Phase II experiences much lower growth rates than phase I, as the berry is focused on seed maturation and lignification (development of a hard outer coating). During phase III, the berry begins to soften, become translucent, and develop color (if it is a red-skinned grape). The beginning of color development, which typically occurs between phases II and III, is denoted by the French word *veraison*. This is a key point in grapevine development, as it also signifies changes inside of the berry. The berry begins to metabolize malic acid, accumulate sugar, and produce varietal flavor and aroma compounds.

There are no universal quantitative parameters for determining berry maturity and readiness for harvest. It is typical to discuss maturity in terms of how suitable the berries are for their intended end use. For example, table grapes are harvested at a sugar content of 17-19 °Brix, whereas wine grapes are harvested at 20-26 °Brix (Puckett 2019). The acid levels in grapes are also commonly measured, as the degree of perceived sweetness is affected by acidity. The harvest date for wine grapes varies based on cultivar and geographical location, but in general,

white grapes reach maturity and are harvested earlier than red grapes. In Arkansas, white wine grapes are typically harvested late July to early August and red wine grapes in late August and early September.

Dormancy. Low temperatures and shortening day lengths in the late growing season (early to mid-autumn) signal the grapevine to accelerate preparation for the dormant season. This includes the yellowing of leaves (leaves become red if it is a red grape cultivar) and eventual leaf-fall. Roots also fall into a quiescent period of dormancy. In warmer areas where leaves do not fall as quickly, another burst of photosynthesis can occur after harvest and roots experience an additional flush of growth prior to dormancy. This means that vines will have increased carbohydrate levels at the start of dormancy, which has been correlated with increased winter hardiness and a 'head start' for the vines coming out of dormancy the following season. In colder climates, there is little to no time between harvest and leaf-fall, so this final flush of root development does not occur (Conradie 2005, Howell 2001, Williams 1996). This could be one of the reasons why grapevines grown in cold climates tend to have lower fruit yields.

Grape berry composition

Compounds in grapes can be classified as either primary or secondary metabolites. Primary metabolites are compounds crucial to the survival of the plant and include sugars and organic acids. Secondary metabolites, like phenolic and aroma compounds, are not needed for basic survival of the vine. Secondary metabolites likely evolved as a means to attract pollinators or seed dispersers or to protect the plant against diseases/pests and physical stressors and are thus only produced when the plant needs them.

Grapes can be divided into three sections: flesh (pulp), skins, and seeds. The flesh contains most of the primary metabolites of the grape, such as water, sugar, acids, and pectin,

whereas skins and seeds contain more secondary metabolites, such as phenolic and aroma compounds.

Primary metabolites. Mature grapes contain 75-85% water, 15-25% sugar, 0.5-1% organic acids (tartaric, malic, and citric acid), and 0.25% pectin. Other nutritional components found in grapes are present in very minor amounts. Sugars (glucose and fructose) make up a majority of grape carbohydrate content. Wine grapes are harvested when they reach specified levels of sugars and acids.

The sugar content of grapes is estimated by measuring the amount of dissolved compounds (soluble solids) in the juice, as the vast majority of dissolved compounds in grape juice are sugars. This is typically done using a refractometer, which measures the extent to which a beam of light is bent when passing through a solution (more bend = more dissolved solids). The percent soluble solids of grapes is often represented as degrees Brix (°Brix), which corresponds to one gram of sugars in 100 grams of solution (percent by mass). Thus, assuming that a majority of the solids in grape juice are sugars, percent soluble solids and °Brix are equivalent. Mature white wine grapes typically have a sugars content of 20-23% soluble solids, and red wine grapes typically have 22-26% soluble solids.

The acidity of grapes is measured using titratable acidity (TA) and pH. TA is a measure of the amount of acid in a solution and is determined by titrating a sample of juice with 0.1 N sodium hydroxide to an endpoint of pH 8.2. Because tartaric acid is the primary acid in wine grapes, the result is expressed as g tartaric acid per liter or as g/100 mL (%). The pH of grapes is important for the microbiological stability of grape juice and wine and effects wine color, while TA is mostly related to the perceived acidity of the juice/wine. Mature white wine grapes typically have > 0.70% TA and < 3.3 pH, and red wine grapes have > 0.65% TA and < 3.4 pH.

Although quantitative measures of sugars and acids are most commonly used to determine when grapes are ready for harvest, other qualitative parameters can be considered as well. For example, if grapes have become susceptible to physical damage or pests/diseases, a grower may decide to harvest before grapes reach optimal levels of sugars and acids to prevent further damage and avoid potential loss of their crop that season. This is a common occurrence for grapes grown in difficult environments, such as Arkansas. Wine grape growers will also sometimes evaluate other qualitative attributes of the grapes, such as color or aroma, to make a decision on harvest timing.

Secondary metabolites: phenolic compounds. The term 'phenolics' (or phenolic compounds) refers to plant compounds that have at least one 6-carbon aromatic ring and one or more hydroxyl groups (Waterhouse et al. 2016). In grapes, phenolics are present in the highest amounts at approximately 50 days post-bloom, and gradually drop in concentration as berries mature (Ristic and Iland 2005). Phenolics are products of phenylalanine metabolism and can be divided into two groups: non-flavonoids and flavonoids. Within the flavonoid category, compounds are further classified as anthocyanins, flavonols, or tannins. Phenolics contribute to the color, bitterness and astringency of grapes.

Non-flavonoids are smaller than the other phenolics found in grapes, and they often interact with flavonoids and are involved in browning reactions in grape juice and wine. Although non-flavonoids can contribute to bitterness, their overall effect is much less than that of the flavonoids. These compounds are typically found in the pulp of the berry and are thus easily extracted during winemaking. Gallic acid, protocatechuic acid, *p*-coumaric acid, and ferulic acid are examples of non-flavonoid compounds found in grapes.

Anthocyanins are responsible for the red color of grapes and wine. These compounds are found primarily in the skin of red grape cultivars, with the exception of teinturier grapes, which also have anthocyanins in the pulp. Anthocyanins make up approximately 0.1% of the grape berry by weight (Brossaud et al. 1999), and there are five anthocyanin aglycones ('anthocyanidins') found in grapes: malvidin, cyanidin, delphinidin, petunidin, and peonidin. However, the anthocyanidin form is unstable and less soluble in water, so only the glycosylated anthocyanin structure is found in grapes. There can be one or two sugar molecules attached to the base structure, and malvidin-3-glucoside is the anthocyanin found in the highest concentrations in *V. vinifera* cultivars. Anthocyanin diglucosides, like malvidin-3,5-diglucoside, are often seen in non-*vinifera* cultivars, such as muscadine and hybrid grapes.

Production of anthocyanins in grapes is affected by environmental factors such as sunlight and temperature. Spayd et al. (2002) determined that exposing Merlot grapes to high temperatures (35-40°C) decreased anthocyanin production but that more exposure to light increased production. Other studies have also confirmed that high temperatures both during the day (37°C) and night (32°C) inhibited the formation of new anthocyanin compounds and decreased their concentration in already-colored berries when plants were transferred to this environment (Kliewer 1977). Yamane et al. (2006) showed that vines are most sensitive to heat-induced inhibition of anthocyanin formation at the point of veraison, when berries are first beginning to develop color.

Flavonols found in grapes include quercetin, kaempferol, myricetin, and isorhamnetin, and these compounds account for only 0.01% (by weight) of the grape berry (Brossaud et al. 1999, Cheynier and Rigaud 1986). Like anthocyanins, flavonols occur as glycosides and are primarily found in the skin of the grape (Singleton and Esau 1969). It has been shown that the

concentration of flavonols in grapes increases in response to sun exposure, and that flavonols decrease significantly when fruit is shaded (Price et al. 1996, Spayd et al. 2002). In fact, this relationship is so strong that the concentrations of flavonol compounds, especially quercetin, can be used as indicators of berry sun exposure during the growing season (Creasy and Creasy 2009). It is theorized that flavonols are produced by berries as a natural form of sunscreen, as they strongly absorb UV light at 360 nm (Waterhouse et al. 2016).

Tannins, or flavan-3-ols, make up 0.5% by mass of the grape berry and are the compounds primarily responsible for grape astringency and bitterness (Brossaud et al. 1999). They are found in both the skins and seeds of grapes, although those from the seeds are very difficult to extract during winemaking due to the hard seed coat (Singleton and Draper 1964). The term tannin is used to describe large polymers (MW 500-3000) of flavan-3-ols, and the most common flavan-3-ol monomers found in grapes are catechin, epicatechin, epicatechin gallate, and epigallocatechin (Souquet et al. 1996, Swain and Bate-Smith 1962).

During the winemaking process, phenolic compounds are extracted from the grape berries at first by the water in the juice and then by alcohol and heat as fermentation begins. Thus, red wines with fermentation on the skins will have higher concentrations of phenolic compounds than white wines. Although the concentration of phenolics in berries is certainly important for the resulting color and flavor of wine, the relationship is not that simple. Various aspects of a wine, such as pH, sulfite usage, oxygen exposure, and reactions among phenolic compounds themselves, can affect the color, bitterness, and astringency.

***Secondary metabolites: aroma compounds*.** Although phenolic compounds are important for the color and flavor of wine grapes, the aromas of grapes are considered their *raison d'être*, or 'reason for being'. Varietal aromas of grapes are those aromas used to distinguish cultivars from

one another, such as the fruity and spicy characteristics of Gewürztraminer, the tropical character of Sauvignon blanc, and the foxy/artificial grape aromas of Concord. There are many compounds responsible for the aroma of grapes, and the collective effect of these compounds may be different than the impression of any single compound. However, there are certain compounds that are known to impart specific, distinguishable aromas that are characteristic of particular grape varieties. These compounds include methyl anthranilate (foxy/artificial grape aroma), terpenes (fruity aromas), norisoprenoids (cooked fruit aromas), and methoxypyrazines (green/unripe aromas).

Methyl anthranilate is the compound primarily responsible for the artificial grape aroma associated with grape species native to North America, like the Concord grape. These grapes are often described as 'foxy', and although the origin of this term is unclear, it may be a derivative of the French word *faux*, meaning false/artificial (Amerine et al. 1959, Nelson et al. 1977).

Terpenes impart fruity and floral aromas to grapes and other plants, and the monoterpenes (linalool, geraniol, nerol, citronellol, etc.) are the most important terpenes for grape aroma. Monoterpenes are associated with aromatic white wine grape cultivars such as Muscat, Sauvignon blanc, Gewürztraminer, and Riesling and impart tropical fruit, orange, rose, and floral aromas. These compounds are found in both the flesh and skin of grapes and accumulate as berries mature. Sun exposure increases monoterpene levels, whereas high temperatures decrease concentrations (Belancic et al. 1997, Ewart 1987).

Norisoprenoids are derived from carotenoid pigments, and beta-damascenone (cooked apple and raspberry aroma) is the most well-known norisoprenoid in grapes. This compound is characteristic of Shiraz, Cabernet Sauvignon, and Chardonnay and its concentration increases with light exposure and temperature.

While methyl anthranilate, terpenes, and norisoprenoids are associated with ripe/fruity aromas and increase in concentration during ripening, methoxypyrazines have green/unripe aromas and decrease as berries ripen. Methoxypyrazines are characteristic of cultivars like Cabernet Sauvignon and Sauvignon Blanc. If high levels of methoxypyrazines are present in wine grapes at harvest, undesirable green bell pepper or canned pea aromas will be imparted to the wine. However, low levels of methoxypyrazines can be considered a positive stylistic choice for some wines, such as the slightly green character of New Zealand 'Marlborough Sauvignon blanc' or the faint green bell pepper aroma of Cabernet Sauvignon.

Deciding when and how to harvest wine grapes

Of all viticultural decisions made during the seasonal lifecycle of the grapevine, the timing of harvest has the largest impact on grape composition. This will depend heavily on the type of wine to be produced. For example, if wine grapes are harvested early, they will be low in flavor and sugar and high in acid, which makes them suited to sparkling wine production. Late-harvest grapes are high in residual sugars and are most often used to produce dessert wine (Creasy and Creasy 2009).

Decisions must also be made on how to harvest grapes, as this will have an influence on their quality. Harvesting can be done either by hand or mechanically. For some styles of wine and for certain trellis systems, the fruit must be hand-harvested. For example, grapes for Champagne production must be intact before processing to ensure minimal extraction of harsh phenolics from the stems and skins (Jackson 2000). However, for most table wines, machine-harvested grapes are used (unless a winemaker wants a hand-selected crop to produce an ultra-premium quality wine).

Once all decisions have been made in the vineyard as to which grapes to plant, how to grow them, and when and how to harvest, work is turned over to the winemaker. Winemakers must have an extensive knowledge of the winemaking process, and the chemistry involved in each of these steps, in order to produce a superb product.

Wine Production and Chemistry

History of wine production

The earliest wine residues date back to the early- to mid-fifth millennium B.C., while the first evidence of intentional winemaking was discovered in Egypt and dated back approximately 5,000 years. Grapes have a natural yeast population that develops as the berries mature, and if grapes are left piled for several days after harvest, they will begin to ferment. It is likely that winemaking was discovered due to this spontaneous fermentation. The rapid production of ethanol by yeasts limits the growth of most bacteria, including pathogens, and the acidity of wine further inhibits microbes. Therefore, wine was a relatively safe beverage to consume in a time before the existence of preservative food storage or water purification technologies. Over time, people expanded their knowledge of winemaking, and in the seventeenth century, wine production shifted towards more modern techniques when the use of sulfur to prevent mold growth during barrel treatments became widespread. During the Industrial Revolution (mid-eighteenth to mid-nineteenth century), cylindrical wine bottles were invented and mass produced. This allowed bottles to be stored on their sides, which kept the corks wet and isolated the wine from oxygen, enabling wines to develop a smooth character and complex fragrance (Jackson 2000).

In the 1860s, Louis Pasteur discovered the importance of yeasts and bacteria for fermentation. Prior to Pasteur's discoveries, very little was known about yeasts or the role they played in converting sugars to alcohol. *Saccharomyces cerevisiae*, the yeast used today for wine and beer production, is not a part of grapes' natural microflora but is highly efficient at converting sugar to ethanol. Once it was known that *S. cerevisiae* was the optimal yeast for wine production, yeast inoculation during fermentation became much more intentional and controlled, leading to wines with greatly improved quality. Therefore, Pasteur's discoveries set in motion a chain of events, propelled by a greater understanding of fermentation and wine science, which produced the huge range of wines known today (Jackson 2000).

Wine production statistics

Worldwide wine production. According to OIV, there were 292 million hL of wine produced worldwide in 2018 (OIV 2019). This was the largest volume of wine produced in the last five years, with a 17% increase reported from 2017 to 2018. Since 2014, 10 countries have been responsible for over 80% of the world's wine production: Italy, France, Spain, USA, Argentina, Chile, Australia, Germany, South Africa, and China. In fact, just three countries, Italy, France, and Spain, produce over 50% of the world's wine.

Over 246 million hL of wine were consumed in 2018, and a steady increase has been seen in worldwide wine consumption since 2000. The United States is the top wine consuming nation, representing 13.4% of the world's wine consumption (by volume) in 2018. Similar to wine production, just 10 countries (the United States, France, Italy, Germany, China, the United Kingdom, Russia, Spain, Argentina, and Australia) were responsible for almost 70% of worldwide wine consumption (OIV 2019).

United States wine production. The United States is the world's fourth largest wine producer, by volume, producing 23.9 million hectoliters of wine in 2018. There were $1.32 billion of wine exported and $5.84 billion of wine imported by the United States in 2018, making it the sixth-largest exporter and the largest importer of wine in the world (OIV 2019).

Just five states (California, Washington, New York, Pennsylvania, and Oregon) are responsible for 95% of wine production in the United States. California alone represents 81% of all wine produced in the United States. (TTB 2015).

Arkansas wine production. There were 12,050 hL of bottled table wine and 7,720 hL of boxed/bulk table wine produced in Arkansas in 2015 (TTB 2015). Unfortunately, data on wine production and sales in Arkansas are sparse, outside of the total produced volumes reported by the TTB. In 2010, Arkansas Technical University commissioned a study on the economic impact of Arkansas grapes and wine through The Wine Business Center in St. Helena, California (Frank 2010). It was determined that the full economic impact of the grape and wine industry in Arkansas, including jobs/wages, wine produced and sold, vineyard revenue, wine-related tourism, and federal, state, and local taxes, was $173.2 million.

There were an estimated 121,913 cases of wine produced (a case is defined as 12 750-mL bottles) and a retail value of $20 million for Arkansas wine in 2010. At the time that this report was published, there were 13 wineries in Arkansas, and today there are at least 14 (Arkansas Department of Parks, Heritage 2019). The majority of Arkansas wineries produce less than 5,000 cases per year, and Arkansas was ranked twenty-first in the United States for total wine production, producing just 0.04% of the country's wine by volume. The Arkansas grape and wine industry was responsible for approximately 1,668 jobs and over $42 million in wages, with the majority of these jobs related to wine tourism. The study by Frank (2010) estimated that

306,000 tourists visited Arkansas wineries in 2010, and that wine tourism brought in $21 million of revenue. The Arkansas grape and wine industry generated $12.5 million in state and local taxes and $11.2 million in federal taxes in 2010 (Frank 2010).

The Arkansas wine industry is a significant benefit to the state economy, in terms of providing jobs and generating revenue from tourism and taxes. Therefore, it would be of interest to explore methods to improve the quality of wine that can be produced from locally-grown grapes.

Overview of wine production

Winemaking can be defined as the techniques and technologies used in the transformation of grapes into wine. *Vinification*, or the conversion of grape sugars into ethanol and carbon dioxide by yeast, is the primary reaction that occurs during winemaking. However, there are a variety of other physical and biochemical changes due to extraction and microbial metabolism of many other grape compounds. Waterhouse et al. (2016) wrote a comprehensive book on wine chemistry, which will be referenced throughout this review. The winemaking process can be separated into four basic steps: (1) obtaining high-quality fruit that has been harvested in optimum condition, (2) fermenting fruit into wine, (3) clarifying, stabilizing, and filtering wine, and (4) bottling and aging the wine. While each of these steps makes a specific contribution to wine characteristics, obtaining high quality fruit has perhaps the greatest influence on wine quality (Eisenman 1998).

Obtaining high-quality fruit. It is often said that wine quality is made in the vineyard, as soil, climate, and viticultural practices are highly influential for the quality of grapes at harvest. Even if the winemaker does a ‘perfect’ job on their end, it will be very difficult to make an excellent

wine from poor quality grapes. This is why many wineries prefer to have their own vineyard, rather than sourcing from an independent grower (Eisenman 1998).

For white wines, grapes should be harvested at 20-23% soluble solids, TA > 0.7%, and pH < 3.3. For red wines, optimal harvest chemistry is slightly different, with 21-24% soluble solids, TA > 0.65%, and pH < 3.4. If grapes must be harvested outside of these parameters, due to weather, pests, disease, etc., it is possible to adjust wines with sugar or acid additions prior to fermentation, depending on state regulations. However, there are also aroma compounds that develop as berries mature, so harvesting early could mean a lack of varietal character in the resulting wine (Eisenman 1998).

Differences between red and white wines. There are differences in the winemaking process for red and white wines. While both red and white grapes are crushed/destemmed immediately after harvest, white grapes do not spend as much time in contact with the grape solids (skins, seeds, and pulp) and are pressed to juice almost immediately. On the other hand, red grapes are crushed/destemmed and then undergo maceration (fermentation with the grape solids present) before pressing. Because of the differences in skin contact time, extraction of polyphenols is mostly avoided with white wine and is encouraged with red wine.

Temperature is a key parameter controlled during winemaking, and different temperatures should be used for red and white wine production. White wines are fermented at lower temperatures to control aroma characteristics, and red wines use warmer temperatures to enhance extraction of compounds from skins/seeds. Malolactic fermentation (the conversion of malic acid to lactic acid by lactic acid bacteria to reduce perceived acidity) and aging on oak are used with the majority of red wines to enhance flavor and mouthfeel, whereas only a few varieties of white wine (ex. Chardonnay and Semillon) employ these techniques. As a result of

the additional red wine processing steps, white wine can usually be bottled and released much earlier than red wines.

Fermenting grapes into wine. Primary fermentation in wine is the conversion of grape sugars (glucose and fructose) into ethanol and carbon dioxide by yeast, as shown in the formula below.

$C_6H_{12}O_6$ (glucose or fructose) → $2C_2H_5OH$ (ethanol) + $2CO_2$ (carbon dioxide)

Although this reaction seems simple, there are many steps in the fermentation process, and yeast must produce several different enzymes for fermentation to occur optimally. There are many types of commercial yeast strains that can be used for wine production. Different strains of yeast will produce wines with specific flavor attributes, so it is important for winemakers to select the appropriate yeast for the style of wine. Some secondary fermentations can occur as well, both intentionally and unintentionally. Malolactic fermentation is intentional inoculation of wine, after primary fermentation is complete, with lactic acid bacteria to reduce acidity and produce specific flavor attributes (ex. 'buttery' diacetyl and acetoin). Malolactic fermentation can also occur spontaneously in some wines. Examples of unintentional negative secondary fermentations include the bacterial fermentation of glycerol into acetic and lactic acids (Eisenman 1998).

Clarifying, stabilizing, and filtering wine. After fermentation is complete, wine contains dead yeast cells, tartaric acid crystals (tartrate crystals), proteins, small pieces of grape tissue, and particles of dirt. Any of these substances will interact with light as it passes through the wine and give an opaque, cloudy appearance. Clarification can remove this haze, and this step is especially important for white wines, as a lack of clarity will be much more apparent in the finished product. Many of these particles will eventually settle the bottom of the storage vessel due to gravity, and the wine can be siphoned/pumped off the sediment. However, some of the smaller particles will take a very long time to settle or may not settle. Various 'fining agents', such as

bentonite clay and gelatin, can be used to bind these particles and pull them to the bottom. In addition, chilling the wine < 0°C for a few days (cold stabilization) will greatly enhance the clarification of wine, both with and without the use of fining agents (Eisenman 1998).

Although wine may look clear and bright after it is clarified, there is a chance it will not stay this way over an extended period. This is because most wines contain tartaric acid and proteins that do not precipitate during initial clarification, and there may be some bacteria and yeasts that cause further haze or produce undesirable aromas. Thus, many commercial wineries sterile filter their wine to eliminate any particles/microbes (Eisenman 1998).

Bottling and aging wine. The final step in winemaking is to bottle and age the wine. This is typically the step during which red wines (and some white wines) are put on oak. Wine can be aged in a tank, barrel, or bottle. During aging, both the bouquet and mouthfeel of wine will transform. Bouquet is defined as the wine aromas produced during the winemaking process by yeast, bacteria, oak barrels, etc. While some bouquet aromas are intense after the completion of fermentation, they will decrease during aging. Others may only become noticeable after several years of aging. These differences in bouquet chemistry are part of the reason why certain wines are best consumed immediately after bottling, but some need years in order to reach their full potential (Eisenman 1998).

In most commercial wineries, a fully automated bottling line is used. Bottles are dosed with liquid nitrogen prior to filling to flush out oxygen and prevent oxidation. The most commonly used closure for wine bottles is cork (both natural and synthetic), although the use of screw caps is increasing in popularity. For smaller winemaking operations, bottles can be filled using gravity siphoning. Regardless of the size of the winery, the most important consideration

during bottling is sanitation- if clean bottles are not used, wine can be re-contaminated by harmful microbes, such as vinegar bacteria (*Acetobacter*) (Eisenman 1998).

There are a variety of processes and parameters that must be controlled by the winemaker in order to produce a quality wine. While an understanding of the steps and 'ingredients' for winemaking is critical, a well-rounded knowledge of wine flavor chemistry is also needed in order to take wine from just acceptable/drinkable to extraordinary.

Overview of wine flavor

Flavor is defined as "the perception resulting from stimulating a combination of the taste buds, the olfactory organs, and chemesthetic receptors within the nasal and oral cavities". Therefore, the flavor of a wine arises from perception of basic taste, volatile aroma compounds, and chemesthetic sensations. Taste is the detection of the five basic tastes by receptors located in the taste buds (Chandrashekar et al. 2006). Although the five basic tastes are sweet, salty, sour, bitter, and umami, only sweet, sour, and bitter are experienced in wine (Hufnagel and Hofmann 2008a). Olfaction is the detection of aroma compounds by olfactory receptors in the nasal cavity. Of the approximately 700 olfactory receptors in the human nasal cavity, about half are functional in any given individual (DeMaria and Ngai 2010). There have been over 10,000 volatile compounds detected in foods, but less than 3% of these are believed to be important to food aroma (Dunkel et al. 2014). Chemesthesis refers to the sensations elicited by the chemical activation of sensors responsible for pain, temperature, and touch (Lawless and Heymann 2010). The chemesthetic sensations most relevant to wine perception, often referred to as the mouthfeel of a wine, are pungency/irritation (caused by ethanol and carbon dioxide) and astringency (caused by condensed tannins and other phenolic compounds) (Schöbel et al. 2014).

The chemical compounds present in wine determine the flavor profile, and these compounds can be classified as either primary, secondary, or tertiary flavors. Primary flavor compounds arise from the berry and are influenced by factors such as grape cultivar and any treatments done in the vineyard. Secondary flavor compounds are produced during fermentation and are influenced by the parameters chosen for fermentation, such as length of fermentation on the skins, yeast variety, and temperature. Tertiary compounds arise during aging and are affected by factors such as oxygen exposure, storage temperature, use of oak, and duration of aging. Therefore, the winemaking parameters chosen for the production of any given wine will influence the flavor of the resulting product.

There are several properties of aroma compounds that can affect their perceived quality/intensity (Waterhouse et al. 2016). If one volatile compound is perceived very intensely, it can 'mask' the presence of another compound. For example, methoxypyrazines (bell pepper, earthy aromas) in red wine have a tendency to mask the perception of fruity aromas (Hein et al. 2009). Even if several similar compounds do not have individual concentrations above their respective sensory thresholds, together they can reach sensory threshold through an 'additive effect'. This is observed in wines with series of alkyl esters or ketones (Guadagni et al. 1963). Differences in the pH, temperature, ethanol concentration, and non-covalent interactions with macromolecules within a wine can affect the volatility and therefore odor activity of flavor compounds (Pozo-Bayón and Reineccius 2009). Synesthetic effects can occur when information from different sensory modalities impact one another. For example, King et al. (2007) found that increasing the sweetness of a fruit beverage increased the perception of fruitiness. Consumers' familiarity with and prior knowledge of a particular wine can lead to a 'confirmation bias'. Delwiche (2004) showed that when white wine was dyed red, it was perceived as having a fuller

body, like that of red wine. Finally, combinations of volatile compounds elicit different perceptions than if each of these compounds were perceived individually: there is no single compound in wine that has the typical ‘wine aroma’ (Ferreira et al. 2002). Therefore, in order to fully characterize the flavor of a wine, both chemical analyses and sensory studies need to be conducted.

Chemical composition of wine

From a macroscopic perspective, wine is a slightly acidic hydroethanolic solution. A typical dry table wine contains 85-89% (w/w) water and 9-13% ethanol, with the remaining composition consisting of glycerol, acids, sugars, polyphenols, polysaccharides, minerals, and volatile odorant compounds.

The compounds that make up a wine can be roughly divided into 10 categories: (1) water and ethanol, (2) carbohydrates, (3) organic acids, (4) nitrogenous compounds, (5) higher alcohols, (6) esters, (7) isoprenoids, (8) aldehydes, ketones, and related compounds, (9) sulfur compounds, and (10) phenolic compounds.

Water and ethanol. Most table wines contain 85-89% (w/w) water and 9-13% ethanol. When ethanol is added to water, it has several effects on the solution matrix. The boiling point and surface tension both decrease because ethanol is less capable of hydrogen bonding than water (Zoecklein et al. 1999). The solution also decreases in polarity, and less polar aroma compounds, like vanillin, have greater solubility and therefore less aroma activity. The viscosity of the solution increases because ethanol disrupts the more ‘open’ lattice structure of water. The presence of ethanol also leads to the formation of aroma-active ethanol aggregates, like ethyl acetate, through chemical reactions with other compounds in wine.

A positive correlation has been reported between the ethanol content and bitterness of a wine (Sokolowsky and Fischer 2012). Fischer and Noble (1994) demonstrated that increasing the ethanol content of a model wine from 8 to 14% resulted in more than a three-point increase in perceived bitterness. Ethanol can also elicit pungent and sweet sensations (Martin and Pangborn 1970) and can interact with volatile aroma compounds to affect their perception. Increasing the ethanol concentration of a wine will decrease the intensity and increase the threshold of some aroma compounds (Escudero et al. 2007). Grosch (2001) showed that wine with 7% ethanol had more intense fruity/floral aromas than wine with 10% ethanol, and that the odor thresholds of these fruity/floral compounds were 10-100 times greater in an ethanolic matrix than they were in water. This altered perception of odor compounds can be explained by both masking and matrix effects. Ethanol can 'mask' the perceived intensity of other odorants (Waterhouse et al. 2016). Most volatile compounds are hydrophobic, so the increased hydrophobicity of an ethanolic matrix (relative to pure water) means that these compounds will be more soluble and less volatile. Athès et al. (2004) showed that the gas-liquid partition coefficient of isoamyl alcohol (solvent/fusel aroma) and ethyl hexanoate (green apple aroma) decreased by a factor of two in a 10% ethanol solution compared to pure water.

Carbohydrates. The most abundant sugars in grapes are glucose and fructose. Their concentrations are negligible before veraison but increase to a concentration of 180-250 g/kg at harvest. The wine industry more commonly reports soluble solids (SS), measured through density or refractometry methods, as opposed to the concentrations of individual sugars. Although the sugars in grape must/juice are almost entirely fermented to ethanol by yeast, there are some residual sugars in wine. Incomplete fermentation can occur, either because fermentation was stopped ('stuck fermentation') or because the must contained unfermentable

sugars, such as arabinose or xylose. Wine can be ‘back-sweetened’ after fermentation with sucrose or grape juice to increase the perceived sweetness, although this practice varies depending on state or country rules. It is also possible for some glycosides to be hydrolyzed during storage or for sugars to be extracted from oak during aging (del Alamo et al. 2000).

Typically, dry table wines have a residual sugar content of 1-4 g/L, whereas sweet wines can have > 100 g/L. Because yeast are glucophilic (prefer to ferment glucose), fructose is found in higher concentrations than glucose at the end of fermentation (Fugelsang and Edwards 2007). While polysaccharides, such as cellulose, pectin, and hemicellulose, can be found in low amounts in wine, their sensory and chemical effects are usually negligible (Brady 2013).

The most notable flavor contribution of sugars to wines is sweetness, and monosaccharides have detection thresholds of 0.2-1.0% w/w. At a concentration of 10% w/w, fructose is perceived as twice as sweet as glucose and 15% sweeter than sucrose (Belitz et al. 2009). In addition to providing sweetness, sugars can also mask sour and bitter taste sensations and astringency and pungency tactile sensations, and can increase the perception of ‘body’ (Lawless and Heymann 2010). McBride and Johnson (1987) demonstrated that increasing the sugar concentration of a citric acid solution resulted in a decrease in perceived sourness. Similar results have been found using artificial wine (Hufnagel and Hofmann 2008b).

Although sugars are nonvolatile, and therefore have no aroma of their own, high sugar concentrations can affect the volatility of other aroma compounds. Sugar binds water, thus decreasing its availability for solvation of volatile compounds. However, Friel et al. (2000) observed less than a 20% increase in volatility of isoamyl acetate (banana aroma), ethyl hexanoate (green apple aroma), and eugenol (clove aroma) in a 15% w/v sucrose solution compared to pure water.

The carbonyl group of sugars can be enzymatically reduced to an alcohol group, and these sugar alcohols can have an effect on the flavor of the wine. Glycerol is the most common sugar alcohol in wines and is the third-most abundant component of dry table wine, after water and ethanol. Wines typically have glycerol concentrations of 7-10 g/L (Mattick and Rice 1970), but the concentration can be over 15 g/L in high-sugar fermentations, like ice wines (Pigeau et al. 2007). Other sugar alcohols, such as sorbitol, arabitol, and mannitol, can also be found in wine, but usually at concentrations below their sensory threshold. Higher concentrations can be an indicator of microbial spoilage, especially by lactic acid bacteria (Bartowsky 2009). While some research has shown that glycerol is an important contributor to the mouthfeel of a wine, other studies have contradicted this. Noble and Bursick (1984) found that it was necessary to add more than 25 g/L of glycerol to a model wine to cause a perceivable change in mouthfeel.

Organic acids. Organic acids are weak acids with a carbon chain and at least one carboxylic acid group, and they may contain other functional groups such as ketones or alcohols. In wine, organic acids determine pH, and pH affects the color and chemical/microbial stability. These acids either come from grapes or are added by the winemaker (Fowles 1992, Swiegers et al. 2005). Six acids represent over 95% of the total organic acids in wine: tartaric acid, malic acid, acetic acid, citric acid, lactic acid, and succinic acid.

With the exception of acetic acid, all wine organic acids are non-volatile and therefore only affect taste, not aroma. Tartaric, malic, and citric acid come from grapes, and tartaric and malic acid are the acids present in the highest concentrations after fermentation. Malic acid has a very high concentration in grapes before veraison (> 20 g/kg) but is metabolized during ripening. Therefore, malic acid levels are typically lower in more mature grapes and grapes grown in warmer regions. Tartaric acid is formed during berry cell division, and unlike malic acid is not

metabolized during berry ripening or winemaking. However, it can be lost through physicochemical processes during winemaking like precipitation. Succinic and acetic acids are formed during alcoholic fermentation, and lactic acid is a product of malolactic fermentation.

Because the primary contribution of organic acids to wine is acidity, there are various properties of acidic solutions that must be considered. pH is the negative log of the free hydrogen ion concentration ($-\log[H^+]$). The typical pH range for a white wine is 3.0-3.4 and for a red wine is 3.3-3.7. Higher pH values can result in decreased microbial stability, decreased effectiveness of sulfur dioxide for mold prevention, decreased anthocyanin pigment color, and decreased rate of acid-catalyzed reactions. Titratable acidity (TA) is the concentration of the free hydrogen ions plus the concentration of the undissociated carboxylic acid groups that are released during titration with sodium hydroxide. Typical TA values are 0.6-0.9% (w/v) (as tartaric acid equivalents) for a white wine and 0.5-0.8% (w/v) for a red wine. The TA of a wine is a very important measurement for winemakers because it is positively correlated with perceived sourness.

Biological deacidification, or malolactic fermentation (MLF), is the conversion of malic acid to lactic acid by lactic acid bacteria. Lactic acid has only one carboxylic acid (–COOH) functional group, whereas malic acid has two. Because TA is calculated as $[H^+] + [COOH]$, a complete MLF will result in a TA decrease equal to the original concentration of malic acid. However, this means that the pH will increase. Winemakers want to keep pH low to increase microbial stability while also keeping TA low to decrease perceived sourness. Although this may seem impossible to achieve, as TA and pH are inversely correlated, their correlation is not perfect. Organic acids vary in pKa: stronger acids will result in a greater decrease in pH for the same contribution to TA. Tartaric acid is the strongest of the wine acids, so it is common practice

for winemakers to make acid additions using tartaric acid rather than weaker acids such as malic or citric acid.

Although the major flavor contribution of organic acids to wine is sourness, Gawel (1998) showed that lowering the pH of solutions increased the perception of astringency. This was most likely due to acid-induced precipitation and functional loss of lubricating salivary proteins (Siebert and Chassy 2004). Acetic acid is the only organic acid in wine that is volatile, and concentrations approaching the threshold (400 mg/L) are often found in wine. Higher concentrations than this are an indication of bacterial spoilage by acetic acid bacteria (Bartowsky 2009). However, it is difficult to evaluate the effect of acetic acid on wine aroma, as it is typically found mostly in its ethyl ester form, ethyl acetate.

Nitrogenous compounds. In grapes and wine, the major nitrogenous compounds are amino acids, oligopeptides, proteins, amines, and imines. These compounds have a lone electron pair on their nitrogen atom(s) and behave as weak bases. However, because this lone pair is typically protonated at wine pH, most of the common food chemistry reactions involving amine groups acting as nucleophiles, such as the Maillard reaction, occur at very slow rates, if at all, in wine.

Yeast require nitrogen for alcoholic fermentation, and free amino acids serve as their primary nitrogen source. While most amino acids are present in concentrations well below their sensory thresholds in wine, proline and glutamate can be close to threshold and can elicit sweet and umami tastes, respectively. However, Hufnagel and Hofmann (2008b) found that eliminating all amino acids in a model wine had no effect on flavor compared to a model wine with the amino acids at their typical concentrations. Skogerson et al. (2009) showed that proline had a positive correlation with the perception of ‘body’ in white wines, but this could have been

because both proline concentration and body naturally increase (unrelated to one another) as a white wine matures.

White musts typically contain 20-250 mg/L proteins and white wines contain 30-275 mg/L (Bayly and Berg 1967, Santoro 1995). While none of the proteins in wine exist above their sensory thresholds (Marchal et al. 2011), many soluble proteins are unstable under cold temperatures (such as those experienced during cold stabilization) and can denature to cause haziness (Waters et al. 1996). Therefore, although proteins do not contribute to wine flavor, they can have undesirable visual sensory effects on wine. Proteins in red wines have not been as well-studied as those in white wines because haze is not as much of an economic concern with red wines. The protein concentration of red wines, 50-100 mg/L (Smith et al. 2011), is lower than that of white wines because proteins bind to the tannins in red wines. This tannin-binding property of proteins is often utilized by winemakers to decrease tannin concentration, thereby decreasing astringency. Springer and Sacks (2014) showed that there is a negative correlation between the protein content of grapes and the tannin content of the finished wine.

Several cyclic amines have been identified as contributors to wine aroma, including indole, 3-isobutyl-2-methoxypyrazine (IBMP), 3-isopropyl-2-methoxypyrazine (IPMP), methyl anthranilate, and *o*-aminoacetophenone. Methoxypyrazines are a class of cyclic amines particularly important to wine aroma. Because methoxypyrazines are primary odorants (derived from the grape), they contribute to varietal aromas and have some of the lowest sensory thresholds (~1 ng/L) of any compounds in wine. The most important methoxypyrazines for wine aroma are IBMP (bell pepper aroma) and IPMP (pea/vegetal aroma) (Botezatu and Pickering 2012, Lacey et al. 1991). While methoxypyrazines give certain wine varieties, such as Sauvignon Blanc and Cabernet Sauvignon, complexity and typical vinous character, excessive

concentrations can mask fruity aromas, especially in red wines (Hein et al. 2009). The maximum methoxypyrazine concentration in grapes occurs 1-2 weeks before veraison and decreases during ripening. This means that lower concentrations are measured in grapes with longer, warmer, and drier growing seasons, and in wine produced from such grapes (Scheiner et al. 2009).

Two aniline derivatives, methyl anthranilate (artificial strawberry aroma) and *o*-aminoacetophenone (artificial grape aroma), are found at suprathreshold concentrations in some large-berried native American grape species. They are responsible for the 'foxy' aroma of *V. labrusca* (ex. Concord and Niagara) and *V. rotundifolia* (muscadine) grapes. While these foxy compounds are present in some *V. vinifera* wines, it is typically at sub-threshold concentrations.

***Higher alcohols*.** Higher alcohols, volatile alcohols with more than two carbon atoms, are secondary aroma compounds produced as a byproduct of yeast amino acid metabolism during fermentation. Higher alcohols are amphiphilic, with a non-polar hydrocarbon chain and a polar (hydrogen-bonding) alcohol group. These compounds can participate in esterification reactions, where they combine with carboxylic acids to form esters, and in oxidation reactions, where they can be oxidized to their corresponding aldehydes.

Because higher alcohols are produced as a byproduct of yeast amino acid metabolism, they typically have a clear structural relationship to a particular amino acid. For example, isobutanol has structural similarities to valine, amyl alcohol has similarities to isoleucine, and isoamyl alcohol has similarities to leucine. All three of these higher alcohols have solvent/fusel aromas. Methionol has a structural relationship to methionine and gives a boiled potato aroma. The compound 2-phenylethanol, which has a structural relationship to phenylalanine, is one of the only higher alcohols found in wine that has a pleasant aroma (rose/honey). The formation of

higher alcohols is dependent on fermentation conditions, yeast nutrient availability, and initial amino acid content.

Makhotkina and Kilmartin (2012) found no significant changes in isoamyl alcohol, isobutyl alcohol, and 2-phenylethanol concentrations in wine after one year of storage at various temperatures ranging from 5-18°C. While higher alcohols usually have unpleasant odors on their own (with the exception of 2-phenylethanol), there have been various reconstitution studies indicating they have only very minor effects on overall wine aroma. Ferreira et al. (2002) concluded that the removal of higher alcohols from a model Grenache rosé wine had a detectable but indescribable effect on aroma, and Guth (1997) determined that removal of higher alcohols from a model Gewürztraminer wine had no detectable effect. Ferreira et al. (2009) found that there was no correlation between red wine quality scores and the concentrations of isoamyl alcohol, 2-phenylethanol, or methionol. Therefore, these higher alcohols are not impact odorants in most wines, although it is likely that they contribute to overall vinous character.

While the previously mentioned higher alcohols are not particularly impactful odorants in wine, they can serve as substrates for the formation of more potent compounds, such as acetate esters and aldehydes. On the other hand, there are some C_6 alcohols, like 1-hexanol and *cis*-3-hexenol, that are impactful odorants with herbaceous/green aromas. C_6 alcohols (and aldehydes) are formed by enzymatic oxidation of polyunsaturated fatty acids in plants that have been subjected to mechanical damage, such as grape crushing. Escudero et al. (2007) found that while these compounds had no significant effect on model wine aroma on their own, when they were added to wines with elevated IBMP concentrations, the previously perceived earthiness of the wines was perceived instead as green bell pepper. Therefore, it is likely that C_6 alcohols can contribute additively with methoxypyrazines to have a significant effect on wine aroma.

Esters. Esters are major contributors to wine aroma, as well as to the aroma of many flowers and ripe fruits. Esters are mostly absent from grapes but are formed during fermentation through enzymatic processes. They can also be synthesized post-fermentation through acid-catalyzed reactions. Therefore, esters are secondary and tertiary wine aroma compounds. Esters are formed through condensation of carboxylic acid and alcohol groups, referred to as an esterification reaction. These reactions are reversible, which means that the relative proportions of the acid, alcohol, and ester forms will move towards equilibrium during wine storage. The two main classes of esters in wine are ethyl esters and acetate esters.

A majority of the esters found in wine are fatty acid ethyl esters (FAEE). The FAEEs are formed by esterification of ethanol and free fatty acids derived from yeast lipid metabolism. For example, ethyl hexanoate (green apple aroma) is formed from ethanol and caproic acid and ethyl-3-methylpentanoate (strawberry aroma) is formed from ethanol and 3-methylpentanoic acid.

After ethyl esters, acetate esters are the next-most prevalent esters in wine. They are formed by enzymatic acetylation of higher alcohols during fermentation. Examples of acetate esters in wine include isoamyl acetate (banana aroma), formed by acetylation of isoamyl alcohol, and 2-phenylethyl acetate (honey, rose aroma), formed by acetylation of 2-phenylethanol. Acetate esters typically decrease in concentration during wine storage, while FAEEs are fairly stable.

Many gas chromatography-olfactometry (GC-O) studies have shown that FAEEs are some of the most odor-active compounds in wine, particularly ethyl butanoate (apple, fruity aroma), ethyl hexanoate (green apple aroma), ethyl octanoate (fruity, peach aroma), and ethyl 2- and ethyl-3-methylbutanoate (apple, fruity aroma). Lytra et al. (2012) found that while single

FAEEs do not have significant effects on wine aroma, in combination they are likely responsible for the red- and dark-fruit aromas of wine. Some ethyl esters can also give undesirable off-odors in wine. For example, high concentrations of ethyl acetate add to the perception of 'volatile acidity', a common wine fault characterized by pungent nail polish remover and vinegar aromas (Fugelsang and Edwards 2007).

While ethyl esters appear to affect wine aroma through combined effects, acetate esters have been shown to have more individualized effects on wine aroma. Ferreira et al. (2002) found that removing isoamyl acetate from a reconstituted Grenache rosé wine lead to a decrease in perceived fruitiness. Escudero et al. (2004) showed that spiking a Maccabeo white wine with isoamyl acetate gave a 200% increase in the perceived banana aroma. However, the acetate ester concentrations of wine decrease during storage to subthreshold levels, due to acid hydrolysis, so that acetate esters have minimal effects on the aroma of aged wines.

Isoprenoids. Isoprenoids are a class of hydrocarbon compounds, and their oxygenated derivatives, that are made of repeating C_5 isoprene units (2-methylbuta-1,3-iene). These compounds can be saturated or unsaturated and cyclic or acyclic, and have functional groups such as alcohols, aldehydes, ketones, esters, ethers, and acetals. Isoprenoids are produced enzymatically via the isoprenoid pathway and are key aroma compounds in a variety of plants (Martin et al. 2012, Schwab et al. 2008). The isoprenoids that are important for wine aroma are monoterpenoids (C_{10}), sesquiterpenoids (C_{15}), and C_{13}-norisoprenoids, and these compounds typically impart pleasant aromas. The terpenoid profile of grapes depends on the variety, so volatile isoprenoids are primary varietal aroma compounds.

The largest concentrations of monoterpenoids are found in white wines made with Muscat variety grapes, where concentrations can exceed threshold values by 100 times. The most

important grape-derived monoterpenoids for wine aroma are linalool (floral, citrus aroma), geraniol (floral, citrus aroma), and *cis*-rose oxide (rose aroma). Linalool and geraniol give the floral character to Muscat wines and *cis*-rose oxide gives the lychee character to Gewürztraminer wines (Ong and Acree 1999). Both grape cultivar and growing conditions will have an effect on the monoterpenoid profile of grapes, with cultivar having the biggest impact. Extraction from grapes and the transformation of aroma compounds during fermentation and storage will also affect monoterpenoid profiles (Marais 1983, Mateo and Jiménez 2000). The parameters chosen for skin contact time, pressing force, temperature, and the use of pectolytic enzymes will affect how monoterpenoids are extracted from grapes during winemaking.

Numerous sesquiterpenoids have been identified in wine, such as farnesol (floral, rose aroma), nerolidol (floral, apple, green aroma), and rotundone (black pepper aroma). These isoprenoid compounds are synthesized in cultivars such as Cabernet Sauvignon, Shiraz, Riesling, and Gewürztraminer (May and Wüst 2012, Parker et al. 2007). The most important flavor-active sesquiterpenoid in wine is rotundone, which has a black pepper aroma and a low sensory threshold of 16 ng/L. The concentration of rotundone in grapes is influenced by grape variety, and levels increase as the grape matures. The highest concentrations of rotundone are found in grapes from cooler environments (Scarlett et al. 2014, Zhang et al. 2015).

Various C_{13}-norisoprenoids have been identified in all major wine varieties, such as Chardonnay, Riesling, Cabernet Sauvignon, Pinot Noir, and Shiraz (Mendes-Pinto 2009). The most important norisoprenoids for wine aroma are β-damascenone (cooked apple, quince, floral aroma), β-ionone (violet, wood, raspberry aroma), and 1,1,6-trimethyl-1,2-dihydronapthalene (kerosene, petrol aroma).

Aldehydes, ketones, and related compounds. Aldehydes and ketones in wine originate as fermentation metabolites and oxidation byproducts. After a wine is microbially stable, they can be formed by non-enzymatic oxidation of their analogous alcohols. The carbon of the carbonyl group in aldehydes and ketones is an electrophile, so it can react with nucleophiles in wine, such as phenolics and sulfites. Because these reactions readily occur, significant amounts of carbonyl compounds are a sign that a wine is highly oxidized. Even small degrees of oxidation can significantly affect the sensory properties of a wine, because volatile aldehydes and ketones have much lower sensory thresholds than their corresponding alcohols.

The most abundant volatile aldehyde in wine is acetaldehyde, the reduced form of ethanol. Less than 1% of the acetaldehyde in wine exists in the volatile form if free SO_2 is present, because acetaldehyde is a very strong SO_2 binder. Therefore, acetaldehyde does not contribute much to the aroma of the finished wine, unless the wine has low free SO_2 content, like sherry (Escudero et al. 2002). At low levels, acetaldehyde can enhance the fruitiness of a wine, but higher levels give wine a rotten apple aroma.

Higher alcohols are easily oxidized to aldehydes/ketones, and some of these odorous aldehydes can reach suprathreshold concentrations. For instance, 2-methylpropanal (oxidized isobutanol), 2-methylbutanal (oxidized amyl alcohol), and 3-methylbutanal (oxidized isoamyl alcohol) are detectable in oxidized aged wines (Culleré et al. 2007). Medium chain (C_8-C_{10}) aldehydes, like octanal, nonanal, and decanal, give citrus aromas to wines and have additive sensory effects. However, they are usually only present in low levels, so any sensory effects are minor (Culleré et al. 2011).

Dicarbonyl compounds in wine are produced by microbial metabolism. Specifically, diacetyl is produced by lactic acid bacteria and gives a buttery aroma to wine. Acetoin is formed

from the reduction of one of the carbonyl groups of diacetyl and has a butter/cream aroma. Typical diacetyl concentrations in wine range from 0.2-2.5 mg/L but can reach concentrations 2-3 times higher if malolactic fermentation is done (Marchand et al. 2000).

Sulfur compounds. There are numerous classes of sulfur-containing compounds in grapes in wine. These include the sulfur dioxide added by winemakers to stabilize wine and various volatile sulfur compounds that are found in grapes themselves or are formed in wine as byproducts of fermentation and aging.

Winemakers have used sulfur dioxide for centuries for its antimicrobial and antioxidant properties (McGovern 2003). The terms 'sulfur dioxide', 'SO_2', and 'sulfites' are used interchangeably in the wine industry. However, from a chemical perspective, SO_2 refers only to the neutral volatile species. This neutral volatile species is referred to as molecular SO_2. When sulfur dioxide is added to wine, it behaves as a weak diprotic acid, undergoes various acid-base chemistry reactions, and can take different forms. The major roles of SO_2 and its derivative compounds in wine include (1) acting as a nucleophile to form covalent adducts with aldehydes and other electrophilic wine components, (2) reacting with byproducts of oxidation, (3) inhibiting activity of various enzymes, including the browning enzyme polyphenoloxidase, and (4) inhibiting the growth of a wide range of microorganisms, such as wild yeasts and bacteria. The various SO_2 species in wine play different roles.

SO_2 is added to most wines exogenously in the form of potassium metabisulfite (KBMS). When added to an aqueous solution (such as wine), the SO_2 in KBMS will act as a weak base and form the conjugate bases bisulfite (HSO_3^-) and sulfite (SO_3^{2-}). These reactions have different pK_a, which change depending on temperature, ethanol concentration, and ionic strength of the

solution. In typical wine pH of 3-4, a majority of the SO_2 species would be present as bisulfite, with a small amount as molecular SO_2 and an even smaller amount as sulfite.

The term 'free SO_2' is often used to refer to the bisulfite (HSO_3^-) content of wine and is the metric most often measured and regulated. Winemakers typically want the bisulfite concentration of wine to be 20-40 mg/L. However, the bisulfite levels added to wine can vary depending on the desired level of molecular SO_2. For dry wines, at least 0.6 mg/L molecular SO_2 is needed to prevent spoilage, whereas sweet wines require at least 0.8 mg/L. The average concentration of total SO_2 (molecular SO_2, bisulfite, and sulfite) is 60 mg/L in a finished red wine and 80 mg/L in a finished white wine. White wines are typically higher in total SO_2 either because they contain more residual sugar or because they are more susceptible to oxidative browning and aroma changes. For example, acetaldehyde, produced from the oxidation of ethanol, is higher in most white wines and can bind SO_2, meaning that more KBMS will need to be added to compensate for this (Jackowetz et al. 2011).

When KBMS is added to wine, it is initially in the bisulfite form. A portion of this is transformed into molecular SO_2 while the majority will remain as bisulfite. The concentration of molecular SO_2 must be carefully considered, as it is the form of SO_2 that acts as an antimicrobial agent. A greater percentage of the total SO_2 will exist in wine as molecular SO_2 at a lower pH than at a higher pH. The Henderson-Hasselbalch equation can be used to predict the amount of bisulfite that must be added to a wine at a given pH, temperature, and ionic strength to achieve the desired molecular SO_2 level: [molecular SO_2] = [bisulfite] / $(1+10^{pH-pKa})$.

Molecular SO_2 has a sensory threshold of 2 mg/L in wine, and above this concentration it causes an irritating/burning sensation in the nose. Bisulfite and sulfite have been shown to have minimal direct sensory effects, but odor-active carbonyl compounds can bind to sulfites and form

non-volatile SO_2 adducts. This reaction could be desirable, as is the case with the binding of acetaldehyde and other oxidative aldehyde aroma compounds. On the other hand, it could cause loss of aroma activity of pleasant smelling compounds, such as fruity β-damascenone (Daniel et al. 2004).

In addition to the sulfur dioxide compounds added to wine for antimicrobial or antioxidative purposes, volatile sulfur compounds originate from the grape itself or can be formed in wine due to fermentative or aging processes.

The sulfur atom has a similar electronic configuration to oxygen, so it forms analogous compounds, such as thiols (RSH, vs. alcohols, ROH). The sulfur compound profile of a wine has significant effects on sensory properties and quality. There are various classes of sulfur compounds important to wine aroma, including sulfides and polyfunctional thiols. Volatile sulfur compounds have a wide range of aroma properties and can contribute either positive or negative sensory properties to a wine, depending on the type of compound and its concentration. These compounds can come from the grape, from yeast metabolism, or can arise during storage and oak aging.

The majority of varietal sulfur compounds found in grapes are polyfunctional thiols, which impart desirable citrus and tropical fruit aromas. These compounds are denoted polyfunctional thiols because they have additional oxygen-containing functional groups. Examples of polyfunctional thiols are 3-mercaptohexan-1-ol (3-MH, grapefruit, passionfruit aroma), 3-mercaptohexyl acetate (3-MHA, passionfruit, box tree aroma), and 4-mercapto-4-methylpentan-2-one (4-MMP, box tree, guava aroma). Polyfunctional thiols typically have low thresholds and high odor activity values, so they can have a major impact on wine aroma. 3-MH, 3-MHA, and 4-MMP are particularly important to the flavor of young Sauvignon Blanc wines,

but have been detected in many other white, red, and rosé wines, including Riesling, Syrah, and Grenache (Coetzee and du Toit 2012, Dubourdieu and Tominaga 2009, Roland et al. 2011). Lund et al. (2009) and Benkwitz et al. (2012) identified a positive correlation between the concentrations of 3-MH and 3-MHA and the tropical/passionfruit characters of Sauvignon Blanc wines.

Phenolic compounds. Phenolic compounds in wine come from the grape berry and from oak or other woods used in production/aging, with a large majority coming from grapes. While most of these phenolic compounds are non-volatile, there are a few volatile, odorous phenols. Phenols are compounds with hydroxyl groups attached to aromatic rings. Compounds with a single aromatic ring and one or more hydroxyl groups, such as catechol and guaiacol, are the simplest phenolic substances.

Polyphenols (polyphenolics) are compounds with multiple phenol rings within a single structure. Most phenolic compounds in wine are polyphenols. Wine phenolics can also be categorized as flavonoids or non-flavonoids. Non-flavonoids, such as hydroxycinnamates, are the major class of phenolics found in white wine.

Flavonoids are polyphenols with a specific C_6-C_3-C_6 ring structure. The central ‘C’ ring of flavonoids is fused to the aromatic ‘A’ ring along one bond and to the aromatic ‘B’ ring through a single bond. In grapes and wine, all flavonoids have the same hydroxyl substitution at positions 5 and 7 on the A ring. Differences in oxidation state and substitution on the C-ring define the different flavonoid classes, and substitutions on the B-ring differentiate compounds within the same class. Flavonoids make up the majority of phenols in red wines. About half of the flavonoids in grape skins and seeds are extracted by ethanol during red wine fermentation.

Flavonols are flavonoids with a keto group at the C-4 position, a hydroxyl group at C3, and a double bond between C2 and C3 of the C-ring. They are found in grape skins and are thought to serve as a sunscreen because their concentration increases in response to high sun exposure. Anthocyanins are flavonoids with a fully aromatic, positively charged C-ring and a red color due to their conjugated structure. Anthocyanins are responsible for the color of red wines, and can complex with flavonols, condensed tannins, and other wine compounds to stabilize and change wine color. Finally, flavan-3-ols have a saturated C-ring and a hydroxyl group at the C3 position. This class of compounds includes monomeric catechins and oligomeric/polymeric proanthocyanidins (condensed tannins) and is responsible for about half of the phenolics in red wine. Flavan-3-ols are found in the skins and seeds of grapes.

Grape variety and viticultural conditions (climate, soil quality, sun exposure) can affect phenolic content of grapes. There is also a high amount of variability in phenolic content among vines, clusters, and berries within the same vineyard (Reynolds 2010, Reynolds and Vanden Heuvel 2009). Phenolics are mostly found in the skins and seeds of grape berries, and red grapes have higher concentrations than white grapes because of the anthocyanins in their skins. Phenolics can have significant sensory impacts on wine, so winemakers try to control the amount of different classes of phenolic compounds present. This can be done by manipulating the amount of phenolics that are extracted from skins/seeds and/or by adding proteins or other agents that can bind and precipitate tannins. White wines typically contain around 200 mg/L total phenolics (as gallic acid equivalents) and red wines contain around 2000 mg/L.

While a large majority of phenolic compounds in grapes and wine are non-volatile, there are some volatile phenols that contribute to the aroma of grapes and wine. These compounds come mostly from oak, where they are extracted during aging. They may also arise due to the

transformation of grape precursors by microbiological or chemical processes, such as contamination by wild yeast *Brettanomyces*. Unlike flavonoids or hydrolysable tannins, volatile phenols are smaller, simpler molecules (hence their volatility), including phenol and its derivatives with alkyl, methoxy, vinyl, allyl, aldehyde, and halide functional groups. They are present in much lower concentrations than other phenolic compounds in wine and are relatively stable compounds.

Phenolic compounds: non-flavonoids. Non-flavonoid phenolics in wine include three classes of compounds: hydroxycinnamates (HCAs), stilbenes, and benzoic acids. HCAs and stilbenes are found in grapes and benzoic acids are found in grapes and in oak. Therefore, oaked wines will have additional benzoic acids.

HCAs are phenolic acids with a conjugated double bond between the phenolic ring and the carboxylate group. These compounds are the first to be enzymatically oxidized during grape crushing and will therefore initiate browning in white wine must if sulfites are not added at crush. The three common HCAs in grapes are coumaric, caffeic, and ferulic acids. Because these acids are found in the flesh of grape berries, not just the skins and seeds, they are present in both red and white wines. Hufnagel and Hofmann (2008a) reported that HCAs were perceived as bitter and astringent in water. However, Verette et al. (Vèrette et al. 1988) showed that these compounds were present in below-threshold levels in wine.

Gallic and ellagic acid (hydroxybenzoic acids) are formed in wine by the hydrolysis of gallate esters in condensed and hydrolysable tannins (Chira et al. 2011). Small amounts of other hydroxybenzoic acids, such as syringic, protocatechuic, and vanillic acids, are also found in wines. Hydrolysable tannins are polymeric phenols composed of gallic acid and ellagic acid

esters of glucose (or other sugars). They are classified as either ‘gallotannins’ or ‘ellagitannins’ based on which benzoic acid they are composed of.

These tannins are classified as ‘hydrolysable’, as opposed to condensed tannins, because their ester linkages are easily hydrolyzed under mild conditions. During wine aging, these tannins are hydrolyzed to their constituent gallic and ellagic acids. Hydrolysable tannins are present in native grape species, such as *V. rotundifolia*, and are extracted into other wines during oaking. These tannins do not have a significant impact on the taste of wines (Glabasnia and Hofmann 2006). However, if a wine has high concentrations of ellagitannins, as is the case with muscadine wine, they will precipitate and cause undesirable haziness/sediment.

Resveratrol is the primary stilbene compound in grapes and is produced by grapes and grapevines in response to *Botrytis* and other fungal infections (Joshi and Devi 2009). Resveratrol forms oligomers, called viniferins, which have antifungal properties. These compounds are found in the skin of grapes and are therefore present in higher concentrations in red wines. There have been popular reports (Jang et al. 1997) implicating resveratrol as a nutraceutical compound that may reduce the risk of heart disease and cancer. However, it has been shown that 10-100 times the concentration found in wine is needed to realize the therapeutic benefits in animals. Resveratrol derivatives have strong antimicrobial properties, particularly against wild yeasts and *Acetobacter*, and this property is often of more interest to winemakers than the potential health benefits (Pastorkova et al. 2013).

Phenolic compounds: flavonols. There have been six flavonol aglycones identified in grapes and wine: quercetin, myricetin, laricitrin, kaempferol, isorhamnetin, and syringetin. In grape berries, flavonols are always present in the glycosidic form, and the position and type of sugar substituent can vary, which gives a wide range of possible flavonols. The 3-*O*-glucosides and 3-

O-glucuronides are the most prevalent. These glycosides are also found in wine, where the concentrations are dependent upon their extraction from the skin. Therefore, red wines have much higher concentrations than white wines (Castillo-Muñoz et al. 2007). Mattivi et al. (2006) conducted a study on 91 grape varieties and found that when all flavonol glycosides were hydrolyzed, quercetin and myricetin were present at about 12 mg/kg and the other four aglycones were present at about 1-2 mg/kg.

It has been shown that sun exposure greatly increases the levels of flavonols in grapes (Price et al. 1995). Spayd et al. (2002) found that the flavonol concentration was increased by 10-fold in Merlot grapes that were exposed to the sun, relative to grapes that were shaded. Because flavonols are found mostly in the outer layer of cells in the grape skin and they absorb UV light strongly at 360 nm, it is believed that plants produce them as a form of sunscreen.

Flavonols are known to have a bitter taste, but it is unclear if, at the concentrations found in wine, they make a contribution to flavor. Sáenz-Navajas et al. (2010) found that there was no correlation between bitterness and flavonol concentration in red wines. However, it was proposed that other compounds could have overpowered their effect. Preys et al. (2006) showed that when phenolic fractions were added back to wine, there was an association between bitterness and the fractions higher in flavonols. Hufnagel and Hofmann (2008a) concluded that flavonols possess a ‘velvety astringency’.

Phenolic compounds: anthocyanins. The color of wine produced from red grapes comes from anthocyanins. The red color of anthocyanins is due to the fully conjugated 10 π-electron flavonoid ring system. Color is lost when this conjugation is disrupted, such as when anthocyanins react with bisulfite or other nucleophiles or when the pH changes. Monomeric anthocyanins in wine can react with carbonyl-containing compounds and tannins to produced

stabilized ‘polymeric pigments’. In fact, after a few years of aging, most wine anthocyanins are present in the form of polymeric pigments.

‘Anthocyanidin’ is the term for the simple, conjugated aglycone. However, anthocyanidins are not found in grapes or wine. Instead, their more stable glycosylated form, anthocyanins, are present. Anthocyanins are often referred to as ‘monomeric pigments’ to distinguish them from polymeric pigments formed through complexation with condensed tannins. The 3-*O*-glucoside is the predominant form of anthocyanin in *V. vinifera* grapes and wine. However, in American and hybrid species, the 3,5-di-*O*-glucoside is also present. The glucose moieties attached to the anthocyanidin flavonoid ring can be substituted through esterification at the 6-position, either by an acetyl or a coumaroyl group (Mattivi et al. 2006, Waterhouse et al. 2016). There are five anthocyanidin aglycones found in red grapes: cyanidin, peonidin, delphinidin, petunidin, and malvidin. These aglycones differ in substitution patterns on the flavonoid B-ring. Because of these differences in the B-ring and the sugar moiety, red grapes can contain between 10-15 different anthocyanins. The predominant anthocyanin in most *V. vinifera* grapes and wine is malvidin-3-glucoside and its derivatives. Therefore, most studies on anthocyanins in grapes and wine focus on the reactivity and interactions of malvidin-3-glucoside.

The form of anthocyanins in wine is dependent on pH, and the relative proportions of the different forms significantly affect the color of the wine. The anthocyanin form that gives wine its typical red color is the flavylium cation. This cation has a positively charged, electrophilic C-ring, and its C2 and C4 positions can react with nucleophiles in wine, such as water and bisulfite. When this reaction occurs, the flavylium ring structure is altered, the double bond conjugation is disrupted, and red color is lost. The disruption of conjugation by electrophilic addition of water occurs as the pH of solution rises above 2.7. This results in the colorless carbinol pseudobase

form of anthocyanins. Because typical wine pH is 3.4-3.7, about 90% of the anthocyanins are in the pseudobase form, and therefore colorless. If the pH of a solution rises above 4.7, the pseudobase form is converted to the quinoidal base form, which has a blue-violet color. Therefore, wine with a high pH value will have small amounts of the quinoidal base.

Anthocyanins in the flavylium cation form will react with the bisulfite nucleophile at the C4 position of the C-ring. This causes the double bond conjugation, and therefore the color, to be lost. This is referred to as 'bisulfite bleaching' (Timberlake and Bridle 1967). The KBMS used in wine, although necessary for microbial and oxidative stability, will noticeably bleach some of the red color immediately when added. If a covalent bond at the C4 position blocks bisulfite addition, the bleaching effect is prevented. This occurs when the anthocyanin form co-pigmentation complexes or polymeric pigments.

Anthocyanins can form stable polymeric pigments, also referred to as co-pigmentation complexes or modified pigments, with other phenolic compounds as wine ages. The aromatic (conjugated) form of anthocyanins is preserved by this mechanism and they are protected from bisulfite bleaching. Thus, the absorbance of aged wines is often greater than what would be predicted by the monomeric anthocyanin concentration and pH alone (Boulton 2001). In general, the best co-factors for color enhancement are planar aromatic structures, as non-planar structures are sterically unfavorable. For example, quercetin, a planar molecule, has a binding constant (K_d) that is over 30 times greater than that of catechin, a non-planar molecule.

Polymeric pigments are more stable than monomeric anthocyanins because they are less prone to degradation during long-term storage, absorb more strongly at wine pH, demonstrate less pH dependence in their absorbance behavior, and are less bleachable by the bisulfite nucleophile. The simplest of the modified pigments are formed through electrophilic aromatic

substitution on the flavonoid A-ring. It is common for a tannin to attach to the A-ring of the anthocyanin to form what is called a T-A (tannin-anthocyanin) wine pigment. This occurs when a proanthocyanidin (tannin) is cleaved at the interflavan bond to form an electrophilic cation, which then attaches to the anthocyanin A-ring. The anthocyanin must be in the neutral pseudobase form, because the pseudobase is a nucleophile and therefore can attack the C4 position of the proanthocyanidin B-ring. T-A pigments are very common in wine since about 90% of wine anthocyanins are in the pseudobase form. When anthocyanins are in the flavylium cation form, they are electrophilic and can react directly at the C4 position with the nucleophilic A-ring of a proanthocyanidin to form an A-T pigment. This disrupts the fully conjugated structure of the anthocyanin, and therefore the resulting flavene product is colorless. The flavene could oxidize to regenerate the aromaticity and recover its color. However, some research shows that this reaction stops with the flavene product, resulting in a net loss of color (Hayasaka and Kennedy 2003).

Phenolic compounds: flavan-3-ols and condensed tannins. Flavan-3-ols are the class of flavonoids present in the largest quantities in grapes. A notable property of flavan-3-ols is that positions 2 and 3 of the central C-ring can have *cis* and *trans* isomers, relative to the B-ring. The *cis* isomers are denoted with the prefix ‘epi’ (i.e. epicatechin). There are two possible substitution patterns on the B-ring for flavanols: the more common 3’,4’-dihydroxy substitution and the 3’,4’,5’- ‘gallo’ pattern. Therefore, the flavan-3-ol with *cis* substitution on the C-ring and three –OH groups on the B-ring is called epigallocatechin. There can also be substitutions at position 3 of the C-ring, forming gallic acid esters. Thus, there are five different monomeric flavanols found in grapes: catechin, epicatechin, gallocatechin, epigallocatechin, and epicatechin

gallate. The distribution of the flavanols in grapes varies with grape variety and between the skins and seeds (Mattivi et al. 2009).

About 25-50% of the phenolic compounds in a typical red wine are oligomers (proanthocyanidins) and polymers (condensed tannins) formed by the biochemical condensation of flavan-3-ol units (Dixon et al. 2004, Manuel et al. 1990). These condensation reactions form covalent bonds between the subunits, and most condensed tannins in grapes and wine are made up of epicatechin monomers, and catechin is the next most abundant monomer.

'Proanthocyanidin' is the broad term given to the class of compounds that includes both procyanidins and prodelphinidins. In the presence of a strong mineral acid, these compounds will break down into either cyanidin or delphinidin anthocyanidins. Catechin and epicatechin flavan-3-ol units will yield cyanidin and gallocatechin and epigallocatechin flavan-3-ol units will yield delphinidin (Porter et al. 1985). Proanthocyanidins are an indication of quality in grapes and wine as they play several key roles. They react with anthocyanins to form stable pigments in aged red wine and can both accelerate the rate of oxygen consumption and react with the products of oxidation, essentially scavenging them and preventing their accumulation. Finally, proanthocyanidins are highly correlated with the perception of astringency in red wines.

Proanthocyanidins are found at concentrations of 0.5-1.5 g/L in grapes. They are only partially extracted from the grape skins and seeds during fermentation, and their concentration in red wines is < 50% of that in grapes. The concentration of proanthocyanidins in white wines is about 10-50 mg/L, much lower than that in red wines. However, the amount of proanthocyanidin measured in a grape or wine sample is dependent on the analytical method used, and it is therefore difficult to compare values across literature (Herderich and Smith 2005). Measurement is difficult both because proanthocyanidins are complex molecules and because there is no

available standard reference material. They absorb strongly at 280 nm but other phenolics also absorb at this value. Therefore, published methods usually include an isolation/separation step (Jeffery et al. 2008) followed by various types of analyses. Some methods involve the precipitation and isolation of proanthocyanidins using proteins (Harbertson et al. 2003) or polysaccharides (Sarneckis et al. 2006) prior to UV-vis detection, and these methods are correlated with perceived astringency. Proanthocyanidins can also be analyzed using direct HPLC measurement with a polar or gel permeation column to separate them based on molecular size (Kennedy and Taylor 2003, Waterhouse et al. 2000). However, these methods all vary in the amount of proanthocyanidin they will detect, even in the same sample. Therefore, absolute proanthocyanidin concentration should not be compared across studies, especially when analytical methods differ.

Flavan-3-ol monomers are perceived as bitter and astringent, and as the degree of polymerization increases, their bitterness decreases and astringency increases (Peleg et al. 1999, Robichaud and Noble 1990). Hufnagel and Hofmann (2008a) identified polymeric proanthocyanidins as the compound group responsible for most of the astringency in wine, and determined that different molecular weight sub-fractions produce different sensory responses. Mercurio and Smith (2008) showed a strong correlation ($r^2 > 0.8$) between the tannin content measured using the protein or carbohydrate precipitation methods and perceived astringency of model wines. When a wine is aged, its tannins are hydrolyzed and can react with other wine components. Because these modified tannins are more hydrophobic and have lower DP values, they are less astringent (McRae et al. 2013). Therefore, the astringency of a wine will decrease as it is aged.

Grapes Grown in Arkansas and the Southeastern United States

The genus *Vitis* contains over 60 species (Reisch et al. 2012, Young and Vivier 2010), but most of the commercially important wine grape varieties belong to the *V. vinifera* species. While *V. vinifera* grapes have traditionally preferred flavor characteristics, they are highly vulnerable to pests, diseases, and extreme temperatures. For example, common *V. vinifera* species, such as Chardonnay and Cabernet Sauvignon, are extremely difficult to grow in much of the United States, including Arkansas, and the high cost of maintaining these grapevines typically exceeds the profit that can be made from producing wine from these popular grapes. Native species, such as *V. rotundifolia* (muscadine) and *V. aestivalis*, are generally better-adapted to surviving stressors that would devastate *V. vinifera* grapes. For instance, the muscadine grape is resistant to several diseases, including Pierce's disease, that would be extremely harmful to *V. vinifera* grapes (Reisch et al. 2012). However, these native species often have a low crop yield and can produce wines with unfavorable characteristics, such as high acidity, low astringency, and excessive herbaceous aromas (Waterhouse et al. 2016).

Other alternatives to *V. vinifera* grapes are hybrid cultivars. Hybrid grapes (*Vitis* spp.) are created by grape breeders to reap advantageous traits from both parents, such as the cold-hardiness from wild species and the desirable yield and flavor characteristics of *V. vinifera*. The Concord (*V. labruscana*) grape, widely used for juice production, was developed as a cross between the native *V. labrusca* and a *vinifera* species. Therefore, it has both the pest resistance of *V. labrusca* and the high yields of *V. vinifera* (Reisch et al. 2012). French-American hybrids are a particular class of grape hybrids that come from breeding efforts conducted in France to combat the Phylloxera epidemic that destroyed much of the European grape population. French-

American hybrids grown in Arkansas include the red wine grape Chambourcin and the white wine grape Vignole.

University of Arkansas System Division of Agriculture wine grape breeding

The University of Arkansas System Division of Agriculture (UA System) has a Fruit Breeding Program established in 1964 and located at the Fruit Research Station in Clarksville, Arkansas. This program has released many cultivars of blackberries, peaches and nectarines, table and juice grapes, and blueberries. The overall program focuses on development of fruit cultivars for Arkansas production of fresh-market fruits and has released about 70 cultivars.

The Fruit Breeding Program began breeding wine grapes over 40 years ago. The goal of this program was to develop new hybrid wine grape cultivars that grow well in Arkansas, have desirable flavor attributes, and are suitable for winemaking. In 2016, the first wine grape cultivars, Enchantment (red-wine cultivar) and Opportunity (white-wine cultivar), were released from the UA System. Two other white-wine advanced breeding selections, A-2359 and A-2574, are being evaluated for potential release and will be named if released. These genotypes (cultivars and advanced selections) are *Vitis* hybrids that show potential for regions that have limited productivity of wine grape cultivars.

Enchantment wine grapes. The first cross for Enchantment was made in 1990. The parents were two other Arkansas crosses Ark. 1628 and Ark. 1481. Ark. 1628 was the female parent, and it resulted from a cross of two *V. vinifera* cultivars Petit Sirah and Alicante Bouschet (a teinturier grape). The male parent, Ark. 1481, was a cross between the *V. vinifera*-derived cultivars Bouschet Petit and Salvador (Clark et al. 2018).

The Enchantment grapevine produces teinturier berries with a dark purple color in the flesh and juice of the grape. Growth, yield, hardiness, and disease resistance data for

Enchantment grapevines were taken from 1998-2015. Vines had an average crop yield of 10.1 kg/vine and average cluster and berry weights of 178.3 g and 1.5 g, respectively. The average soluble solids, pH, and TA at harvest were 18.9%, 3.4, and 0.8%, respectively. Although sugar levels were lower than the target value of 20%, grapes were usually harvested before this could be reached to prevent any further decrease in acid levels (Clark et al. 2018).

Enchantment was able to survive the cold winter climate in Clarksville. During the time period in which the vines were evaluated, winter low temperatures ranged from -17°C to -9°C. There was very little, if any, winter damage observed. Therefore, it was determined that Enchantment wine had good hardiness for growth in the Arkansas climate. In addition, there was minimal observation of common diseases on vines during the years they were evaluated, which reflected Enchantment's potential to manage disease pressures presented in Arkansas (Clark et al. 2018).

Wines were produced from Enchantment berries at the University of Arkansas Department of Food Science from 1998-2015 using small-scale winemaking techniques. The quality of Enchantment wine was consistently good, as indicated by both composition measurements and sensory analyses. The average ethanol content, pH, and TA of Enchantment wine was 11.2%, 3.4, and 0.86%, respectively. These values were within acceptable ranges for a finished red wine. The primary anthocyanin in Enchantment was identified as the *V. vinifera*-like malvidin-3-glucoside, which is more stable relative to the anthocyanin diglucosides typically found in Arkansas red wines. This led researchers to believe that Enchantment wine would perform well if aged in the bottle or on oak. In sensory studies, panelists noted the deep, red color of Enchantment and determined it had Syrah-like fruit notes. A general consensus was that

Enchantment could either be used to produce a single varietal table wine or added to other wines as a color/flavor enhancer (Clark et al. 2018).

Opportunity wine grapes. The first cross for Opportunity wine grapes was made in 1987 between Cayuga White and Ark. 1754. Cayuga White, a cross between *V. labrusca* L. and *V. vinifera* L. from New York, was the female parent. Ark. 1754 was the male parent and was derived from two *V. vinifera* cultivars- Semillon (from France) and Rkatsiteli (from the country of Georgia).

The growth, yield, hardiness, and disease resistance of Opportunity grapevines was evaluated from 1994-2015. Average yield was 10.9 kg/vine, average cluster weight was 234.3 g, and average berry weight was 2.7 g. Opportunity produced a slightly larger crop yield than Enchantment, with larger, more compact clusters and bigger berries. The average soluble solids, pH, and TA at harvest were 17.3%, 3.5, and 0.5%, respectively. These numbers were less optimal than those of Enchantment. Similar to Enchantment grapevines, Opportunity did not experience any winter damage and there was minimal observation of disease (Clark et al. 2018).

Wines were produced from Opportunity grapes at the University of Arkansas Department of Food Science from 1995-2015 using small-scale white winemaking procedures. The average ethanol content, pH, and TA of these wines was 12.1%, 3.0, and 0.66%, respectively. A sensory analysis was also conducted in which it was determined that Opportunity has distinct fruit flavors, honey aroma, and a light gold color. It was also noted that the wine had Semillon-like spice notes and a bouquet similar to that of Cayuga White. Researchers determined that Opportunity would be a complement to other white wine grape cultivars grown in Arkansas and similar regions (Clark et al. 2018).

Unreleased advanced selection white wine grapes. A-2359 and A-2574 are white wine grape advanced selections pending release from the University of Arkansas Fruit Research Station. A-2574 has Gewürztraminer characteristics and A-2359 has Muscat characteristics. These advanced selections have shown good climatic adaptation and consistent productivity in Arkansas. A-2359 had an average of 18.6% soluble solids, 3.4 pH, and 0.6% TA at harvest, whereas A-2574 had 20.2% soluble solids, 3.3 pH, and 0.6% TA. Wines produced from these grapes are soft white wines with fruit-forward flavors. A-2574 berries have pink skins and have shown potential for the production of late-harvest wines. A-2359 wines have a distinct Muscat-like aroma (Threlfall et al. 2019).

Muscadine grapes (*Vitis rotundifolia*)

Muscadine grapes (*V. rotundifolia*) are the most widely-grown grape in the Southeastern United States because they are well-accustomed to warm, humid climates that would be unsuitable for the growth of other grapes, such as *V. vinifera*. The berries are approximately 2.5-3.8 cm in diameter and have thick, tough skins that protect them from heat, UV radiation, humidity, insects, and fungi (Sandhu and Gu 2010, Sims and Morris 1985). Muscadines can be either light-skinned (green or bronze) or dark-skinned (red to almost black) (Ector et al. 1996, Lee and Talcott 2002, Pastrana-Bonilla et al. 2003) and are marketed in fresh and processed forms such as juice, wine, and jam/jelly. A majority of the commercial muscadine crop is used to produce wine (Sims and Morris 1985).

Research has shown that muscadine grapes contain a wide variety of antioxidant polyphenolic compounds, such as hydroxybenzoic acids, ellagic acid, resveratrol, anthocyanins, quercetin, myricetin, and kaempferol (Ector et al. 1996, Huang et al. 2009, Lee et al. 2005). These phenolic compounds have been linked to many positive human health benefits, including

protection against cancer and cardiovascular disease (Arts and Hollman 2005, Djoussé et al. 2004, Kaur and Kapoor 2001). In addition, some cell culture studies (Mertens-Talcott et al. 2006, Yi et al. 2005) have indicated that muscadine polyphenols can inhibit proliferation of colon cancer cells and induce apoptosis. As consumers have become aware of these muscadine health benefits, the demand for fresh and processed muscadine products has increased. In fact, the muscadine grape industry is experiencing its greatest growth in decades (Striegler et al. 2005).

Muscadine grape research. The polyphenolic profiles of fresh muscadine grapes have been researched. Sandhu et al. (2010) compared the total phenolic content and antioxidant capacity in the seeds, skin, and pulp of eight cultivars of Florida-grown muscadine grapes. Total phenolics and antioxidant capacity were the highest in the seeds, followed by the skin and the pulp. On average, 87.1% of the phenolics were in the seeds, 11.3% were in the skins, and 1.6% were in the pulp. Lee et al. (2005) isolated and identified several ellagic acid derivatives in muscadine grapes using HPLC-ESI-MS. Grapes contained phenolic acids, flavonols, anthocyanins, ellagic acid, and numerous ellagic acid derivatives. Pastrana-Bonilla et al. (2003) separated the skins, seeds, and pulp of 10 muscadine cultivars grown in southern Georgia, and the seeds had the greatest total phenolics, followed by the skins then the pulp. Overall, ellagic acid was the most prevalent phenolic compound.

In terms of anthocyanins, muscadine grapes contain only non-acylated 3,5-diglucosides of delphinidin, petunidin, cyanidin, malvidin, and peonidin (Ballinger et al. 1973). Muscadine grapes with large amounts of malvidin-3,5-diglucoside produce wines and juices with the best color quality (Ballinger et al. 1974, Flora 1978, Nesbitt et al. 1974). Processed products made from muscadine grapes, such as juices and wine, brown more quickly than juices/wines produced from other grapes, likely because diglucoside anthocyanins are more susceptible to degradation

than the monoglucoside anthocyanins found in other grape varieties (Robinson et al. 1966). Huang et al. (2009) identified and quantified the anthocyanins in both bronze- and purple-skinned muscadine grapes using HPLC-ESI-MS. Approximately 90% of the total anthocyanins were 3,5-diglucosides of delphinidin, cyanidin, and petunidin, while the remaining 10% were 3,5-diglucosides of peonidin and malvidin. The purple-skinned muscadines had significantly higher total anthocyanins than the bronze varieties, and the anthocyanins were concentrated mainly in the skins of the grapes. Because research (Huang et al. 2009, Pastrana-Bonilla et al. 2003, Sandhu and Gu 2010) has shown that a majority of the phenolic compounds in muscadine grapes are concentrated in the skins and seeds, the extraction and solubility of these compounds during wine and juice making are greatly influenced by the time and temperature of extraction (Baderschneider and Winterhalter 2001).

There have been a few sensory studies conducted on muscadine grapes. For example, Breman et al. (2007) evaluated quality characteristics and eating quality of 11 muscadine grape cultivars grown in northern Florida. Grapes were evaluated for pH, titratable acidity (TA), soluble solids (Brix), and water activity (A_w). Consumers evaluated cultivars based on color, taste (sweetness and sourness), muscadine flavor, firmness, and overall preference. The pH, TA, Brix, Brix/acid ratio, and consumer evaluations were different among the 11 cultivars.

Muscadine juice research. Talcott and Lee (2002) compared the antioxidant properties of flavonoids and ellagic acid in eight juices and wines produced by various processing methods from red and white muscadine cultivars. Juices and wines were subjected to both hot- and cold-pressing techniques and wine was produced by fermentation on the skins for 3, 5, and 7 days. Changes in anthocyanins, ellagic acid, flavonols, and overall antioxidant capacity (AOX) were measured after storage for 60 days at 20°C and 37°C. The red and white wines had higher AOX

values than juices produced with identical processing methods. Therefore, it was concluded that processing methods for muscadine juices and wines are important factors in determining the concentrations of antioxidant flavonoids and ellagic acid.

Similar to fresh muscadine grapes, there has been limited sensory research conducted on muscadine juice. Threlfall et al. (2007) evaluated juice from five black muscadine cultivars and three bronze cultivars for basic composition, nutraceutical content, and sensory characteristics. Overall consumer liking was positively correlated with sweetness and caramelized flavor and correlated negatively with sour and green/unripe flavor. Consumers showed a preference for juice with a SS content of approximately 14% and a SS to acids ratio of 26-31.

There have been several studies examining the volatile aroma profile. Baek et al. (1997) identified the predominant aroma compounds in muscadine grape juice from two different locations and different harvest dates using GC-mass spectrometry (MS) and GC-O aroma extract dilution analysis. Furaneol was the most intense aroma in the juice, with a burnt sugar-like aroma. Other prevalent compounds included 2,3-butanedione (buttery, cream cheese aroma), ethyl butanoate (bubblegum, fruity aroma), ethyl 2-methylbutanaote (green apple, fruity aroma), 2-phenylethanol (rose aroma), and *o*-aminoacetophenone (foxy aroma). It was proposed that furaneol and *o*-aminoacetophenone were responsible for the characteristic candy and foxy aroma of muscadine juice. In a separate study, Baek and Cadwallader (1999) isolated and identified free and glycosidically-bound volatile compounds in muscadine grape juice. The most abundant compound in both the free and bound form was furaneol. *o*-Aminoacetophenone and 2-phenylethanol were found in free and bound forms as well.

Muscadine wine research. Because a majority of the commercial muscadine harvest is used to produce wine (Sims and Morris 1985), there have been several studies focusing on the

composition, flavor, and color of muscadine wines. Lamikanra (1997) determined the organic acid composition of muscadine wines during fermentation and aging. In non-muscadine wines, tartaric and malic acids usually account for > 90% of organic acids. However, tartaric and succinic acids were the most predominant in this study. The concentration of succinic acid was negligible at the onset of fermentation but increased over time, while the concentration of tartaric acid decreased very gradually. Wine produced from *V. rotundifolia* grapes has a characteristic increase in acidity during fermentation, which is not seen with other grape varieties. Lamikanra (1997) attributed this increase in acidity during vinification to the increase in succinic acid.

Fining agents such as polyvinyl-polypyrrolidone (PVPP), gelatin, egg albumen, and casein have been shown to reduce phenolic levels and alter the color and sensory characteristics of non-muscadine wines (Chris Somers and Evans 1977, Ough 1960, Zoecklein et al. 1990). Sims et al. (1995) treated white muscadine wine (cv. Welder) with fining agents PVPP, casein, and gelatin before or after fermentation. Red muscadine wine (cv. Noble) was treated the same after fermentation. In the white wines, PVPP and casein, added both pre- and post-fermentation, reduced total and flavonoid phenols, lightened the color, and improved resistance to browning. Gelatin reduced the total phenols and altered sensory characteristics but did not affect color. PVPP added post-fermentation altered sensory characteristics, but casein did not. In the red wine, post-fermentation addition of casein and PVPP reduced total and polymeric phenols and lightened the color, and PVPP reduced brown color. Gelatin had little effect on the phenols, color, or sensory characteristics, and only PVPP altered sensory characteristics.

Sims and Morris (1984) investigated browning and color degradation in muscadine wine. This study examined the effects of three pH levels (2.90, 3.20, and 3.80), three sulfur dioxide levels (25, 50, and 75 ppm free SO_2), three storage temperatures (20, 30, and 40°C), and three

storage times (0, 3, and 9 months). A higher pH resulted in a loss of color intensity and red color and increased browning over storage for 9 months. Wines with lower pH values had a greater loss of free anthocyanins, which indicated a greater degree of anthocyanin-tannin polymerization and therefore a more stable color. Sulfur dioxide levels higher than 25 ppm severely bleached the color and lessened browning in wine with a higher pH. Higher storage temperatures greatly increased browning and anthocyanin loss during 9-months of storage, and wine stored at 30 or 40°C had unacceptable color after 9 months. A similar study was conducted by Sims and Morris (1985) to compare the color stability of Noble muscadine and Cabernet Sauvignon wines at various storage times and pH levels. Noble wine browned to a much greater extent and lost more color over 16 months of aging than did Cabernet. It was proposed that this was because of a lack of tannin-anthocyanin polymerization in muscadine wine.

There has been limited research on the volatile aroma profile of muscadine wine. Lamikanra et al. (1996) analyzed flavor development in Noble muscadine wine during fermentation and aging using GC-MS. The complexity of the aroma profile increased with time, especially after fermentation was complete. It was determined that 2-phenylethanol was a major aroma compound and that it was biosynthesized during the vinification process. Anaerobic formation of fatty acid esters occurred after active fermentation had ceased, and these compounds were determined to be major aroma components of aged muscadine wine.

Research has shown that the skins and seeds of muscadine grapes contain a majority of the nutraceutical phenolic compounds (Pastrana-Bonilla et al. 2003, Sandhu and Gu 2010). Thus, longer periods of fermentation on the skin (‘skin contact time’) for muscadine wines will affect the phenolics content of the wine significantly. It has also been determined that muscadine juice contains significant amounts of glycosidically-bound aroma compounds (Baek and

Cadwallader 1999), and it is likely that muscadine wines follow a similar trend. Glycosidic enzymes, such as β-glucosidase, could be used to release these bound compounds.

Use of Inactivated Dry Yeasts in Wine Production

The use of inactivated dry yeasts (IDYs) for winemaking has been popularized in recent years. These products are typically used to enhance or preserve wine aroma and to improve mouthfeel. IDYs are *S. cerevisiae* byproducts from various manufacturing processes (Šuklje et al. 2016), and they can be divided into four categories of commercially available products: inactive yeasts, yeast autolysates, yeast hulls, and yeast extracts (Pozo-Bayón et al. 2009, Rodríguez-Bencomo et al. 2014). IDYs are typically added to juice before, during, or after fermentation (Del Barrio-Galán et al. 2011, Comuzzo et al. 2012) and are used as fermentation enhancers to promote yeast resistance to osmotic stress, improve nitrogen compound assimilation, and enhance sensory profiles of wine (Del Barrio-Galán et al. 2011, Pozo-Bayón et al. 2009, Pozo-Bayón et al. 2009a). It is also believed that IDY products will decrease perceived bitterness and increase perceived sweetness of wines, improve tartaric acid stabilization, provide antioxidant properties, and improve the mouthfeel of wines (Del Barrio-Galán et al. 2011, Pozo-Bayón et al. 2009a, 2009b). IDYs can be used to enhance malolactic fermentation by providing nutrients for bacteria, enhancing bacterial growth and malolactic fermentation rate, and reducing the risk of contamination by undesirable bacteria (Pozo-Bayón et al. 2009a, 2009b).

There have been multiple theories for the modifications of wine by IDYs, based on the type of IDY product used and/or the timing of addition to must or wine. Saerens et al. (2008) proposed that IDYs could modify yeast metabolism and therefore its by-products. Other explanations include the release of amino acids, mannoproteins, lipids, peptides, vitamins,

minerals, and volatile compounds from IDYs (Andújar-Ortiz et al. 2014, Guadalupe et al. 2010, Pozo-Bayón et al. 2009, Pozo-Bayón et al. 2009a, 2009b), retention of wine aroma compounds by IDY mannoproteins and peptides (Chalier et al. 2007, Comuzzo et al. 2012, 2006, Pozo-Bayón et al. 2009, Pozo-Bayón et al. 2009a, 2009b), and antioxidant effects of IDYs (Del Barrio-Galán et al. 2011, Kritzinger et al. 2013b, Rodríguez-Bencomo et al. 2014). In addition, some IDYs are glutathione-enriched, and these products are claimed to increase reduced glutathione (GSH) concentrations in wine by directly liberating GSH into juice/wine or by providing GSH synthesis precursors during fermentation (Kritzinger et al. 2013a). It has been reported that GSH can act as an antioxidant to prevent browning in juice/wine, can increase production of volatile thiols during fermentation, and can protect against the loss of certain terpenes, esters, and thiols during wine aging (Andújar-Ortiz et al. 2014, Kritzinger et al. 2013a, Makhotkina et al. 2014).

Application of inactivated yeasts to grapevines

Although IDYs are commonly used in the wine industry, they are typically added to juice/wine during the vinification process. LalVigne® (Lallemand, Inc., Montreal, Canada) is a specific inactivated dry yeast that is rehydrated and applied foliarly to grapevines in the vineyard at veraison. It is promoted to enhance fruit ripening, encourage even ripeness, increase phenolic maturity, concentrate and increase aroma precursors, and improve mouthfeel and overall quality of resulting wine. There are two commercially available forms of LalVigne®: LalVigne® Aroma, intended for use with white wine grapes, and LalVigne® Mature, intended for use with red wine grapes. Despite use of these products in the wine industry, there have only been three published studies evaluating their use (Giacosa et al. 2019, Šuklje et al. 2016, Villangó et al. 2015).

Effect of inactivated yeast foliar application on wine grapes. Villangó et al. (2015) evaluated the use of LalVigne® Mature on Syrah (*V. vinifera*) grapevines grown in a cool climate (Eger,

Hungary). The ability of the yeast spray to create a balance between sugar development and phenolic maturity was of special interest. It was determined that grapes from treated vines had thicker skins and greater phenolic potential, particularly anthocyanin concentrations and extractability, than grapes from untreated vines. Therefore, it was concluded that phenolic ripening of red wine grapes can be enhanced using LalVigne® Mature.

Giacosa et al. (2019) evaluated LalVigne® Aroma application on Chardonnay and Cortese (*V. vinifera* white-wine grapes), and LalVigne® Mature application on Nebbiolo (*V. vinifera* red-wine grape) grown in Italy. In general, grapes from sprayed vines had increased skin thickness and anthocyanin content at harvest. However, the effects of treatment varied among cultivars and growing season.

Effect of inactivated yeast foliar application on wine. Šuklje et al. (2016) evaluated LalVigne® Aroma application on Sauvignon Blanc (*V. vinifera*) grapes to create wines with improved aroma. Use of the inactivated yeast lead to increased GSH concentrations in juices and corresponding wines, differences in individual higher alcohol acetate (HAA) and fatty acid ethyl ester (FAEE) concentrations at the end of fermentation, and significantly slower degradation of FAEEs and HAAs after two months of storage. In addition, sensory analysis demonstrated that wines produced from treated grapes had greater perceived fruitiness, whereas control wines were more commonly described as green/unripe. Correlations were found between chemical compositions and sensory properties. For example, HAAs and thiols were positively correlated with tropical fruit, pear, and artificial banana flavor descriptors.

These studies (Giacosa et al. 2019, Šuklje et al. 2016, Villangó et al. 2015) provide some evidence that the use of IDY foliar sprays can enhance wine aroma and overall quality, but the *V. vinifera* cultivars used in these studies are very difficult to grow in Arkansas and similar regions.

It would be of interest to determine how LalVigne® products would perform if used with a non-*vinifera* cultivar, such as Chambourcin, a French-American hybrid widely grown in the midwestern and eastern United States.

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CHAPTER I

Effect of specific inactivated yeast vineyard spray application on physical, composition, and phenolic attributes of Chambourcin grapes

Abstract

Chambourcin is an interspecific French-American *Vitis* spp. hybrid red wine cultivar grown throughout the eastern and midwestern United States. Some regions growing Chambourcin struggle with delayed or uneven ripening and lack of color development in the grapes. LalVigne® (Lallemand, Inc., Montreal, Canada) is a specific inactivated yeast that is sprayed foliarly on grapevines during ripening and has been shown to increase phenolic content and skin thickness of *V. vinifera* grapes. As *V. vinifera* grapevines are difficult to grow in Arkansas and similar regions, the objective of this study was to evaluate the effects of inactivated yeast application on Chambourcin grapevines on physical, composition, and phenolic attributes of Chambourcin grapes. In 2018 and 2019 at a commercial vineyard in Hindsville, AR, four rows of Chambourcin grapevines were sprayed (spray treatment) with LalVigne® Mature, and an additional four rows were unsprayed (control treatment). Berries were sampled from each treatment once per week from veraison to harvest and clusters were sampled at harvest. Cluster attributes (232-233 g and 87-101 berries/cluster in both years) were not impacted by the Spray treatment. The physical attributes (berry weight, length, width, skin color, and skin elasticity), composition attributes (soluble solids, pH, titratable acidity, sugars, and organic acids), and phenolic attributes (individual and total anthocyanins and total flavonols) were evaluated during ripening and at harvest. The impact of the inactivated yeast spray on the grape attributes varied during ripening and at harvest. In both years, berries from sprayed vines had higher skin

elasticity (thicker skins). The berries weighed 2.4-2.7 g at harvest, but the inactivated yeast application did not impact the other physical attributes. The harvest composition of the 2018 Chambourcin berries (21% soluble solids, 3.6 pH, and 0.6% titratable acidity) was more ideal for winemaking than in 2019 (19% soluble solids, 3.8 pH, and 0.5% titratable acidity). Berries from sprayed vines had lower pH than berries from control vines during ripening and at harvest in both years, but other composition attributes were not consistently impacted by the Spray treatment. Malvidin-, delphinidin-, and petunidin-3-glucoside and malvidin-3,5-diglucoside were the predominant anthocyanins. At harvest, Chambourcin berries had higher total anthocyanins (634 mg/100g) and total flavonols (25 mg/100g) in 2019 than in 2018 (251 and 16 mg/100g, respectively). Berries from sprayed vines had higher malvidin- and petunidin-3-glucoside than berries from control vines in 2018 across all sampling dates. In 2019, berries from sprayed vines had higher levels of individual anthocyanins and total anthocyanins and lower levels of total flavonols than berries from control vines at harvest. Therefore, application of a specific inactivated yeast to Chambourcin grapevines lead to better attributes for winemaking, including higher levels of red-colored anthocyanins that could be extracted during winemaking.

Acknowledgements

This research was funded by Danstar Ferment AG (Zug, Switzerland) and Lallemand, Inc. (Montreal, Canada).

Introduction

Grapevines (*Vitis* spp.) are one of the most widely-planted horticultural crops in the world. In the United States, 95% of grape and wine production occurs in California, Washington,

New York, Pennsylvania, and Oregon, but production is focused mostly on *V. vinifera*, which is the most popular species of grapevines (Creasy and Creasy 2009, OIV 2000, TTB 2015, USDA NASS 2019). *V. vinifera* grapevines are highly vulnerable to pests, diseases, and extreme temperatures and are difficult to grow in much of the United States, including Arkansas. The high cost of maintaining *V. vinifera* grapevines in non-ideal climates offsets the profit from producing these wines.

Hybrids (a cross of two or more *Vitis* species) and native species, such as *V. rotundifolia*, are better-adapted to surviving stressors that devastate *V. vinifera* grapes (Reisch et al. 2012). Despite the challenges, grape and wine production contribute significantly to the Arkansas economy. In 2010, the Arkansas grape and wine industry was responsible for 1,700 jobs and over $42 million in wages, and wine-related tourism generated $21 million in revenue (Frank 2010).

Grapes grown in Arkansas include mostly native species and hybrids. Hybrid grapes are created by grape breeders to reap advantageous traits from both parents, such as the cold-hardiness of native species and the desirable yield and flavor of *V. vinifera*. French-American hybrids originate from breeding efforts conducted in France to combat the Phylloxera epidemic (a pest that attacks the roots of grapevines) that destroyed much of the European grape industry (Jackson 2000). Chambourcin (Seyve-Villard 12-417 x Chancellor) is an interspecific French-American hybrid red wine grape, created by French grape breeder Joannes Seyve, that is grown throughout the midwestern and eastern United States, including Arkansas (Homich et al. 2016, Prajitna et al. 2007). Chambourcin has higher disease and winter resistance than *V. vinifera* grapevines, and is considered one of the best red wine hybrid cultivars for producing quality wine (Dami et al. 2006).

Inactivated yeasts are *Saccharomyces cerevisiae* byproducts used during winemaking to enhance or preserve wine aroma and improve mouthfeel (Šuklje et al. 2016). Inactivated yeast products are typically added to juice or wine before, during, or after fermentation and are used as fermentation enhancers to promote yeast resistance to osmotic stress, improve nitrogen compound assimilation, and enhance sensory profiles of wine (Del Barrio-Galán et al. 2011, Comuzzo et al. 2012, Pozo-Bayón et al. 2009). LalVigne® Mature and LalVigne® Aroma (Lallemand, Inc., Montreal, Canada) are foliar specific inactivated yeast spray developed for use on grapevines in the vineyard at the point of veraison (when berries begin to develop color and ripening quickens). These products are promoted to quicken fruit ripening, encourage even ripeness, increase phenolic maturity, concentrate and increase aroma precursors, and improve mouthfeel and overall quality of resulting wine.

Despite use of these products in the viticulture industry, there has been little published research on effects on grapes and wine, with most studies focused on *V. vinifera*. Villangó et al. (2015) evaluated the use of LalVigne® Mature on Syrah grapevines (a red-wine cultivar) grown in Hungary, and it was determined that grapes from treated vines had thicker skins and greater anthocyanin content and extractability than grapes from untreated vines. Similar results were found by Giacosa et al. (2019), where LalVigne® Aroma application was evaluated on white-wine cultivars Chardonnay and Cortese and LalVigne® Mature application was evaluated on red-wine cultivar Nebbiolo grown in Italy. In general, grapes from sprayed vines had increased skin thickness, and Nebbiolo grapes from sprayed vines had higher anthocyanin content at harvest. However, the effects of treatment varied among cultivars and growing season. Šuklje et al. (2016) applied LalVigne® Aroma to Sauvignon Blanc grapevines (a white-wine cultivar) and produced wines from both treated and control grapes. There were differences in fatty acid ethyl

ester concentration after fermentation among wines from sprayed and control vines, and wines from sprayed vines had slower degradation of fatty acid ethyl esters during storage. Sensory analysis demonstrated that Sauvignon blanc wine from sprayed vines had greater perceived fruitiness, whereas wine from control vines was more green/unripe.

There have been several studies evaluating the yield and quality of Chambourcin grapevines for wine production (Dami et al. 2006, Ferree et al. 2004, Mikami et al. 2017, Miller et al. 1997, Prajitna et al. 2007, Xu et al. 2016, Zhang and Dami 2012, Zhu et al. 2012). Chambourcin grapevines tend to overcrop, which can lead to uneven ripeness and underripe berries at harvest. Therefore, a longer growing season relative to other red wine grapes is required for Chambourcin in some areas for berries to reach desirable compositions for wine production (Dami et al. 2006, Ferree et al. 2004). However, this longer growing season can make vines vulnerable to early fall frosts in cool seasons (Zhang and Dami 2012). It has been shown that cluster thinning can help produce a more balanced crop (Dami et al. 2006, Ferree et al. 2004, Prajitna et al. 2007), but this means that overall crop yield is lower. Although Chambourcin grapevines grown in Arkansas do not experience as many issues with uneven/delayed ripening as those grown in cooler climates, they are subjected to the typical disease pressures of the area, such as powdery mildew and downey mildew (Creasy and Creasy 2009, Urbez-Torres et al. 2012), which can affect the quality of grapes for wine production.

Therefore, further exploration of techniques to improve the properties of Chambourcin grapes for wine production would be of interest. While previous studies on LalVigne® Mature application provide some evidence that the use of inactivated yeast grapevine foliar sprays can enhance wine aroma and overall quality, research has mainly focused on *V. vinifera* cultivars. As *V. vinifera* grapevines are difficult to grow in Arkansas and similar regions, the objective of this

study was to evaluate the effects of specific inactivated yeast application to Chambourcin grapevines on the physical, composition, and phenolic attributes of grapes.

Materials and Methods

Vineyard treatments

Chambourcin grapevines (Seyve-Villard 12-417 x Chancellor) were grown at a commercial vineyard in Hindsville, AR (USDA hardiness zone 6b). The soil type was Linker fine sandy loam (fine-loamy, siliceous, semi active, thermic Typic Hapludults). The grapes were grown on a single bilateral cordon system on 8-10-year-old vines. The vines were rooted on 3309 Couderc rootstock, commonly known as 3309 or C-3309, which is a hybrid of *V. riparia* and *V. rupestri* and is the most commonly-used rootstock in the eastern United States. Each row of grapevines was approximately 200-m long and oriented east to west. Eight consecutive rows of grapevines were sprayed with LalVigne® Mature specific inactivated yeast spray (Lallemand, Inc., Montreal, Canada) at approximately 5% veraison and again 10 days later. The first spray application at 5% veraison in 2018 was July 20, and in 2019 was July 25. The LalVigne® Mature was dissolved in water and applied at the manufacturer's recommended rate of 1.0 kg/ha at each application date using a Rears air-blast sprayer (Rears Manufacturing Company, Coburg, OR). An additional eight rows were left unsprayed. There were a total of 16 rows of grapevines in this study. Of the eight sprayed rows, the four middle rows (rows 3-6, Figure 1) of grapevines were sampled as the sprayed treatment. Of the eight unsprayed rows, the last four rows (rows 13-16, furthest from sprayed rows) were sampled as the control treatment.

Weekly berry sampling

Two-hundred individual berries were hand harvested from Chambourcin grapevines in triplicate across all four rows from each treatment once per week from veraison to harvest. Harvest date was determined by the vineyard owner based on ideal composition attributes for Chambourcin, as well as past harvest data, weather, and quality of the fruit. Average daily temperature and rainfall for January-August 2018 and 2019 were recorded near Hindsville, AR (Figure 2). To ensure random samples of the berries, the same sampling protocol was done each time. Twenty-five berries were collected from each side of each of the four rows in each treatment. Sampling was initiated near the beginning of each row. One berry was selected from the "shoulder" of a cluster, one berry from the middle of a different cluster, and one berry from the tip of a cluster. Locations of the clusters on the vine and selection of berries from the front/back of clusters varied. Ten steps were taken down the row, and the same three-berry sampling procedure was repeated. The number of steps between sampling zones was determined based on the total number of steps needed to walk the entire row. The ten-step and three-berry sampling procedure was repeated across the row, until 25 berries were collected. Then, the next side of the row was sampled until 200 berries were collected from the four rows.

In 2018, there were seven sampling dates: week 0 (veraison, July 20), week 1 (July 27), week 2 (August 3), week 3 (August 10), week 4 (August 17), week 5 (August 24), and harvest (August 27). In 2017, there were six sampling dates: week 0 (veraison, July 25), week 1 (August 1), week 2 (August 8), week 3 (August 15), week 4 (August 22), and harvest (August 29). The grapes were taken to the University of Arkansas System Division of Agriculture (UA System) Food Science Department in Fayetteville, AR and used for analysis. The 200-berry sample was used to evaluate the physical, composition, and phenolic attributes. Five berries were used for

most of the physical attributes analysis on the day the berries were harvested. The rest of the berries were frozen (-10°C) for remaining analyses. One hundred berries were used for composition and berry weight attributes. Five berries were used for phenolic attributes.

Harvest cluster sampling

Ten clusters were sampled in triplicate across all four rows for each treatment at harvest. A similar sampling procedure in terms of number of steps and sampling zones for berry sampling was used for cluster sampling. The clusters were harvested into large freezer bags, taken to the UA System Food Science Department in Fayetteville, AR, and frozen at -10°C for analysis of cluster attributes.

Physical attributes analysis

Physical attributes were evaluated for each triplicate sample for Spray treatment and sampling date (Week). The physical attributes analysis of berry samples included berry size (weight, length, and width), berry skin color (L*, chroma, and hue angle), and skin elasticity. For berry weight, the average berry weight of each 100-berry sample was determined. For length, width, L*, chroma, hue angle, and skin elasticity, each berry in the five-berry sample was evaluated individually. The cluster attributes (cluster weight and berries/cluster) for each triplicate sample for Spray treatment at harvest were also evaluated.

Berry weight. Each 100-berry sample was weighed, in grams (g), using an Ohaus Pioneer® PA224 analytical balance (Ohaus Corporation, Parsippany, NJ), and average berry weights were calculated.

Berry length and width. Berry dimensions were measured in millimeters (mm) using VWR® Traceable® digital calipers (VWR International, Radnor, PA). Length of the berry was the

measured from the stem scar to the bottom of the berry, and width of the berry was measured across the center of the berry.

Berry skin L*, hue angle, and chroma. Berry skin color analysis was conducted using a Konica Minolta Chroma Meter CR-400 (Konica Minolta, Inc., Tokyo, Japan) to measure the color of each berry at the location opposite the stem scar for Commission Internationale de l'Eclairage (CIE) Lab transmission values of L*=100, a*=0, and b*=0, hue angle, and chroma (Commission Internationale de l'Eclairage (CIE) 1986). The CIELAB system describes color variations as perceived by the human eye. CIELAB is a uniform three-dimensional space defined by colorimetric coordinates, L*, a*, and b*. The vertical axis L* measures lightness from completely opaque (0) to completely transparent (100), while on the hue-circle, +a* red, -a* green, +b* yellow, and -b* blue are measured. Hue angle described color in angles from 0 to 360°: 0° is red, 90° is yellow, 180° is green, 270° is blue, and 360° is red. For samples with hue angles <90°, a 360° compensation (hue + 360°) was used to account for discrepancies between red samples with hue angles near 0° and 360° (McLellan et al. 2007). Chroma identified color by which a berry differed from gray of the same lightness and corresponded to saturation (intensity/purity) of the perceived color.

Berry skin elasticity. The skin elasticity of each berry was determined using a Stable Micro Systems TA.XTPlus® texture analyzer (Texture Technologies Corp., Hamilton, MA) fitted with a TA-52 2-mm probe. Berries were placed horizontally on the plate, and the probe was lowered at a rate of 2 mm/sec until it contacted the berry (trigger force 0.02 N). The skin elasticity was calculated as the distance traveled before the berry was penetrated with the probe, measured in millimeters (mm). The point of penetration occurred when a sharp drop in force was detected.

Cluster weight. The total weight of each 10-cluster sample was measured using a Mettler Toledo PE3600 Delta Range precision balance (Mettler Toledo, Columbus, OH), and average cluster weight was calculated and expressed in grams (g).

Berries per cluster. The number of total berries were counted in each 10-cluster sample, and the average number of berries/cluster was calculated.

Composition attributes analysis

Composition attributes were evaluated for each triplicate sample for Spray treatment and sampling date (Week). The composition analysis of the berries included soluble solids (SS), pH, titratable acidity (TA), individual sugars, total sugars, individual organic acids, and total organic acids. Berries were frozen (-10°C) then thawed overnight at 4°C. Each 100-berry sample was squeezed through cheese cloth to extract the juice. The juice of each triplicate sample was analyzed in duplicate for composition attributes.

Soluble solids. The SS (expressed as %) of juice from the grapes was determined using a Bausch & Lomb Abbe Mark II refractometer (Scientific Instruments, Keene, NH).

pH. The pH of juice from the grapes was measured using a Metrohm 862 Compact Titrosampler (Metrohm AG, Herisau, Switzerland) fitted with a pH meter. The probe was left in the juice for two minutes to equilibrate before recording the pH value.

Titratable acidity (TA). The TA of juice from the grapes was expressed as % w/v (g/100 mL) tartaric acid and measured using a Metrohm 862 Compact Titrosampler. Six grams of juice was added to 50 mL degassed, deionized water and titrated with 0.1 N sodium hydroxide to an endpoint of pH 8.2.

Sugars and organic acids. The sugars and organic acids in juice from the grapes were identified and quantified according to the high performance liquid chromatography (HPLC) procedure of

Walker et al. (2003). Juices were diluted with deionized water as needed to avoid overloading the detector. Diluted samples were passed through a 0.45 μm polytetrafluoroethylene (PTFE) syringe filter (Varian, Inc., Palo Alto, CA) before injection onto an HPLC system consisting of a Waters 515 HPLC pump, a Waters 717 plus autosampler, and a Waters 410 differential refractometer detector connected in series with a Waters 996 photodiode array (PDA) detector (Water Corporation, Milford, MA). Analytes were separated with a Bio-Rad HPLC Organic Acids Analysis Aminex HPX-87H ion exclusion column (300 x 7.8 mm) connected in series with a Bio-Rad HPLC column for fermentation monitoring (150 x 7.8 mm; Bio-Rad Laboratories, Hercules, CA). A Bio-Rad Micro-Guard Cation-H refill cartridge (30 x 4.5 mm) was used as a guard column. Columns were maintained at a temperature of 65 ± 0.1°C by a temperature control unit. The isocratic mobile phase consisted of pH 2.28 aqueous sulfuric acid at a flow rate of 0.45 mL/min. An injection volume of 4 μL was used and the total run time per sample was 45 minutes.

Citric, tartaric, and malic acids were detected at 210 nm by the PDA detector, and glucose and fructose were detected at 410 nm by the differential refractometer detector. Analytes in samples were identified and quantified using external calibration curves based on peak area estimation with baseline integration. Total sugars were calculated as the sum of glucose and fructose, and total organic acids was calculated as the sum of citric, tartaric, and malic acids. Results were expressed as grams (g) per liter (L) juice.

Phenolic attributes analysis

Phenolic attributes were evaluated for each triplicate sample for Spray treatment and sampling date (Week). The phenolic analysis of berries included individual and total anthocyanins and total flavonols. Five frozen berries for each triplicate sample were used for

phenolic extraction, and the extraction was repeated with an additional five berries. The extracts were analyzed in duplicate for phenolic attributes.

Phenolic extraction. Prior to phenolic analysis, phenolic compounds were extracted using two solvents: a flavonol extraction solvent (methanol/water/formic acid, 60:37:3) and a procyanidin extraction solvent (acetone/water/acetic acid, 70:29.5:0.5). The five berries in each sample were weighed into a 50-mL centrifuge tube, and the weight was recorded. Enough of the flavonol solvent was added to the tube to cover the berries. An IKA® T18 Basic Ultra-Turrax homogenizer (IKA-Works, Staufen im Breisgau, Germany) was used to homogenize the sample for approximately 30 seconds. The flavonol solvent was used to rinse the remaining sample from the homogenizer. The sample was centrifuged at 10,000 rpm for five minutes, and the supernatant was decanted into a volumetric flask fitted with a funnel and filter paper. Procyanidin solvent was added to the tube with the centrifuge pellet, and the homogenization, centrifugation, and filtration step was repeated. The extraction process was repeated, alternating between the flavonol and procyanidin solvents, until there was no longer any visible red color in the centrifuge pellet. The filtered supernatant was brought up to volume in the volumetric flask using either of the extraction solvents, and the final volume was recorded. The final volumes differed depending on the amount of color in the sample but were factored into the calculations.

For anthocyanins analysis, 10 mL of extract was dried in a 50-mL centrifuge tube using a Savant® SpeedVac® Plus SC210A High Capacity Concentrator fitted with a Savant® RVT 400 Refrigerated Vapor Trap and a Thermo Scientific® OFP400-115 Oil Free Vacuum Pump (ThermoFischer Scientific, Waltham, MA) and reconstituted with 2 mL 5% (v/v) formic acid in water. For flavonols analysis, 40 mL of extract was dried and reconstituted with 50% (v/v) methanol in water.

Anthocyanin quantification. The anthocyanin content of reconstituted extracts was analyzed using the HPLC-PDA method of Cho et al. (2004). Samples were passed through a 0.45 μm PTFE syringe filter before injection onto a Waters Alliance HPLC system equipped with a Waters model 996 PDA detector and Millennium version 3.2 software. A 4.6 x 250 mm Symmetry® C_{18} column (Waters Corporation) with a 3.9 mm x 20 mm Symmetry® C_{18} guard column was used to separate analytes. The mobile phase consisted of a binary gradient with 5% (v/v) formic acid in water (solvent A) and methanol (solvent B) at a flow rate of 1.0 mL/min. A gradient was used with 2% to 60% B from 0-60 minutes, 60% to 2% B from 60-65 minutes, then holding at 2% B from 65-80 minutes. A 50 μL injection volume was used and the total run time per sample was 80 minutes. Anthocyanins were detected at 510 nm.

Anthocyanins were quantified as the anthocyanidin-3-glucoside of their major aglycone (cyanidin, delphinidin, peonidin, petunidin, or malvidin) using external calibration curves based on peak area estimation with baseline integration. Unknown anthocyanin peaks were quantified as delphinidin-3-glucoside equivalents. Total anthocyanins were determined by summing the concentrations of individual anthocyanin compounds. Results were expressed as mg anthocyanin per 100 g berries.

Anthocyanin identification. An HPLC-electrospray ionization (ESI)-mass spectrometry (MS) system equipped with an analytical Hewlett Packard 1100 series HPLC instrument (Hewlett-Packard Enterprise Company, Palo Alto, CA), an autosampler, a binary HPLC pump, and a UV/VIS detector interfaced to a Bruker Esquire LC/MS ion trap mass spectrometer (Bruker Corporation, Billerica, MA) was used to identify anthocyanin compounds according to the method of Cho et al. (2004). Reverse-phase separation of anthocyanins was conducted using the same HPLC conditions previously described, and absorption was recorded at 510 nm. Mass

spectral analysis was operated in positive ion electrospray mode with a capillary voltage of 4000 V, a nebulizing pressure of 30.0 psi, a drying gas flow of 9.0 mL/min, and a temperature of 300°C. Data was collected with the Bruker software in full scan mode over a range of *m/z* 50-1000 at 1.0 seconds per cycle. Characteristic ions were used for peak assignment. Any peaks that could not be identified by ESI-MS but had a maximum absorbance at 510 nm were classified as "unknown anthocyanins".

Flavonol quantification. The total flavonol content of reconstituted extracts was analyzed using the HPLC-PDA method of Cho et al. (2004). Samples were passed through a 0.45 μm PTFE syringe filter before injection onto a Waters Alliance HPLC system equipped with a Waters model 996 PDA detector and Millennium version 3.2 software. A 4.6 x 250 mm Phenomenex Aqua 5μm C_{18} column (Phenomenex, Torrance) was used to separate analytes. The mobile phase consisted of a binary gradient with 2% (v/v) acetic acid in water (solvent A) and 0.5% (v/v) acetic acid in water/acetonitrile (1:1, v/v) (solvent B) at a flow rate of 1.0 mL/min. A gradient was used with 10% to 55% B from 0-50 minutes, 55% to 100% B from 50-60 minutes, then 100% to 10% B from 60-65 minutes. A 50 μL injection volume was used, and the total run time per sample was 80 minutes. Flavonols were detected at 360 nm.

Total flavonols were quantified as rutin equivalents, using external calibration curves based on peak area estimation with baseline integration. Results were expressed as mg flavonols per 100 g berries.

Flavonol identification. An HPLC-ESI-MS system equipped with an analytical Hewlett Packard 1100 series HPLC instrument (Hewlett-Packard Enterprise Company, Palo Alto, CA), an autosampler, a binary HPLC pump, and a UV/VIS detector interfaced to a Bruker Esquire LC/MS ion trap mass spectrometer (Bruker Corporation, Billerica, MA) was used to identify

flavonol compounds according to the method of Cho et al. (2004). Reverse-phase separation of analytes was conducted using the same HPLC conditions previously described, and absorption was recorded at 360 nm. Mass spectral analysis was operated in negative ion electrospray mode with a capillary voltage of 4000 V, a nebulizing pressure of 30.0 psi, a drying gas flow of 9.0 mL/min, and a temperature of 300°C. Data was collected with the Bruker software in full scan mode over a range of *m/z* 50-1000 at 1.0 seconds per cycle. Characteristic ions were used for peak assignment. Any peaks that could not be identified by ESI-MS but had a maximum absorbance at 360 nm were classified "unknown flavonols".

Design and statistical analysis

Each triplicate 200-berry sample was taken across all four rows of each Spray treatment, from different vines, locations within the vine, and locations in the cluster. Berries were sampled once per week from veraison (week 0) to harvest in 2018 and 2019. In 2018, there were seven sampling dates and a total of 42 samples (2 Spray treatments x 7 Weeks of sampling x 3 replications). In 2019, there were six sampling dates and a total of 36 samples (2 Spray treatments x 6 Weeks of sampling x 3 replications). For cluster attributes, clusters were sampled from different vines and different locations within the vines for each Spray treatment at harvest. In each year, there were six samples for cluster attributes (2 Spray treatments x 3 replications). Triplicate samples were treated as individual experimental units in a full factorial design. Statistical analyses were conducted using JMP® Pro statistical software (version 15.0.0, SAS Institute, Cary, NC).

For the 2018 and 2019 berry samples, a univariate analysis of variance (ANOVA) was used to determine the significance of the main factors (Spray and Week) and their interaction. Tukey's Honest Significant Difference (HSD) test and student's t-test were used to detect

differences among means ($p<0.05$). For the 2018 and 2019 cluster samples, a univariate ANOVA was used to determine the significance of the Spray treatment at harvest, and Student's t-test was used to detect significant differences among means ($p<0.05$). All factors were treated as categorical. Figures were created in JMP®, and error bars represented one standard error from the mean.

Results and Discussion

The 2018 and 2019 wine grape production seasons in the Hindsville, AR area were relatively mild in terms of temperature and rainfall (Figure 2). The high and low temperatures were similar from January to August in both years. There was higher rainfall in 2019 than 2018 from April (bud emergence on grapevines) to August (harvest). In August of 2018 and 2019, the average daily high temperature was 35.8°C and 37.2°C, respectively. In August of 2019, there was over twice as much cumulative rainfall (153.7 mm) than in August of 2018 (62.7 mm).

The composition of Chambourcin grapes varied slightly in 2018 and 2019 (Table 1). In 2018, berries from control vines had 21.6% SS, 3.6 pH, and 0.6% TA, and berries from sprayed vines had 20.6% SS, 3.5 pH, and 0.6% TA. Grapes had slightly lower SS (18.8-19.2%) and TA (0.5-0.6%) and higher pH (3.7-3.8) in 2019. The 2018 grapes had more ideal composition attributes for wine production than the 2019 grapes. Homich et al. (2016) reported 21% SS, 3.4 pH, and 0.9% TA at harvest for Chambourcin grapes grown in Pennsylvania, and Zhang and Dami (2012) reported 22.2% SS, 3.3 pH, and 1.1% TA for Chambourcin grapes grown in Ohio. While the SS levels reported in this study were similar to those in the literature, pH was higher and TA was lower for Chambourcin grapes grown in Arkansas. This is likely because higher temperatures during the growing season, like those experienced in Arkansas, tend to yield grapes

with lower acid levels than those grown in cooler climates such as Ohio and Pennsylvania (Mira de Orduña 2010).

Berries from the sprayed and control rows were sampled weekly from veraison to harvest. Cluster samples were taken from both treatments at harvest. Samples were analyzed for physical, composition, and phenolic attributes.

During ripening, Chambourcin berries increased in size, skin red color, SS, pH, sugars, and anthocyanins and decreased in TA and organic acids. These observations were typical for maturing wine grapes. In both 2018 and 2019, berries from sprayed vines had higher skin elasticity, possibly indicating thicker, more flexible skins and thus greater potential phenolic extractability during winemaking and increased protection against fungal pathogens and physical damage. Berries from sprayed vines had a lower pH than berries from control vines at harvest. Chambourcin grapes from sprayed vines had higher levels of anthocyanins than grapes from control vines in both years. The cluster attributes at harvest were not impacted by the treatment in both years. The cluster weights in 2018 and 2019 were similar (233.0 g and 231.7 g, respectively), and berries/cluster were higher in 2018 than 2019 (101 and 87 berries/cluster, respectively). Cluster weights in both years were similar to those found by Dami et al. (2006), who reported an average cluster weight of 225 g for Chambourcin grapevines during a five-year study conducted in Ohio. The Chambourcin grapes attributes were evaluated during ripening and at harvest in 2018 and 2019.

Analysis of physical attributes

Chambourcin berries from 2018 and 2019 were analyzed during ripening and at harvest for berry weight, berry length, berry width, L*, hue angle, chroma, and skin elasticity. Chambourcin cluster samples from 2018 and 2019 were analyzed at harvest for cluster weight

and berries/cluster. The average minimum and maximum values for physical attributes were determined at harvest in both years (data not shown). At harvest in 2018, berries had 2.3-2.4-g berry weight, 15.2-15.5-mm length, 15.7-16.2-mm width, 24.7-25.2 L*, 344-359° hue angle, 0.9 chroma, and 6.4-mm skin elasticity and clusters had 233-g cluster weight and 100-101 berries/cluster. At harvest in 2019, berries had 2.7-g berry weight, 16.6-17.0-mm length, 17.1-17.4-mm width, 25.6-26.0 L*, 341-346° hue angle, 0.5-0.6 chroma, and 2.1-2.5-mm skin elasticity and clusters had 211-252-g cluster weight and 81-93 berries/cluster.

In a general comparison of physical attributes from 2018 and 2019 harvest samples, the 2018 Chambourcin berries were slightly smaller than 2019 berries in terms of berry weight and dimensions. Berry weights in both years were similar to those found by Sommer and Cohen (2018), Zhang and Dami (2012), and Zhu et al. (2012), who reported berry weights of about 2.4 g for Chambourcin grapes at harvest. In addition, 2018 berries had lower L* and higher hue angle and chroma values at harvest, indicating that 2018 berries had a darker, more saturated red color than 2019 berries. The 2018 berries had almost three times the skin elasticity of 2019 berries, which could mean that 2018 berries had thicker, more flexible skins and thus greater potential phenolic extractability during winemaking and increased protection against fungal pathogens and physical damage. The Spray x Week interaction was not significant in either year for any attributes, except hue angle in 2018 (Table 2). In both years, the Week main effect was significant for berry weight, berry length, berry width, L*, chroma, and skin elasticity, and the Spray main effect was significant for skin elasticity. There was no effect of Spray treatment on cluster attributes.

2018 Berries. The Spray main effect was significant for Chambourcin berry weight, and berries from control vines (2.12 g) had a higher berry weight than berries from sprayed vines (2.05 g).

There was no effect of Spray on berry length or width. The Week main effect was significant for berry weight, length, and width. Berries increased in weight, length, and width from week 0 (1.53 g, 13.32 mm, and 14.09 mm, respecitively) to harvest (2.38 g, 15.34 mm, 15.94 mm, respectively). This observation was expected for ripening grapes.

There was no effect of Spray on L* or chroma. The Week main effect was significant for L* and chroma. The color of berries became significantly darker from week 0 (L* 39.82) to harvest (L* 24.94). Chroma decreased from week 0 (17.68) to harvest (0.91). In general, Chambourcin berries were a solid green color at week 0, with a small amount of red color. As berries ripened and developed red color, berry skins became more varied in color, and therefore less saturated for any one color. The Spray x Week interaction was significant for hue angle. Regardless of Spray treatment, the hue angle increased from week 0 to week 1 but remained steady from week 1 to harvest (Figure 3). There were no differences between Spray treatments for hue angle at any of the sampling dates.

The Spray and Week main effects were significant for skin elasticity, and skin elasticity fluctuated week-to-week. Berries from sprayed vines (7.07 mm) had greater skin elasticities than berries from control vines (6.38 mm). Various studies have found correlations between skin elasticity and anthocyanin extractability during maceration (fermentation of red wine on the skins, seeds, and pulp). Zouid et al. (2010) determined that berries with higher skin elasticity released more anthocyanins during extraction in a model hydroalcoholic solution, and Rolle et al. (2012b, 2008, 2009) concluded that tougher berry skins led to an increase in anthocyanin extraction during winemaking. In order to produce high-quality red wines, winemakers must assess both the phenolic compound concentration and the extractability of these compounds

(Sacchi et al. 2005). Therefore, application of an inactivated yeast spray to grapevines could lead to berries with greater anthocyanin extractability during winemaking.

It was also proposed that higher skin elasticity could be correlated with thicker, more flexible skins. Giacosa et al. (2019) found that application of LalVigne® increased the skin thickness of Chardonnay, Cortese, and Nebbiolo (*V. vinifera*) grapes at harvest in Italy, and Šuklje et al. (2016) observed increased skin thickness in LalVigne®-treated Syrah grapes in Hungary. Thicker, more flexible skins were correlated with increased resistance against fungal pathogens (Rosenquist and Morrison 1988), physical damage during harvest and transport (Kök and Çelik 2004), and berry splitting from fluctuations in berry water content (Lang and During 1990). Therefore, application of an inactivated yeast spray to grapevines could provide increased protection against fungal pathogens that impact berry quality.

2019 Berries. Similar to 2018, the Week main effect was significant for Chambourcin berry weight, length, and width in 2019. Berries increased in weight, length, and width from week 0 (1.93 g, 14.26 mm, and 14.84 mm, respecitively) to harvest (2.71 g, 16.79 mm, 17.28 mm, respectively). The Spray main effect was significant for berry width, and berries from sprayed vines (16.29 mm) had greater berry width than berries from control vines (15.95 mm). There was no effect of Spray on berry weight or length.

The Week main effect was significant for L*, hue angle, and chroma of berry skins. During ripening, Chambourcin berries developed a darker, redder color, from L* and hue angle of 38.70 and 201.43°, respectively, in week 0 to 25.77 and 343.40°, respectively, at harvest. There was no effect of Spray on L*, hue angle, or chroma of the berry skins.

The Spray and Week main effects were significant for skin elasticity in 2019. Unlike 2018, there was a consistent decrease in skin elasticity observed week-to-week in 2019, from

6.87 mm in week 0 to 2.31 mm at harvest. The difference in skin elasticity behavior during ripening in 2018 and 2019 was likely because berry skin texture is highly influenced by climate, and in particular water availability and rainfall (Zsófi et al. 2014). There was 60% more rainfall in 2019 as compared to 2018 (Figure 2). The berries from sprayed vines (4.65 mm) had a greater skin elasticity across all weeks relative to berries from control vines (3.99 mm), similar to 2018.

Analysis of composition attributes

Chambourcin berries from 2018 and 2019 were analyzed during ripening for SS, pH, TA, sugars, and organic acids. The average minimum and maximum values for composition attributes were determined at harvest in both years (data not shown). At harvest in 2018, berries had 20.6-21.6% SS, 3.5-3.6 pH, 0.6% TA, 104-110 g/L glucose, 109-117 g/L fructose, 214-227 g/L total sugars, 0.5 g/L citric acid, 2.0-2.3 g/L tartaric acid, 3.6-4.5 g/L malic acid, and 6.4-6.9 g/L total organic acids. At harvest in 2019, berries had 18.8-19.2% SS, 3.7-3.8 pH, 0.5-0.6% TA, 112-113 g/L glucose, 134-135 g/L fructose, 246-248 g/L total sugars, 0.8 g/L citric acid, 4.3 g/L tartaric acid, 4.1-4.3 g/L malic acid, and 9.2-9.3 g/L total organic acids.

In a general comparison of 2018 and 2019 harvest values, 2018 Chambourcin berries had higher SS and lower pH values. TA was similar in both years. However, 2019 berries had higher total sugars and total organic acids. Individual sugars and organic acids were determined but followed similar trends as total sugars and organic acids. Therefore, only total sugars and organic acids were discussed in this study. In both years, there was a significant Spray x Week interaction for pH (Table 3).

2018 Berries. The Spray x Week interaction was significant for SS, pH, and TA. In general, SS and pH increased and TA decreased during ripening, which was expected for maturing grapes (Figure 4). There were no differences in SS between Spray treatments at each sampling date

during ripening or at harvest. Berries from sprayed vines had a lower pH than berries from control vines at week 1 (2.84 and 2.92, respectively), week 3 (3.12 and 3.18, respectively), and week 4 (3.25 and 3.35, respectively) and at harvest (3.52 and 3.64, respectively). These results were similar to those of Giacosa et al. (2019), who found that LalVigne® application decreased the pH of Cortese wine grapes at harvest. This indicated that inactivated yeast application lead to Chambourcin berries with a lower pH more desirable for winemaking. Grape must (skins, seeds, juice, and pulp of crushed grapes) should have a pH of 3.2-3.5 prior to fermentation. If the pH is too high, winemakers can add tartaric acid or other acids or juice/wine with a higher acid content to decrease the pH ("27 CFR § 24.182 - Use of acid to correct natural deficiencies"). Therefore, Chambourcin grapes from sprayed vines would be more appealing to winemakers based on their pH, especially since wine grapes grown in warmer climates, such as Arkansas and the mid-South United States, tend to have low acid levels (Mira de Orduña 2010). Berries from sprayed vines had a lower TA than berries from control vines at week 0 (2.25% and 2.59%, respectively) and week 2 (1.19% and 1.29%), but a higher TA at week 1 (1.56% and 1.38%, respectively). However, differences in TA among Spray treatments were not seen in subsequent weeks or at harvest.

The Spray x Week interaction was significant for total sugars and total organic acids of Chambourcin grapes. Total sugar levels increased from week 0 to week 1, week 2 to week 3, and week 3 to week 4 (Figure 5). In general, sugars remained constant from week 4 to harvest. There was no effect of Spray treatment on total sugar levels during ripening or at harvest. At harvest, the total sugars of the berries from the sprayed vines was 213.45 g/L, and the total sugars of the berries from the control vines was 226.79 g/L. Total organic acids decreased during ripening but remained constant from week 5 to harvest. Berries from the control vines (23.54 g/L) had higher

acids than berries from the sprayed vines (21.41 g/L) in week 0, but berries from the sprayed vines (14.62 g/L) had higher acid levels than berries from the control vines (11.80 g/L) in week 1. There were no differences among Spray treatments from week 2 to harvest. At harvest, the total organic acids of the berries from the sprayed vines was 6.93 g/L, and the total organic acids of the berries from the control vines was 6.43 g/L.

2019 Berries. The Week main effect was significant for SS and TA, and the Spray x Week interaction was significant for pH. SS increased from week 0 (10.68%) to harvest (18.98%), and TA decreased from week 0 (2.26%) to harvest (0.54%). There was no effect of Spray on SS or TA. In general, the pH increased during ripening (Figure 6). Similar to 2018, berries from sprayed vines had a lower pH than berries from control vines at week 1 (3.09 and 3.14, respectively), week 4 (3.63 and 3.68, respectively), and harvest (3.71 and 3.80, respectively). The pH values were less desirable for winemaking in 2019 than in 2018.

Only the Week main effect was significant for total sugars and total organic acids of 2019 Chambourcin grapes. Sugars increased from week 0 (117.99 g/L) to harvest (247.14 g/L) and acids decreased from week 0 (28.28 g/L) to harvest (9.28 g/L). There was no effect of Spray treatment to grapevines on total sugars or total organic acids of the berries.

Analysis of phenolic attributes

Individual and total anthocyanin compounds and total flavonols were identified and quantified in Chambourcin grapes during ripening and at harvest. Anthocyanins identified in grape extracts included malvidin-3-glucoside, delphinidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3,5-diglucoside, delphinidin-3,5-diglucoside, petunidin-3,5-diglucoside, cyanidin-3,5-diglucoside, cyanidin-3-glucoside-pyruvate, and peonidin-3-galactoside pyruvate (Figure 7). Malvidin-, delphinidin-, and petunidin-3-glucoside and

malvidin-3,5-diglucoside made up 70% and 80% in 2018 and 2019, respectively, of the total grape anthocyanin content at harvest, and thus only these four individual compounds and total anthocyanins were discussed in this study. Delphinidin-3-glucoside was the most prevalent anthocyanin in both 2018 and 2019 Chambourcin grapes at harvest. Malvidin-3-glucoside was the second-most prevalent in 2018, but malvidin-3,5-diglucoside was the second-most prevalent in 2019. The mixture of monoglucoside and diglucoside anthocyanins found in Chambourcin is typical of hybrid wine grapes. Zhu et al. (2012) determined the anthocyanin profile of Chambourcin wines and found malvidin and petunidin monoglucosides and diglucosides, with malvidin-3,5-diglucoside present in the greatest amount. These results do not coincide with the wider range of anthocyanins identified in Chambourcin grapes in the present study. However, complex wine chemistry, including the formation of polymeric pigments and anthocyanin-phenolic complexes, could explain this discrepancy. Most studies on Chambourcin grape/wine anthocyanins have used the pH-differential method (Giusti and Wrolstad 2001) to determine total monomeric anthocyanins, rather than classifying and quantifying individual anthocyanins.

The average minimum and maximum values for phenolic attributes were determined at harvest in both years (data not shown). At harvest in 2018, berries had 44-54 mg/100g malvidin-3-glucoside, 56-61 mg/100g delphinidin-3-glucoside, 39-45 mg/100g petunidin-3-glucoside, 24 mg/100g malvidin-3,5-diglucoside, 239-264 mg/100g total anthocyanins, and 13-18 mg/100g total flavonols. At harvest in 2019, berries had 98-149 mg/100g malvidin-3-glucoside, 136-169 mg/100g delphinidin-3-glucoside, 86-121 mg/100g petunidin-3-glucoside, 120-134 mg/100g malvidin-3,5-diglucoside, 556-713 mg/100g total anthocyanins, and 22-28 mg/100g total flavonols.

In a general comparison of the phenolics in 2018 and 2019 Chambourcin grapes, 2019 grapes had over twice the amount of each individual anthocyanin and total anthocyanins relative to 2018 grapes. Environmental factors during the growing season could explain this difference between the two years. For example, Kliewer (1977) and Spayd et al. (2002) showed that high temperatures decreased grapevine anthocyanin production. However, the average daily temperature for August 2018 and 2019 (Figure 2) was relatively the same, so other environmental stressors, such as pests or rain, could explain the higher anthocyanin levels in 2019. Sommer and Cohen (2018) reported 137 mg/100g total extractable anthocyanins in Chambourcin grapes at harvest in North Carolina. The concentrations determined in the present study were much higher than these reported values in both 2018 and 2019. The 2019 Chambourcin grapes had higher total flavonols at harvest than the 2018 grapes. Flavonols can increase in response to sunlight exposure/intensity (Price et al. 1996, Spayd et al. 2002), so this could explain the slight difference between the two years.

2018 Berries. The Week main effect was significant for malvidin-3-glucoside, delphinidin-3-glucoside, petunidin-3-glucoside, malvidin-3,5-diglucoside, and total anthocyanins (Table 4). The levels of all anthocyanin compounds increased from week 0 to harvest: malvidin-3-glucoside increased 1.51 to 49.31 mg/100g, delphinidin-3-glucoside increased 1.36 to 58.59 mg/100g, petunidin-3-glucoside increased 0.96 to 41.55 mg/100g, malvidin-3,5-diglucoside increased 0.07 to 23.63 mg/100g, and total anthocyanins increased 5.19 to 251.39 mg/100g. There was no effect of Spray on delphinidin-3-glucoside, malvidin-3,5-diglucoside, or total anthocyanins. The Spray main effect was significant for malvidin-3-glucoside and petunidin-3-glucoside. Berries from sprayed vines had higher malvidin-3-glucoside and petunidin-3-glucoside (26.38 and 23.29 mg/100g, respectively) than berries from control vines (19.94 and

18.01 mg/100g, respectively). Giacosa et al. (2019) also found that LalVigne® application increased the malvidin-3-glucoside content of Nebbiolo wine grapes. This was notable, as malvidin-3-glucoside and petunidin-3-glucoside are characteristic anthocyanins of *V. vinifera* grapes and are known to display good color stability in red wines (Waterhouse et al. 2016). Therefore, inactivated yeast application could give Chambourcin grapes a better anthocyanin content for winemaking.

The Spray x Week interaction was significant for total flavonols. The flavonol concentration fluctuated during ripening (Figure 8a). Berries from control vines had higher total flavonols than berries from sprayed vines at week 0 (18.75 and 11.46 mg/100g, respectively), but there were no differences among Spray treatments at later sampling dates.

2019 Berries. The Spray x Week interaction was significant for all anthocyanin attributes, and concentrations of all anthocyanins increased during ripening (Figure 9). At harvest, berries from sprayed vines had higher concentrations of malvidin-3-glucoside (149.23 mg/100g), delphinidin-3-glucoside (169.49 mg/100g), petunidin-3-glucoside (121.29 mg/100g), and total anthocyanins (712.98 mg/100g) than berries from control vines (98.10, 135.52, 85.55, and 555.59 mg/100g, respectively). Although there was no difference at harvest, berries from sprayed vines had higher levels of malvidin-3,5-diglucoside than berries from control vines at week 3 (75.25 and 56.74 mg/100g, respectively) and week 4 (107.73 mg/100g and 88.83 mg/100g, respectively). These higher individual and total anthocyanin levels in berries from sprayed vines were consistent with the 2018 results.

The increase in anthocyanin compounds during ripening of Chambourcin grapes from grapevines treated with inactivated yeast relative to unsprayed vines could be related to the stress responses of the grapevine. Although LalVigne® is an inactivated yeast and poses no actual

threat to the health of the grapevine, it contains yeast cellular material. It is possible that the grapevine is detecting this cellular material and perceiving it as a biotic stressor. It is known that biotic stressors induce an accumulation of anthocyanins and stilbenes, such as resveratrol and viniferin, in grapevines and other plants (Timperio et al. 2012). More specifically, biotic stressors can upregulate the *CHS* and *UFGT* genes, which have been shown to be related to a significant increase in anthocyanin production (Belhadj et al. 2008, Petrussa et al. 2013).

The Spray x Week interaction was significant for total flavonols. Similar to 2018, flavonol levels fluctuated during ripening (Figure 8b). There was no difference in total flavonol concentration Spray treatments during ripening, but berries from the control vines (28.12 mg/100g) had higher total flavonols than berries from the sprayed vines (21.91 mg/100g) at harvest.

Conclusions

In both 2018 and 2019, Chambourcin grapes had acceptable compositions for winemaking, but berries from sprayed vines had lower pH values than berries from control vines at harvest. This was especially significant for wine grapes in Arkansas and the mid-South region, as high temperatures during ripening tend to yield grapes with lower acid levels that require more acid additions prior to fermentation.

In both years, berries from sprayed vines had higher skin elasticity than berries from control vines across all sampling dates. This indicated that sprayed berries had thicker, more flexible skins that could lead to increased protection against fungal pathogens and physical damage and greater phenolic extractability during winemaking. Malvidin, delphinidin, and petunidin monoglucosides and malvidin diglucoside made up the majority of anthocyanin

compounds in Chambourcin grapes, with delphinidin-3-glucoside as the predominant anthocyanin in both years. Berries from sprayed vines had greater concentrations of malvidin-3-glucoside and petunidin-3-glucoside than berries from control vines across all sampling dates in 2018, and greater concentration of malvidin-3-glucoside, delphinidin-3-glucoside, petunidin-3-glucoside, and total anthocyanins at harvest in 2019. Therefore, application of an inactivated yeast lead to higher levels of red-colored anthocyanin compounds in Chambourcin grapes that could be extracted at a higher rate during winemaking.

In general, specific inactivated yeast application appeared to improve the quality of Chambourcin grapes for subsequent winemaking. Because the ultimate purpose of wine grapes is wine production, the impact of LalVigne® Mature foliar application should be assessed on Chambourcin wine composition, anthocyanin, aroma, and sensory attributes.

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Tables

Table 1. Composition at harvest of Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with an inactivated yeast[a] (2018 and 2019).

Harvest date	Spray treatment	Soluble solids (%)	pH	Titratable acidity (%)
27 August 2018	Control	21.6	3.64	0.58
	Sprayed	20.6	3.52	0.57
29 August 2019	Control	18.8	3.80	0.53
	Sprayed	19.2	3.71	0.56

[a] LalVigne® MATURE (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

Table 2. Main and interaction effects from ANOVA for Spray and Week on berry size, berry skin color, and skin elasticity attributes during ripening and at harvest of Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with an inactivated yeast[a] (2018 and 2019).

Effects	Berry weight (g)	Berry length (mm)	Berry width (mm)	L*	Hue angle (°)[b]	Chroma	Skin elasticity (mm)
2018							
Spray							
Control	2.12 a[c]	14.49 a	15.10 a	29.04 a	322.25 a	4.96 a	6.38 b
Sprayed	2.05 b	14.63 a	15.08 a	28.93 a	319.62 a	5.26 a	7.07 a
P value	***0.0001***	*0.7608*	*0.8997*	*0.8502*	*0.8016*	*0.6096*	***0.0085***
Week							
0	1.53 e	13.32 d	14.09 c	39.82 a	190.82 b	17.68 a	6.16 b
1	1.78 d	13.84 cd	14.34 bc	32.13 b	338.57 a	7.18 b	7.93 a
2	1.95 c	14.39 c	14.68 bc	29.49 b	335.21 a	5.85 b	8.29 a
3	2.18 b	14.59 bc	14.92 b	25.28 c	344.36 a	2.07 c	8.23 a
4	2.36 a	15.39 a	15.84 a	25.89 c	343.19 a	1.19 c	5.43 bc
5	2.41 a	15.39 a	15.81 a	25.35 c	342.96 a	0.90 c	4.65 c
Harvest	2.38 a	15.34 ab	15.94 a	24.94 c	351.44 a	0.91 c	6.39 b
P value	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
Spray x Week (P value)	*0.0638*	*0.6640*	*0.6344*	*0.0520*	***0.0230***	*0.0983*	*0.6967*
2019							
Spray							
Control	2.43 a	15.55 a	15.95 b	29.38 a	303.67 a	5.00 a	3.99 b
Sprayed	2.42 a	15.80 a	16.29 a	28.58 a	310.88 a	4.48 a	4.65 a
P value	*0.6299*	*0.1117*	***0.0389***	*0.2456*	*0.5262*	*0.4901*	***0.0265***
Week							
0	1.93 d	14.26 c	14.84 d	38.70 a	201.43 b	16.18 a	6.87 a
1	2.13 c	14.95 c	15.43 c	30.95 b	307.87 a	8.01 b	5.89 ab
2	2.50 b	15.85 b	16.19 b	26.34 c	335.95 a	1.43 c	5.05 b
3	2.65 a	15.82 b	16.25 b	26.23 c	325.84 a	1.34 c	2.78 c
4	2.65 a	16.38 ab	16.73 ab	25.89 c	329.17 a	0.91 c	3.04 c
Harvest	2.71 a	16.79 a	17.28 a	25.77 c	343.40 a	0.56 c	2.31 c
P value	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
Spray x Week (P value)	*0.4248*	*0.9852*	*0.7331*	*0.9304*	*0.9222*	*0.9935*	*0.6376*

[a] LalVigne® MATURE (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

[b] Hue angles <90° were subjected to a 360° compensation to account for discrepancies between red samples near 0° and those near 360°.

[c] Means with different letters for each attribute within effects and years are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

Table 3. Main and interaction effects from ANOVA for Spray and Week on composition attributes during ripening and at harvest of Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with an inactivated yeast[a] (2018 and 2019).

Effects	Soluble solids (%)	pH	Titratable acidity (%)	Total sugars (g/L)	Total organic acids (g/L)
2018					
Spray					
Control	17.20 a[b]	3.17 a	1.19 a	169.08 a	11.17 a
Sprayed	17.26 a	3.11 b	1.14 b	172.05 a	11.30 a
P value	*0.7536*	***<0.0001***	***<0.0001***	*0.2145*	*0.1758*
Week					
0	9.52 e	2.63 g	2.42 a	70.34 e	22.47 a
1	12.70 d	2.88 f	1.47 b	113.51 d	13.21 b
2	14.25 c	2.96 e	1.24 c	134.51 c	11.45 c
3	19.46 b	3.15 d	0.99 d	198.36 b	9.36 d
4	21.95 a	3.30 c	0.81 e	229.35 a	8.34 e
5	21.67 a	3.47 b	0.66 f	227.75 a	7.12 f
Harvest	21.08 a	3.58 b	0.57 g	220.12 a	6.68 f
P value	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
Spray x Week (P value)	***0.0234***	***<0.0001***	***<0.0001***	***0.0012***	***<0.0001***
2019					
Spray					
Control	16.20 a	3.40 a	1.19 a	211.05 a	16.70 a
Sprayed	16.58 a	3.37 b	1.19 a	217.24 a	16.44 a
P value	*0.1930*	***<0.0001***	*0.7835*	*0.1772*	*0.2459*
Week					
0	10.68 d	3.03 f	2.26 a	117.99 d	28.28 a
1	14.13 c	3.11 e	1.69 b	173.96 c	22.20 b
2	17.36 b	3.29 d	1.18 c	238.11 b	16.41 c
3	18.33 ab	3.48 c	0.84 d	262.59 a	13.53 d
4	18.85 a	3.66 b	0.63 e	245.08 ab	9.72 e
Harvest	18.98 a	3.76 a	0.54 e	247.14 ab	9.28 e
P value	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
Spray x Week (P value)	*0.9150*	***<0.0001***	*0.5849*	*0.6136*	*0.1896*

[a] LalVigne® MATURE (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

[b] Means with different letters for each attribute within effects and years are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

Table 4. Main and interaction effects from ANOVA for Spray and Week on phenolic attributes during ripening and at harvest of Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with an inactivated yeast[a] (2018 and 2019).

Effects	Malvidin-3-glucoside (mg/100 g)	Delphinidin-3-glucoside (mg/100 g)	Petunidin-3-glucoside (mg/100 g)	Malvidin-3,5-diglucoside (mg/100 g)	Total anthocyanins (mg/100 g)	Total flavonols (mg/100g)
2018						
Spray						
Control	19.94 b[b]	26.61 a	18.01 b	8.82 a	109.87 a	13.97 a
Sprayed	26.38 a	33.21 a	23.29 a	12.13 a	137.79 a	12.11 b
P value	***0.0319***	*0.0796*	***0.0491***	*0.1384*	*0.0508*	***0.0144***
Week						
0	1.51 d	1.36 d	0.96 e	0.07 c	5.19 d	15.10 a
1	3.77 d	5.88 d	3.72 e	1.67 bc	23.85 d	9.55 c
2	7.39 cd	11.93 cd	7.51 de	2.10 bc	46.14 cd	8.55 c
3	21.32 bc	28.41 bc	19.53 cd	7.66 bc	113.37 bc	10.33 bc
4	31.00 b	41.56 ab	28.15 bc	12.91 ab	169.17 b	14.43 ab
5	48.18 a	61.65 a	43.12 a	25.26 a	257.76 a	17.53 a
Harvest	49.31 a	58.59 a	41.55 ab	23.63 a	251.39 a	15.78 a
P value	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
Spray x Week (P value)	*0.3898*	*0.4547*	*0.4612*	*0.0727*	*0.4203*	***0.0027***
2019						
Spray						
Control	57.66 b	74.12 b	50.41 b	51.58 b	295.46 b	18.44 a
Sprayed	70.01 a	84.73 a	59.68 a	59.98 a	346.53 a	15.90 b
P value	***0.0005***	***0.0046***	***0.0011***	***0.0002***	***0.0004***	***0.0006***
Week						
0	2.08 e	4.63 e	2.63 f	1.34 e	18.35 f	16.37 c
1	17.51 e	29.29 d	18.86 e	8.49 e	100.12 e	13.42 cd
2	55.21 d	74.80 c	52.22 d	33.38 d	277.42 d	12.67 d
3	82.65 c	99.79 b	68.99 c	66.00 c	395.29 c	15.00 cd
4	101.90 b	115.53 b	84.15 b	98.28 b	500.47 b	20.55 b
Harvest	123.67 a	152.51 a	103.42 a	127.22 a	634.28 a	25.02 a
P value	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
Spray x Week (P value)	***<0.0001***	***0.0017***	***<0.0001***	***0.0062***	***0.0003***	***0.0006***

[a] LalVigne® MATURE (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

[b] Means with different letters for each attribute within effects and years are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

Figures

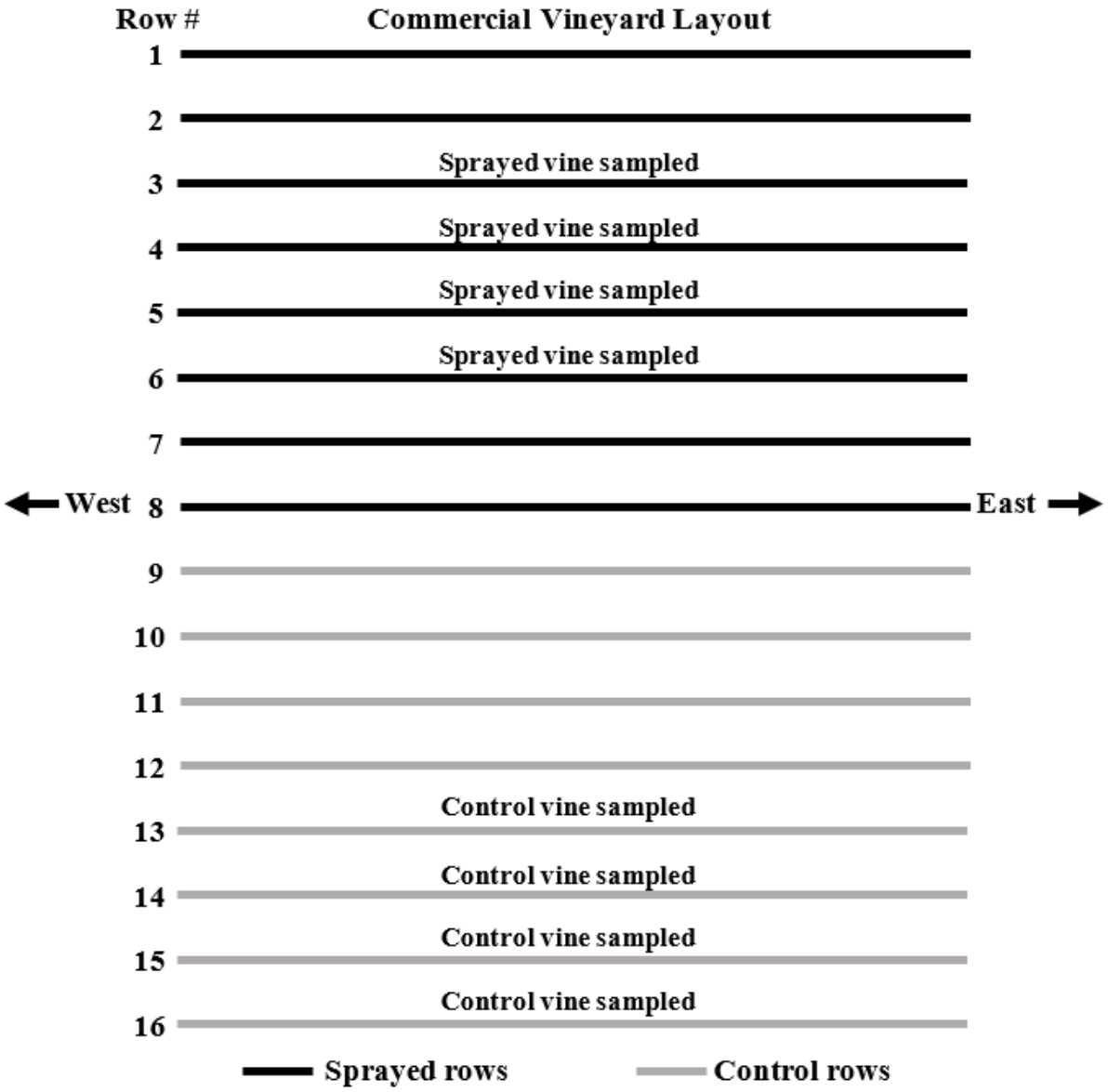

Figure 1. Vineyard layout of Chambourcin grapevine rows[a] sampled from a commercial vineyard in Arkansas unsprayed (control) and sprayed with an inactivated yeast[b] (2018 and 2019).

[a] Rows were approximately 200-m long with east-west orientation.

[b] LalVigne® MATURE (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

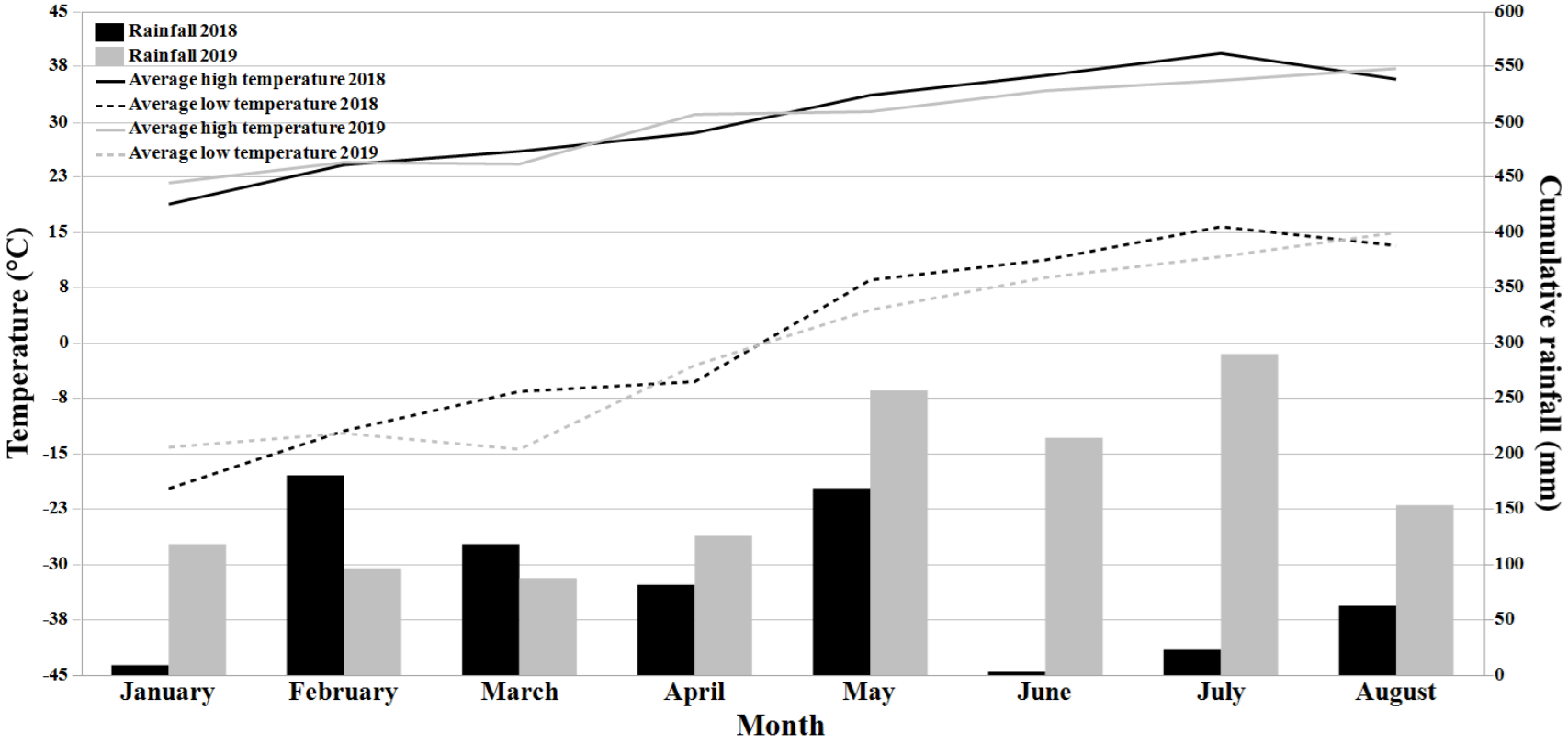

Figure 2. Average monthly high and low temperatures and cumulative rainfall[a] from January-August 2018 and 2019 near Hindsville, AR.

[a]Data was gathered from a personal weather station in Huntsville, AR (https://www.wunderground.com/dashboard/pws/K).

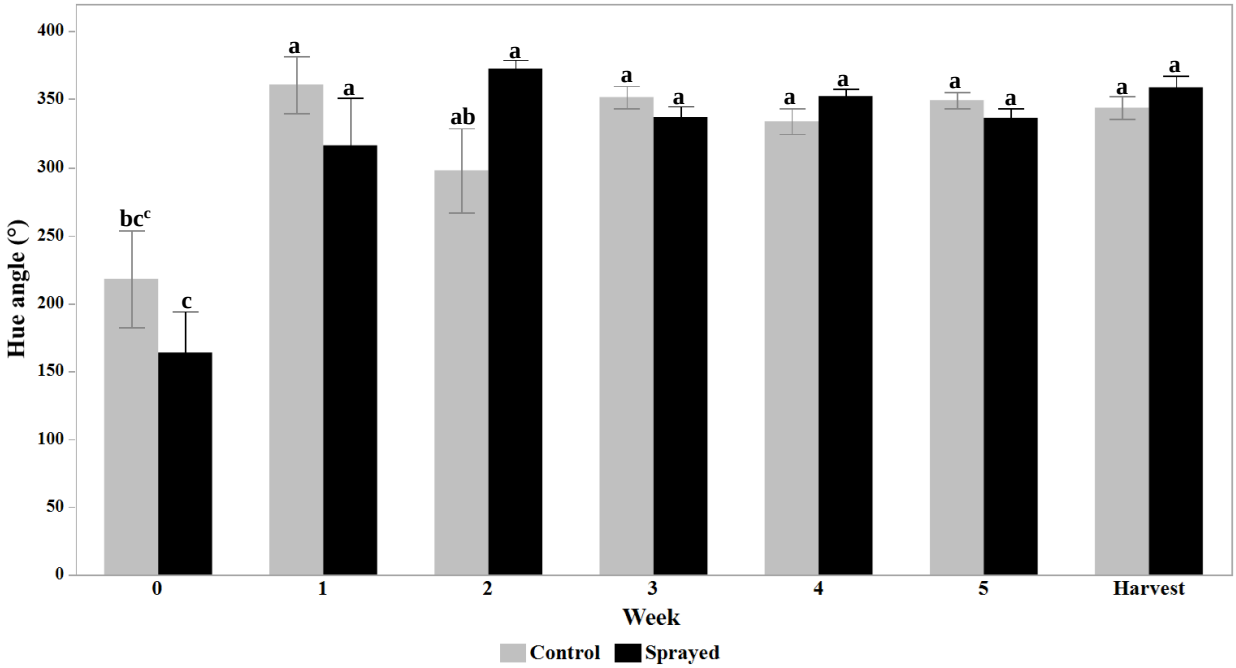

Figure 3. Effect of Spray and Week on hue angle (°)[a] during ripening and at harvest of Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with an inactivated yeast[b] (2018).

[a] Hue angles <90° were subjected to a 360° compensation to account for discrepancies between red samples near 0° and those near 360°.

[b] LalVigne® MATURE (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

[c] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

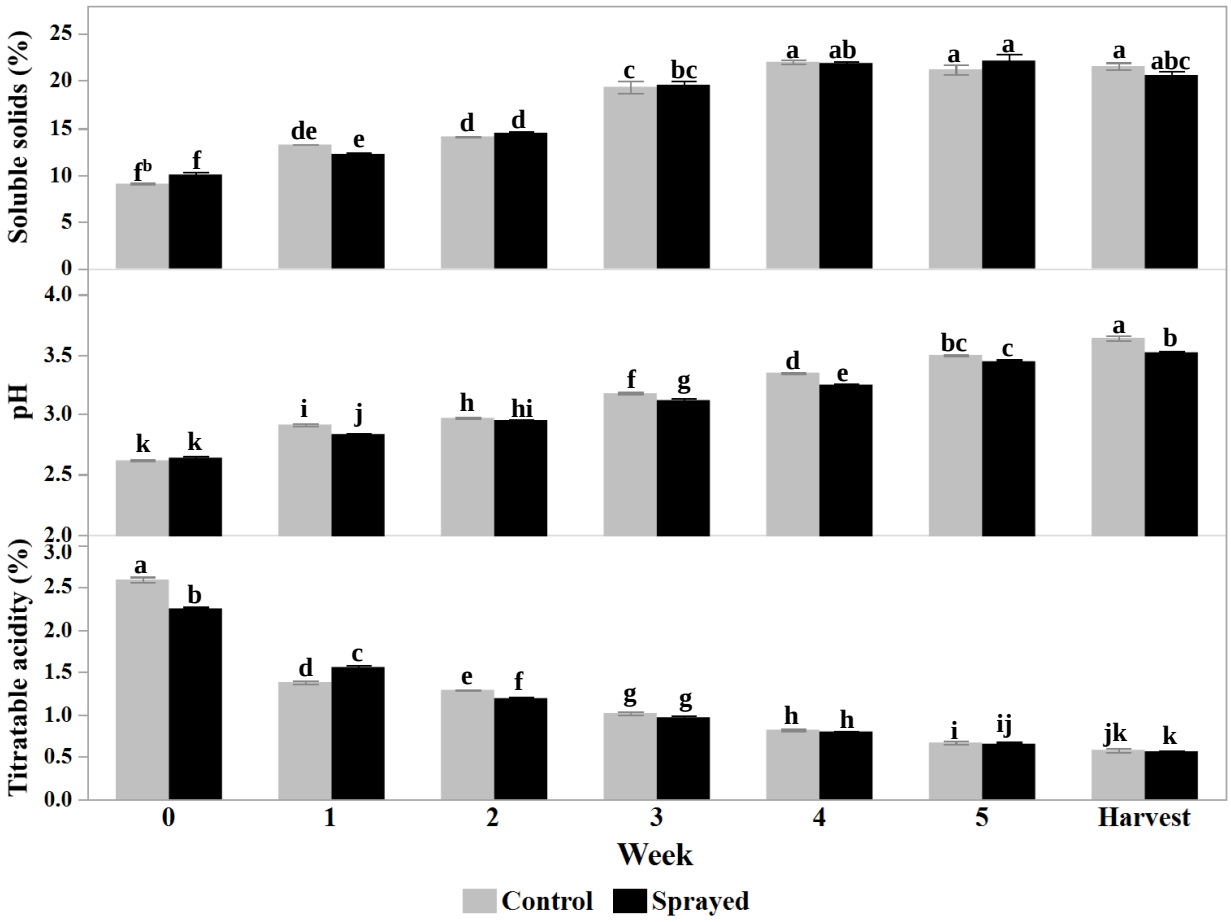

Figure 4. Effect of Spray and Week on soluble solids, pH, and titratable acidity during ripening and at harvest of Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with an inactivated yeast[a] (2018).

[a] LalVigne® MATURE (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

[b] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters for each attribute are were significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

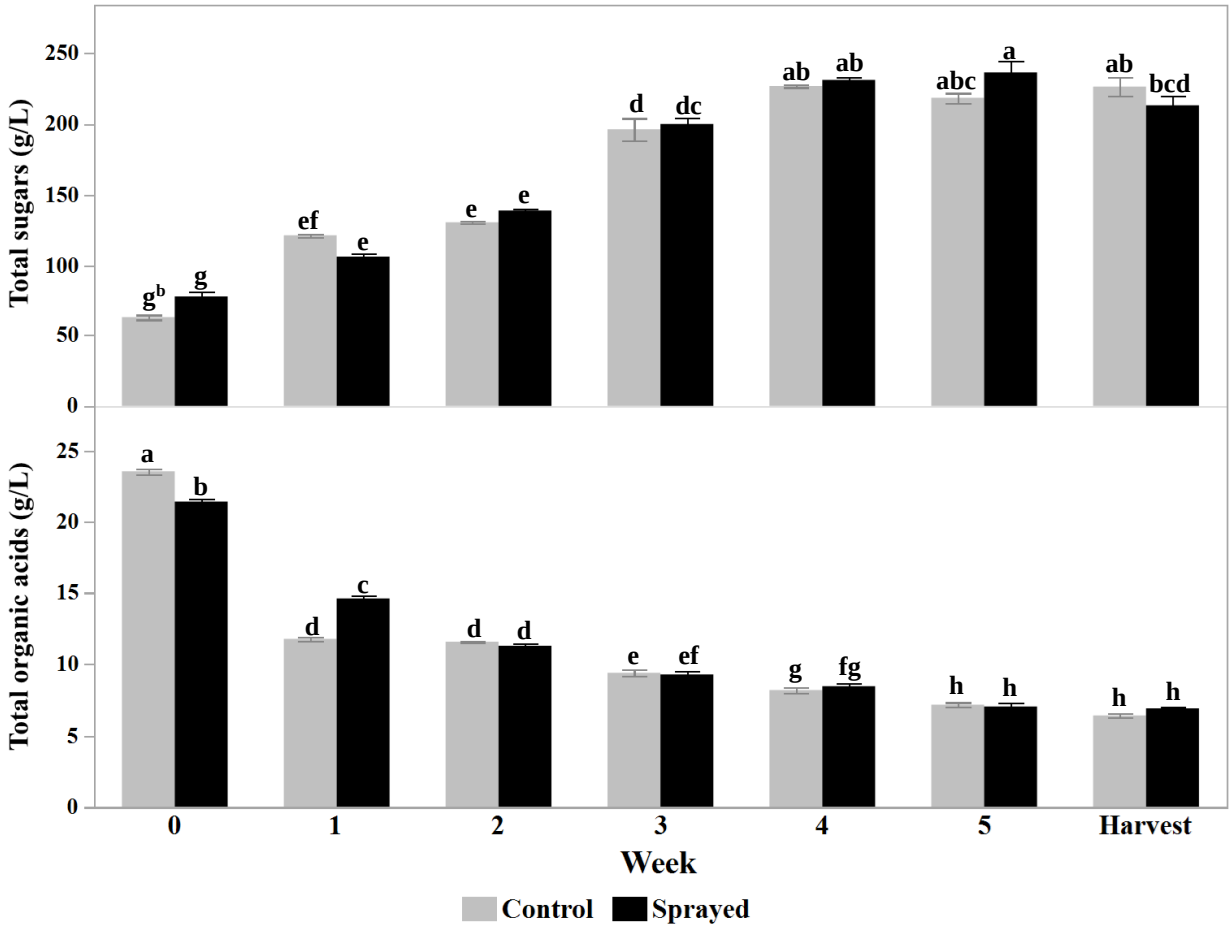

Figure 5. Effect of Spray and Week on total sugars and total organic acids during ripening and at harvest of Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with an inactivated yeast[a] (2018).

[a] LalVigne® MATURE (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

[b] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters for each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

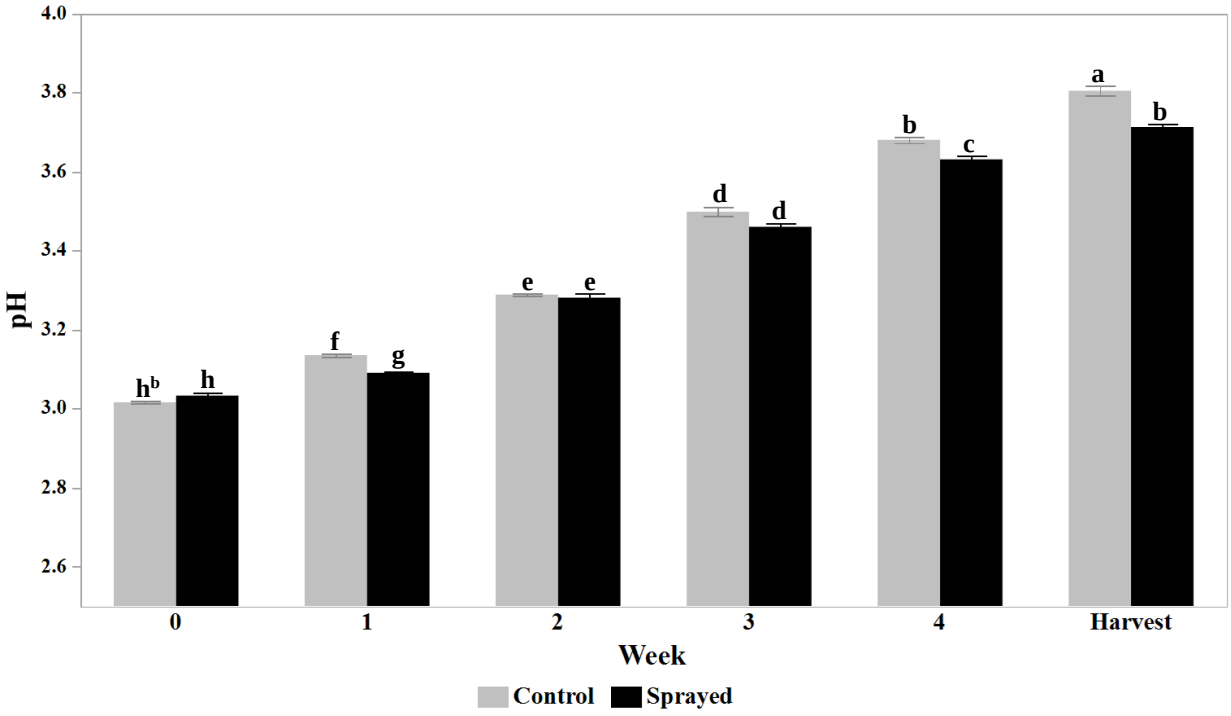

Figure 6. Effect of Spray and Week on pH during ripening and at harvest of Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with an inactivated yeast[a] (2019).

[a] LalVigne® MATURE (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

[b] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

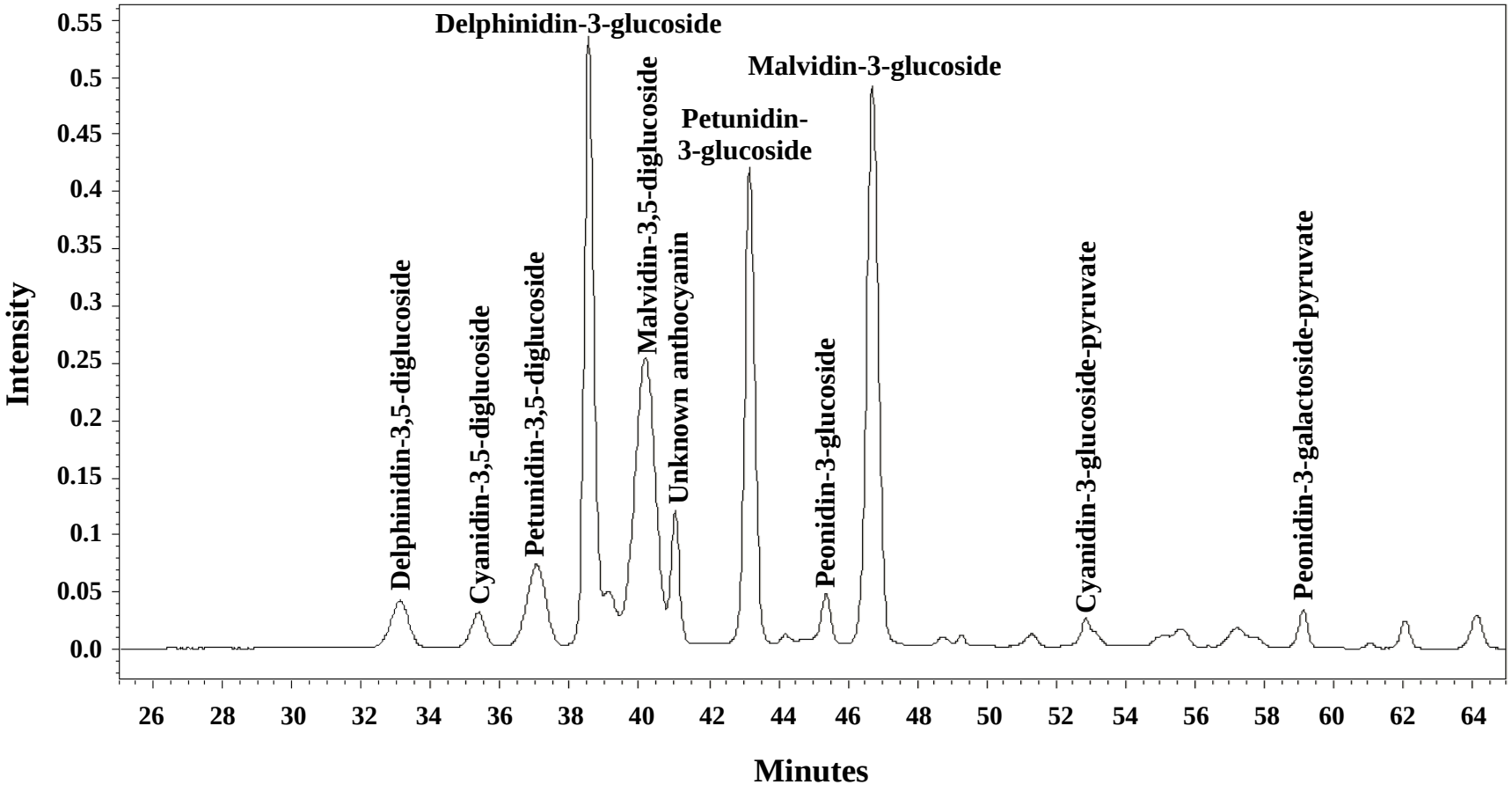

Figure 7. High performance liquid chromatography (HPLC) chromatogram for anthocyanins positively identified in Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with an inactivated yeast[a] (2019).
[a] LalVigne® MATURE (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

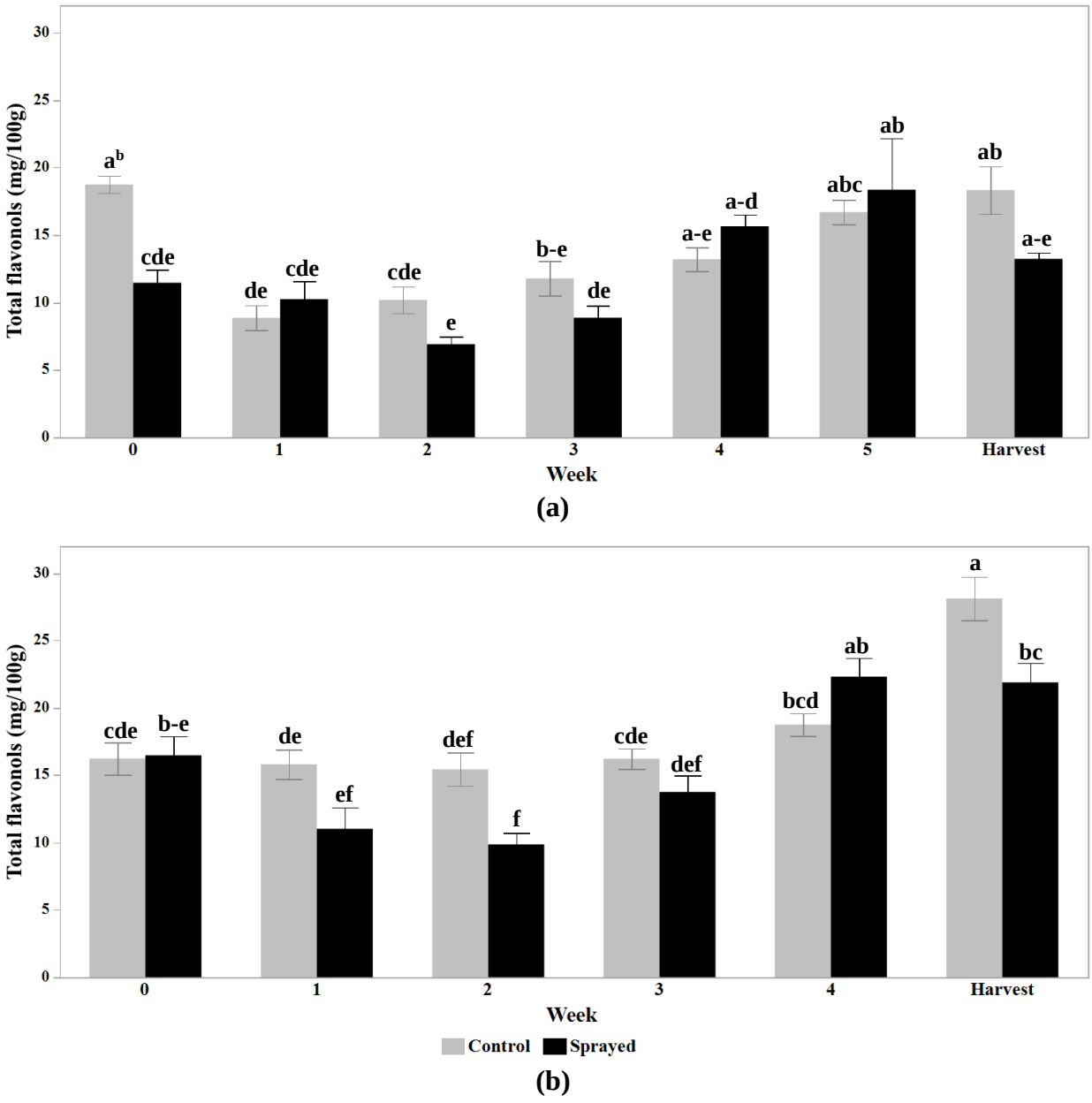

Figure 8. Effect of Spray and Week on total flavonols during ripening and at harvest in 2018 (a) and 2019 (b) of Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with an inactivated yeast[a].

[a] LalVigne® MATURE (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

[b] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters within each year are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

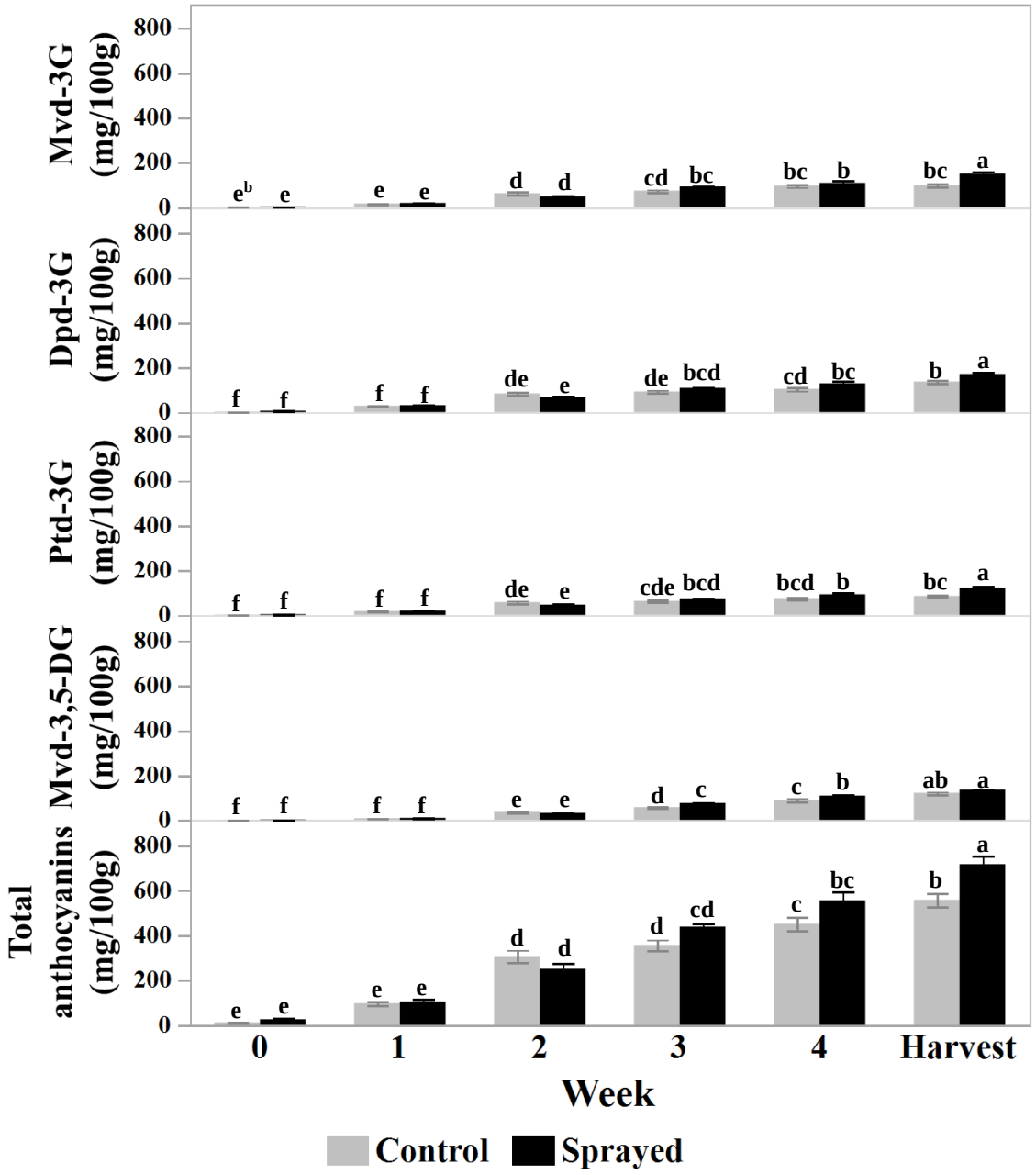

Figure 9. Effect of Spray and Week on malvidin-3-glucoside (mvd-3G), delphinidin-3G (dpd-3G), petunidin-3G (ptd-3G), malvidin-3,5-diglucoside (mvd-3,5-DG), and total anthocyanins during ripening and at harvest of Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with an inactivated yeast[a] (2019).

[a] LalVigne® MATURE (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

[b] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters for each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

CHAPTER II

Effect of specific inactivated yeast vineyard foliar spray on composition, anthocyanin, color, aroma, and sensory attributes of Chambourcin wine

Abstract

Chambourcin is an interspecific French-American *Vitis* spp. hybrid red wine cultivar grown throughout the eastern and midwestern United States, but some regions struggle with delayed or uneven ripening and lack of color development in the grapes. LalVigne® (Lallemand, Inc.) is a specific inactivated yeast that is sprayed foliarly on grapevines during ripening and has been shown to improve ripening and harvest parameters of *V. vinifera* grapes and enhance sensory properties of wines. The effects of inactivated yeast application on composition, anthocyanin, color, aroma, and sensory attributes of wines produced from Chambourcin grapes grown in Arkansas were evaluated in 2018 and 2019. Four rows of Chambourcin grapevines at a commercial vineyard were sprayed with LalVigne®, and an additional four rows were unsprayed. In August of both years, 100 kg of grapes were hand harvested from each treatment for wine production in duplicate. In 2018, grapes had 20% soluble solids (SS), 3.4 pH, and 1.0% titratable acidity (TA), and in 2019 grapes had 19% SS, 3.6 pH, and 0.9% TA. Wines from each Spray treatment ("sprayed wines" and "control wines") were produced at the University of Arkansas System Division of Agriculture Department of Food Science. The 2018 and 2019 Chambourcin wines were analyzed at 0-months storage for composition, anthocyanin, and color attributes. The 2018 wines were analyzed for composition, anthocyanin, and color attributes during storage (0, 6, and 12 months at 15°C) and volatile aroma and sensory attributes at 3-months storage at 15°C. Both 2018 and 2019 wines had initial compositions typical for dry red wines (3.4-3.5 pH and

0.7% TA). In both years, sprayed wines had higher tartaric acid and lower citric and lactic acid at 0-months storage than control wines. Monoglucoside and diglucoside anthocyanins and their coumaroyl derivatives, typical of hybrid grapes, were identified in Chambourcin wines, with malvidin-3,5-diglucoside as the predominant anthocyanin. In 2018 at 0-months storage, Chambourcin wines had 111.14-111.52 mg/L total anthocyanins, but there were no differences between Spray treatments for individual or total anthocyanins. However, at 0-months storage in 2019, sprayed wines had higher concentrations of individual and total anthocyanins (96.20 mg/100 mL) than control wines (83.25 mg/100 mL). The composition of 2018 Chambourcin wines remained commercially acceptable during storage (0, 6, and 12 months at 15°C), and control wines had higher total organic acids than sprayed wines across all storage times. Total anthocyanins decreased 65% in Chambourcin wines during storage, but there was no difference between Spray treatments. Wines developed a darker, more complex color over 12-months storage, and sprayed wines had a higher red color than control wines during storage. Of the 56 volatile compounds identified in 2018 Chambourcin wines at 3-months storage, 10 were determined to be odor active using gas chromatography-olfactometry, with the ethyl esters (red fruit, apple, grape-like, and fermented aromas) as the largest class of compounds. Sprayed wines contained higher concentrations of ethyl butanoate (0.73 mg/L), ethyl hexanoate (0.74 mg/L), ethyl octanoate (0.55 mg/L), and ethyl decanoate (0.55 mg/L) than control wines (0.61, 0.61, and 0.39 mg/L, respectively). The descriptive sensory and grape/wine industry sensory evaluations of Chambourcin were done at 6-8-months storage in 2018. The descriptive panelists (9-11 panelists) evaluated wine appearance, aroma, aromatic, basic taste, and mouthfeel attributes and rated sprayed wines as having a higher red color, lower floral aroma, and lower acetone aromatics than control wines. The industry sensory panel (106 panelists from North America and

Europe) evaluated the liking and intensity of wine color, aroma, flavor, mouthfeel, and overall impression as well as overall preference. Sprayed wines had higher mouthfeel liking, and panelists did not prefer either the sprayed or control wine more than the other. Both sensory panels were unable to identify differences in a majority of the sensory attributes evaluated between sprayed and control wines. This is the first data on wine from the use of a specific inactivated yeast on Chambourcin grapevines, but it shows potential for wines with higher anthocyanins, deeper red color, higher amounts of fruity, fresh ester aromas, and improved sensory attributes.

Acknowledgements

This research was funded by Danstar Ferment AG (Zug, Switzerland), Lallemand, Inc. (Montreal, Canada) and the Austrian Marshall Plan Foundation, with additional support from Hindsville Farm (Hindsville, AR) and the Graz Technical University Institute of Analytical Chemistry and Food Chemistry (Graz, Austria).

Introduction

Grapevines (*Vitis* spp.) are one of the most widely-planted horticultural crops in the world. In the United States, 95% of grape and wine production occurs in California, Washington, New York, Pennsylvania, and Oregon, but production is focused mostly on *V. vinifera*, which is the most popular species of grapevines (Creasy and Creasy 2009, OIV 2000, TTB 2015, USDA NASS 2019). *V. vinifera* grapevines are highly vulnerable to pests, diseases, and extreme temperatures and are difficult to grow in much of the United States, including Arkansas. The

high cost of maintaining *V. vinifera* grapevines in non-ideal climates offsets the profit from producing these wines.

Hybrids (a cross of two or more *Vitis* species) and native species, such as *V. rotundifolia*, are better-adapted to surviving stressors that devastate *V. vinifera* grapes (Reisch et al. 2012). Despite the challenges, grape and wine production contribute significantly to the Arkansas economy. In 2010, the Arkansas grape and wine industry was responsible for 1,700 jobs and over $42 million in wages, and wine-related tourism generated $21 million in revenue (Frank 2010).

Grapes grown in Arkansas include mostly native species and hybrids. Hybrid grapes are created by grape breeders to reap advantageous traits from both parents, such as the cold-hardiness of native species and the desirable yield and flavor of *V. vinifera*. French-American hybrids originate from breeding efforts conducted in France to combat the Phylloxera epidemic (a pest that attacks the roots of grapevines) that destroyed much of the European grape industry (Jackson 2000). Chambourcin (Seyve-Villard 12-417 x Chancellor) is an interspecific French-American hybrid red-wine grape, created by French grape breeder Joannes Seyve, that is grown throughout the midwestern and eastern United States, including Arkansas (Homich et al. 2016, Prajitna et al. 2007, Robinson et al. 2012). Chambourcin has higher disease and winter resistance than *V. vinifera* grapevines and is considered one of the best red-wine hybrid cultivars for producing quality wine (Dami et al. 2006). Chambourcin wines are characterized as full-flavored and aromatic, lacking the less-desirable flavors of some other red-wine hybrids (Robinson et al. 2012).

Inactivated yeasts are *Saccharomyces cerevisiae* byproducts used during winemaking to enhance or preserve wine aroma and improve mouthfeel (Šuklje et al. 2016). Inactivated yeast products are typically added to juice or wine before, during, or after fermentation and are used as

fermentation enhancers to promote yeast resistance to osmotic stress, improve nitrogen compound assimilation, and enhance sensory profiles of wine (Del Barrio-Galán et al. 2011, Comuzzo et al. 2012, Pozo-Bayón et al. 2009). LalVigne® Mature and LalVigne® Aroma (Lallemand, Inc., Montreal, Canada) are foliar specific inactivated yeast sprays developed for use on grapevines in the vineyard at the point of veraison (when berries begin to develop color and ripening quickens). These products are promoted to enhance even ripeness, increase phenolic maturity, concentrate and increase aroma precursors, and improve mouthfeel and overall quality of resulting wine.

Despite use of these products in the viticulture industry, there has been little published research on effects on grapes and wine, with most studies focused on *V. vinifera*. Villangó et al. (2015) evaluated the use of LalVigne® Mature on Syrah grapevines (a red-wine cultivar) grown in Hungary, and it was determined that grapes from treated vines had thicker skins and greater anthocyanin content and extractability than grapes from untreated vines. Similar results were found by Giacosa et al. (2019), where LalVigne® Aroma application was evaluated on white-wine cultivars Chardonnay and Cortese and LalVigne® Mature application was evaluated on red-wine cultivar Nebbiolo grown in Italy. In general, grapes from sprayed vines had increased skin thickness, and Nebbiolo grapes from sprayed vines had higher anthocyanin content at harvest. However, the effects of treatment varied among cultivars and growing season. Šuklje et al. (2016) applied LalVigne® Aroma to Sauvignon Blanc grapevines (a white-wine cultivar) and produced wines from both treated and control grapes. There were differences in fatty acid ethyl ester concentration after fermentation among wines from sprayed and control vines, and wines from sprayed vines had slower degradation of fatty acid ethyl esters during storage. Sensory

analysis demonstrated that Sauvignon blanc wine from sprayed vines had greater perceived fruitiness, whereas wine from control vines was more green/unripe.

There have been several studies examining the attributes and quality of Chambourcin wine (Auw et al. 1996, Homich et al. 2016, Prajitna et al. 2007, Sánchez-Moreno et al. 2003, Sommer and Cohen 2018, Spayd et al. 2015, Zhu et al. 2012). Chambourcin wines typically have good compositions and deeper red color than other red hybrid wines (Zhu et al. 2012). However, Chambourcin wines can have high acid retention and sourness (Homich et al. 2016) and, like other hybrid wines, have lower tannin concentrations and therefore less complex mouthfeel than traditional *V. vinifera* wines (Norton et al. 2020). Chambourcin is one of the most economically-important hybrid wine grapes in the United States and Canada and is the most successful hybrid in Australia (Robinson et al. 2012). However, research is still lacking on the effects of vineyard and/or winemaking treatments on the quality and sensory attributes of Chambourcin wine.

Therefore, further exploration of techniques to improve the properties of Chambourcin wines would be of interest. While previous studies on LalVigne® Mature application provide some evidence that the use of inactivated yeast grapevine foliar sprays can enhance wine aroma and overall quality, research has mainly focused on *V. vinifera* cultivars. As *V. vinifera* grapevines are difficult to grow in Arkansas and similar regions, the objective of this study was to evaluate the effects of specific inactivated yeast application on the composition, anthocyanin, color, aroma, and sensory attributes of Chambourcin wines.

Materials and Methods

Vineyard treatments

Chambourcin grapevines (Seyve-Villard 12-417 x Chancellor) were grown at a commercial vineyard in Hindsville, AR (USDA hardiness zone 6b). The soil type was Linker fine sandy loam (fine-loamy, siliceous, semi active, thermic Typic Hapludults). The grapes were grown on a single bilateral cordon system on 8-10-year-old vines. The vines were rooted on 3309 Couderc rootstock, commonly known as 3309 or C-3309, which is a hybrid of *V. riparia* and *V. rupestri* and is the most commonly-used rootstock in the eastern United States. Each row of grapevines was approximately 200-m long and oriented east to west. Eight consecutive rows of grapevines were sprayed with LalVigne® Mature specific inactivated yeast spray at approximately 5% veraison and again 10 days later. The first spray application at 5% veraison in 2018 was July 20 and in 2019 was July 25. The LalVigne® Mature was dissolved in water and applied at the manufacturer's recommended rate of 1.0 kg/ha at each application date using a Rears air-blast sprayer (Rears Manufacturing Company, Coburg, OR). An additional eight rows were left unsprayed. There were a total of 16 rows of grapevines in this study (Figure 1). Of the eight sprayed rows, the four middle rows (rows 3-6) of grapevines were harvested as the sprayed treatment. Of the eight unsprayed rows, the last four rows (rows 13-16, furthest from sprayed rows) were harvested as the control treatment.

Grape harvest and wine production

One-hundred kg of Chambourcin grapes were hand harvested across all four rows from each treatment in 2018 and 2019. Harvest date was determined by the vineyard owner based on ideal composition attributes for Chambourcin, as well as past harvest data, weather, and quality of the fruit. Average daily temperature and rainfall for January-August 2018 and 2019 were

recorded near Hindsville, AR (Figure 2). Grapes were hand harvested on August 27 in 2018 and August 28 in 2019. Approximately 25 kg of grapes were harvested from each of the four rows within each treatment. The grapes were taken to the University of Arkansas System Division of Agriculture (UA System) Food Science Department in Fayetteville, AR. Chambourcin grapes from each Spray treatment were randomized into two 50-kg batches and stored overnight at 4°C for wine production.

Wines from each Spray treatment ("sprayed wines" and "control wines") were produced in duplicate using a traditional red-wine style. Each batch of grapes was passed twice through a crusher/destemmer, and 30 mg/L sulfur dioxide (SO_2) as potassium metabisulfite (KBMS) was added at crush. The musts (juice, skins, seeds, and pulp after crushing) were kept at room temperature (21°C) for 6-8 hours, then 20 mL/ton Scottzyme® PEC5L pectinase enzyme (Scott Laboratories, Petaluma, CA) was added to each batch to increase juice yield at pressing. The composition of the musts were evaluated prior to, during, and at the end of fermentation, and adjustments were made to the must to ensure a complete fermentation. The free SO_2 levels of the wines were evaluated using the aeration-oxidation method (Iland et al. 1993) and adjusted as needed. Soluble solids (SS), pH, and titratable acidity (TA) of must were evaluated prior to fermentation. The SS (expressed as %) of juice from the must was determined using a Bausch & Lomb Abbe Mark II refractometer (Scientific Instruments, Keene, NH). The pH and TA were measured using a Metrohm 862 Compact Titrosampler (Metrohm AG, Herisau, Switzerland) fitted with a pH meter.

The harvest dates of the grapes and initial composition of the musts for 2018 and 2019 wine production are shown in Table 1. The winemaking procedures were similar for both years. Soluble solid levels of the musts were adjusted to 22% using table sugar (sucrose) in both years.

Musts were inoculated with Lalvin ICV D254® wine yeast (Lallemand, Inc.) at a rate of 0.26 g/L estimated juice in the must. At the onset of fermentation, 20 g/hL Fermaid® O yeast nutrient (Lallemand, Inc.) was added to the musts, and an additional 20 g/hL was added when the SS had decreased by one-third. Musts were fermented on the skins until they had reached dryness (0% SS) and were pressed with a 70-L Enoagricola Rossi Hydropress (Calzolaro, Italy) using three 10-minute press cycles at a pressure of 207 kPa. The wines were collected into 22.7 L and 11.4 L glass carboys fitted with fermentation locks filled with SO_2 solution to allow release of carbon dioxide and limit oxygen exposure. After pressing, wines were inoculated with Lalvin MBR VP41® malolactic fermentation culture (Lallemand, Inc.) at a rate of 1 g/hL to induce malolactic fermentation. When malic acid levels had decreased <10 mg/L, as determined by the high performance liquid chromatography (HPLC) method of Walker et al. (2003), the free SO_2 level was adjusted to 0.8 ppm molecular SO_2 based on the pH. Wines were racked (wines removed from the sediment) several times as fermentation continued at 15°C for approximately four months. After fermentation completion, the free SO_2 content of the wines was determined and adjusted to 0.8 ppm molecular SO_2 based on the pH.

Sprayed and control wines were bottled into 375-mL and 750-mL glass bottles sealed with plastisol-lined screw caps and stored at 15°C until analysis. The ethanol content of all wines was 11.5-12.3% (v/v) at bottling, measured by HPLC (Walker et al. 2003). Wines were stored at 15°C for one week prior to the first analysis (month 0). The 2018 and 2019 Chambourcin wines were analyzed at 0-months storage at 15°C for composition, anthocyanin, and color attributes. The 2018 Chambourcin wines were analyzed during storage (0, 6, and 12 months at 15°C) for composition, anthocyanin, and color attributes, at 3-months storage for volatile aroma attributes, and at 6-8 months storage for sensory attributes.

Composition attributes analysis

The composition attributes analysis of the wines included pH, TA, glycerol, ethanol, residual sugars, and organic acids. Analysis was done on each wine sample (Spray treatment and replicate) in both years, and samples were measured in analytical duplicates. The 2018 and 2019 wines were analyzed for composition attributes at 0-months storage at 15°C, and the 2018 wines were analyzed during storage (0, 6, and 12 months at 15°C).

pH. The pH of wines was measured using a Metrohm 862 Compact Titrosampler fitted with a pH meter. The probe was left in the samples for two minutes to equilibrate before recording the pH value. Wine was degassed prior to analysis.

Titratable acidity (TA). The TA of wines were expressed as % w/v (g/100 mL) tartaric acid and measured using a Metrohm 862 Compact Titrosampler. Six grams of sample was added to 50 mL degassed, deionized water and titrated with 0.1 N sodium hydroxide to an endpoint of pH 8.2. Wine was degassed prior to analysis.

Glycerol, ethanol, residual sugars, and organic acids. The glycerol, ethanol, residual sugars, and organic acids in wines were identified and quantified according to the HPLC procedure of Walker et al. (2003). Samples were passed through a 0.45 μm polytetrafluoroethylene (PTFE) syringe filter (Varian, Inc., Palo Alto, CA) before injection onto an HPLC system consisting of a Waters 515 HPLC pump, a Waters 717 plus autosampler, and a Waters 410 differential refractometer detector connected in series with a Waters 996 photodiode array (PDA) detector (Waters Corporation, Milford, MA). Analytes were separated with a Bio-Rad HPLC Organic Acids Analysis Aminex HPX-87H ion exclusion column (300 x 7.8 mm) connected in series with a Bio-Rad HPLC column for fermentation monitoring (150 x 7.8 mm; Bio-Rad Laboratories, Hercules, CA). A Bio-Rad Micro-Guard Cation-H refill cartridge (30 x 4.5 mm)

was used as a guard column. Columns were maintained at a temperature of 65 ± 0.1°C by a temperature control unit. The isocratic mobile phase consisted of pH 2.28 aqueous sulfuric acid at a flow rate of 0.45 mL/min. Injection volumes of both 10 μL (for analysis of organic acids and sugars) and 5 μL (for ethanol and glycerol) were used to avoid overloading the detector. The total run time per sample was 60 minutes.

Citric, tartaric, malic, lactic, and succinic acids were detected at 210 nm by the PDA detector, and fructose, ethanol, and glycerol were detected at 410 nm by the differential refractometer detector. Analytes in samples were identified and quantified using external calibration curves based on peak area estimation with baseline integration. Results were expressed as milligrams analyte per 100 mL wine for organic acids and residual sugars, grams per liter wine for glycerol, and % v/v (alcohol by volume, ABV) for ethanol. Fructose was the only residual sugar detected in Chambourcin wines. Total organic acids was calculated as the sum of citric, tartaric, malic, lactic, and succinic acids.

Anthocyanin attributes analysis

The anthocyanin attributes analysis of the wines included individual and total anthocyanins. Analysis was done on each wine sample (Spray treatment and replicate) in both years, and samples were measured in analytical duplicates. The 2018 and 2019 wines were analyzed for anthocyanin attributes at 0-months storage at 15°C, and the 2018 wines were analyzed during storage (0, 6, and 12 months at 15°C).

Anthocyanin quantification. The anthocyanin content of wines was analyzed using the HPLC-PDA method of Cho et al. (2004). Samples were passed through a 0.45 μm PTFE syringe filter before injection onto a Waters Alliance HPLC system equipped with a Waters model 996 PDA detector and Millennium version 3.2 software. A 4.6 x 250 mm Symmetry® C_{18} column (Waters

Corporation) with a 3.9 mm x 20 mm Symmetry® C_{18} guard column was used to separate analytes. The mobile phase consisted of a binary gradient with 5% (v/v) formic acid in water (solvent A) and methanol (solvent B) at a flow rate of 1.0 mL/min. A gradient was used with 2% to 60% B from 0-60 minutes, 60% to 2% B from 60-65 minutes, then holding at 2% B from 65-80 minutes. A 50 μL injection volume was used, and the total run time per sample was 80 minutes. Anthocyanins were detected at 510 nm.

Anthocyanins were quantified as the anthocyanidin-3-glucoside of their major aglycone (cyanidin, delphinidin, peonidin, petunidin, or malvidin) using external calibration curves based on peak area estimation with baseline integration. Total anthocyanins were determined by summing the concentrations of individual anthocyanin compounds. Results were expressed as mg/100 mL wine.

Anthocyanin identification. An HPLC-electrospray ionization (ESI)-mass spectrometry (MS) system equipped with an analytical Hewlett Packard 1100 series HPLC instrument (Hewlett-Packard Enterprise Company, Palo Alto, CA), an autosampler, a binary HPLC pump, and a UV/VIS detector interfaced to a Bruker Esquire LC/MS ion trap mass spectrometer (Bruker Corporation, Billerica, MA) was used to identify anthocyanin compounds according to the method of Cho et al. (2004). Reverse-phase separation of anthocyanins was conducted using the same HPLC conditions previously described, and absorption was recorded at 510 nm. Mass spectral analysis was operated in positive ion electrospray mode with a capillary voltage of 4000 V, a nebulizing pressure of 30 psi, a drying gas flow of 9.0 mL/min, and a temperature of 300°C. Data was collected with the Bruker software in full scan mode over a range of *m/z* 50-1000 at 1.0 seconds per cycle. Characteristic ions were used for peak assignment.

Color attributes analysis

The color attributes analysis of the wines included L*, chroma, hue angle, red color, and color density. Analysis was done on each wine sample (Spray treatment and replicate) in both years, and samples were measured in analytical duplicates. The 2018 and 2019 wines were analyzed for color attributes at 0-months storage at 15°C, and the 2018 wines were analyzed during storage (0, 6, and 12 months at 15°C).

L*, hue angle, and chroma. Wine color analysis was conducted using a ColorFlex system (HunterLab, Reston, VA). The ColorFlex system uses a ring and disk set (to control liquid levels and light interactions) for measuring translucent liquids in a 63.5-mm glass sample cup with an opaque cover to determine Commission Internationale de l'Eclairage (CIE) Lab transmission values of L*=100, a*=0, and b*=0 (CIE 1986). The CIELAB system describes color variations as perceived by the human eye. CIELAB is a uniform three-dimensional space defined by colorimetric coordinates, L*, a*, and b*. The vertical axis L* measures lightness from completely opaque (0) to completely transparent (100), while on the hue-circle, +a* red, -a* green, +b* yellow, and -b* blue are measured. Hue angle, calculated as $\tan^{-1}\frac{b*}{a*}$, described color in angles from 0 to 360°: 0° is red, 90° is yellow, 180° is green, 270° is blue, and 360° is red. For samples with hue angles <90°, a 360° compensation (hue + 360°) was used to account for discrepancies between red samples with hue angles near 0° and those near 360° (McLellan et al. 2007). Chroma, calculated as $\sqrt{a*^2 + b*^2}$, identified color by which a wine appeared to differ from gray of the same lightness and corresponded to saturation (intensity/purity) of the perceived color.

Red color and color density. Red color of wines was measured spectrophotometrically as absorbance at 520 nm, and color density was measured as red color + yellow/brown color (420

nm) (Iland et al. 1993). Absorbance values were measured using a Hewlett-Packard 8452A Diode Array spectrophotometer equipped with UV-Visible ChemStation software (Agilent Technologies, Inc., Santa Clara, CA). Samples were diluted 10 times with deionized water prior to analysis and were measured against a blank sample of deionized water. A 1-cm cell was used for all spectrophotometer measurements.

Aroma attributes analysis

Aroma attributes analysis of the 2018 wines was conducted at Graz University of Technology (Graz, Austria) Institute of Analytical Chemistry and Food Chemistry and included identification of odor-active compounds by gas chromatography-olfactometry (GC-O) and GC-MS and quantitation of ethyl esters by GC-MS. Wines were packaged in 20-mL clear glass vials, sealed with a polypropylene cap with a polytetrafluoroethylene-line silicon septum, wrapped with Parafilm® flexible film (Bemis Company, Inc., Neenah, WI), and shipped to Graz University of Technology for analysis of aroma attributes. Odor-active compounds were identified and ethyl esters were quantified in the wines at 3-months storage at 15°C.

Identification of volatile aroma compounds. To identify the volatile aroma compounds, the volatile compounds were extracted from 1 mL of wine in a 10-mL glass vial using solid-phase microextraction (SPME) with a 2-cm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber for 30 minutes at 40°C. A gas chromatography (GC)-MS system equipped with a Shimadzu GC 2010 (Shimadzu Corporation, Kyoto, Japan), Shimadzu QP 2010 MS, and a PAL HTX autosampler (CTC Analytics AG, Zwingen, Switzerland) was used to separate and identify volatile compounds. Samples were extracted/injected in analytical triplicate. Volatiles were separated on a nonpolar Restek Rxi 5MS column (30 m x 0.25 mm x 1 μm; Restek, Bellefonte, PA) 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 were recorded in the scan mode (*m/z* 35-350) with a 9.8 minute solvent cut time and a detector voltage relative to the tuning result.

Data was analyzed using the Shimadzu GCMS Postrun Analysis software. Compounds were identified using comparison of mass spectra with NIST14 (National Institute of Standards and Technology, Gaithersburg, MD), Flavors and Fragrances of Natural and Synthetic Compounds (FFNSC3, John Wiley & Sons, Inc., Hoboken, NJ), and Adam's Essential Oils (Adams 2007) mass spectral libraries and comparison of calculated Kovats retention indices (Kováts 1958) with values reported in the Flavornet (Acree and Arn 2004) and Pherobase (Sayed 2003) databases. A matching library result and a retention index 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.

Determination of odor-active compounds. To determine which volatile compounds were odor-active in Chambourcin wine, GC-O was performed using a Hewlett Packard HP5890 gas chromatograph equipped with a flame ionization detector (FID) and an olfactory detection port (ODP). Initial exploration of GC-MS chromatograms indicated that the sprayed and control wines had similar chromatogram peaks. Therefore, only the sprayed wines were used for GC-O analysis, to avoid panelist fatigue. The volatiles were extracted from 500 μL, 100 μL, 50 μL, and 10 μL of sample for each panelist to determine which compounds were the most odor-active even at lower concentrations. The designated amount of wine was placed in a 10-mL glass vial, and volatiles were extracted using SPME with a 2-cm DVB/CAR/PDMS fiber for 30 minutes at 40°C. Volatile compounds were separated using an Agilent HP5 nonpolar column (30 m x 0.32 mm x 0.25 μm) with a temperature gradient: 35°C to 280°C at 10°C/min with a constant helium

flow of 35 cm/min. At the end of the column, a splitter was used to divide the effluent 1:1 between the FID and ODP. GC effluents were combined with humidified air in the ODP to avoid nasal dehydration, and panelists used the ODP to sniff the effluents.

Five trained, panelists from Graz Technical University were used to evaluate the wines. Panelists evaluated each sample level (500 μL, 100 μL, 50 μL, and 10 μL) one time and order was randomized among panelists. Panelists sniffed each sample for 15 minutes and indicated, through the press of a button, when they perceived an odor. They described the perceived 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 the percentage of panelists that perceived a particular odorant. A NIF of 60% (3 out of 5 panelists) was considered an odor-active compound. Kovats retention indices were calculated for each identified compound and compared with GC-MS spectra to identify the compound.

Quantitation of ethyl esters. Ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate were quantified in Chambourcin wines using a standard additions procedure with the same SPME-GC-MS procedure described above for volatile compounds identification. Four standard solutions were prepared with either 0, 5, 10, or 25 ng/L of each ethyl ester, and 10 ng/L hexyl butanoate internal standard was added to each solution. An artificial wine matrix was prepared by adding 350 mg/L tartaric acid to 12% (v/v) ethanol in water, and 100 μL of each wine sample and 10 μL of ethyl ester standard solution were added to 890 μL of the artificial wine matrix. The resulting mixtures corresponded to 0, 0.5, 1.0, and 2.5 additional mg/L of each ethyl ester in the wine. Each mixture was extracted/injected in triplicate using SPME-GC-MS

and ethyl esters in Chambourcin wines were quantified using the TIC peak areas, corrected for the internal standard.

Sensory attributes analysis

Sensory attributes analysis of the 2018 wines involved a descriptive sensory evaluation conducted at the UA Sensory Science Center and an industry sensory panel conducted at multiple locations across North America and Europe. For descriptive sensory evaluation, 9-11 trained panelists evaluated the intensity of Chambourcin wine appearance, aroma, aromatics, basic taste, and mouthfeel attributes and wine preference. For the industry sensory panel, 106 panelists from the grape/wine industry or related academia evaluated liking and intensity of Chambourcin wine color, aroma, flavor, mouthfeel, and overall impression. The descriptive sensory evaluation was conducted at 6-months storage at 15°C, and the industry sensory panel analyses were conducted at 6-8-months storage at 15°C. For the descriptive and industry sensory analysis, two replications of each wine treatment were combined so that 50% of each replication was used for analysis.

Descriptive sensory evaluation. Descriptive sensory analysis was performed at the Sensory Science Center at the UA System Food Science Department (Institutional Review Board protocol #1903181159; Figure 3). Some of the panelists were not able to consume wine and only evaluated the appearance and aroma attributes. Eleven trained panelists evaluated wines for appearance and aroma (orthonasal) attributes, and nine trained panelists evaluated the wines for aromatics (retronasal), basic tastes, and mouthfeel attributes. Each panelist evaluated 30 mL of each wine in duplicate. The wines were served monadically (one at a time) at room temperature (25°C) in wine glasses labeled with three-digit codes in a randomized complete block design with replications. Serving order was randomized across each replication to prevent presentation

order bias. Panelists were instructed to cleanse their palates with unsalted crackers and water between samples. Expectorant cups were provided. The panelists were trained to use the Sensory Spectrum method, a method for describing the intensity of product attributes using references for the attributes. The descriptive panelists developed a lexicon of sensory terms for the Chambourcin wines through consensus during training and practice sessions (Table 2). The descriptive panel evaluated the wines for appearance (n = 1), aroma (n = 19), aromatics (n = 19), basic tastes (n = 2), and mouthfeel (n = 2). The attributes were evaluated using a scale where 0 = less of an attribute and 15 = more of an attribute.

Industry sensory panel. Industry sensory panels were conducted at various locations across North America and Europe. "Sensory evaluation kits" were sent to each location, and Table 3 shows the supplies included in each kit. Everything needed to conduct the sensory panel, with the exception of the wine glasses, was included in each kit. In total, 106 panelists evaluated the wines for liking and intensity of Chambourcin wine attributes. The industry sensory panelists were located in Arkansas (n = 11), California (n = 27), New Mexico (n = 13), Pennsylvania (n = 10), Texas (n = 10), Austria (n = 10), Canada (n = 15), and Spain (n = 10). Overall, 59% of panelists were female, 41% of panelists were male, 2% were 18-21 years of age, 30% were 22-34, 21% were 35-44, 13% were 45-54, 27% were 55-64, and 7% were 65 or older. Bottles of wine were labeled with random three-digit codes, and ballots were pre-labeled with codes, so that the panel leader at each location did not know the identity of the samples. Each panelist evaluated 30-mL of wine, and each wine was evaluated one time. The wines were served at the same time at room temperature (25°C) in wine glasses labeled with three-digit codes in a randomized complete block design. Serving order was randomized across panelists to prevent presentation order bias. Panelists were instructed to cleanse their palates with unsalted crackers

and water between samples. Expectorant cups were provided. The panelists used a nine-point hedonic scale (1 = dislike extremely; 9 = like extremely) to indicate their liking of wine color, aroma, flavor, mouthfeel, and overall impression and a five-point just-about-right (JAR) scale (1 = much too low; 3 = just-about-right; 5 = much too much) to indicate their impression of wine color, aroma, flavor, and mouthfeel intensity. After evaluating both wines, panelists were instructed to indicate which wine they preferred. An example of a ballot presented to industry sensory panelists is shown in Figure 4.

Design and statistical analysis

After harvest, Chambourcin grape clusters from each Spray treatment were randomized into two batches for wine production in duplicate (sprayed and control). The wines were bottled and stored at 15°C. The 2018 and 2019 wines were analyzed at 0-months storage at 15°C for composition, anthocyanin, and color attributes, and the 2018 wines were analyzed during storage (0, 6, and 12 months at 15°C) for these attributes. There were four wine samples in 2018 and 2019 when the wines were analyzed 0-months storage, and there were 12 wine samples in 2018 when the wines were analyzed at 0-, 6-, and 12-months storage. The 2018 wines were analyzed at 3-months storage at 15°C for aroma attributes, and there were four wine samples in this analysis. At each storage time for composition, anthocyanin, color, and aroma attributes, samples were taken from one 375-mL bottle, which was treated as an individual experimental unit in a full factorial design. The two replications of each wine treatment were combined and used for sensory evaluation. The 2018 wines were analyzed at 6-months storage at 15°C for descriptive sensory attributes in duplicate. For the industry sensory panel, 2018 wines were analyzed at 6-8-months storage at 15°C. Statistical analyses were conducted using JMP® Pro statistical software

(version 15.0.0, SAS Institute, Cary, NC). Additional information on the statistical analyses is provided below.

Composition, anthocyanin, and color attributes. For the 2018 and 2019 wines at 0-months storage, a univariate analysis of variance (ANOVA) was used to determine the significance of the Spray main effect. For the 2018 wines at 0-, 6-, and 12-months storage, a univariate ANOVA was used to determine the significance of the main factors (Spray and Storage) and their interaction. All factors were treated as categorical. Tukey's Honest Significant Difference (HSD) test and student's t-test were used to detect differences among means ($p<0.05$). Figures were created in JMP®, and error bars represented one standard error from the mean.

Aroma attributes. Odor-active compounds in 2018 Chambourcin wine were identified using GC-O at 3 months storage at 15°C, and a compound was considered odor-active if it had NIF > 60%. Peak areas (TIC) from GC-MS for each odor-active compound in sprayed and control Chambourcin wines were used as a semi-quantitative measure for principal components analysis (PCA). A PCA, based on the TIC peak areas of odor-active compounds at 3-months storage at 15°C, was used to explore the relationship between Spray treatment and odor-active compound profiles. It was determined that the ethyl esters (ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate) gave the clearest distinction among the Spray treatments, and therefore these compounds were chosen for quantitation at 3-months storage at 15°C. A univariate ANOVA was used to determine the significance of the Spray main effect. All factors were treated as categorical, and student's t-test was used to detect significant differences among means ($p<0.05$).

Sensory attributes. For the descriptive sensory evaluation of 2018 wines at 6-months storage at 15°C, a univariate ANOVA was used to detect the significance of the Spray main effect for each

appearance, aroma, aromatics, basic tastes, and mouthfeel attribute. The Panelist main effect and Spray x Panelist interaction were also included in the model to account for the error explained by between-panelist and within-panelist variation. A student's t-test was used to detect significant differences among means ($p<0.05$). For the industry sensory panel of 2018 wines at 6-8-months storage at 15°C, a univariate ANOVA was used to detect the significance of the Spray main effect for each hedonic-scaled attribute, and the Panelist main effect was included to account for between-panelist variation. Nine-point hedonic scales were 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 JAR-scaled attributes, a collapsed scale was used (too low, JAR, and too much), and the percent of responses for each wine were tabulated. For the preference-scaled data, an ordinal logistic model was used to assess the likelihood of preferring the sprayed wine ($\chi^2 < 0.05$). Figures were created in JMP® and Microsoft Excel® (version 16, Microsoft Corporation, Redmond, WA), and error bars represented one standard error from the mean.

Results and Discussion

The 2018 and 2019 wine grape production seasons in the Hindsville, AR area were relatively mild in terms of temperature and rainfall (Figure 4). The high and low temperatures were similar from January to August in both years. There was higher rainfall in 2019 than 2018 from April (bud emergence on grapevines) to August (harvest). In August of 2018 and 2019, the average daily high temperature was 35.8°C and 37.2°C, respectively. In August of 2019, there was over twice as much cumulative rainfall (153.7 mm) than in August of 2018 (62.7 mm).

The grapes were harvested in August in both years for wine production. The composition of Chambourcin grape musts at crush varied slightly in 2018 and 2019 (Table 1). In 2018, musts from control vines had 20.4% SS, 3.4 pH, and 1.0% TA, and musts from sprayed vines had 20.2% SS, 3.3 pH, and 1.0% TA. Musts had slightly lower SS (18.5-19.0%) and TA (0.9%) and higher pH (3.5-3.6) in 2019. The 2018 grape musts had more ideal composition attributes for wine production than the 2019 musts. Homich et al. (2016) reported 21% SS, 3.4 pH, and 0.9% TA at harvest for Chambourcin grapes grown in Pennsylvania, and Zhang and Dami (2012) reported 22.2% SS, 3.3 pH, and 1.1% TA for Chambourcin grapes grown in Ohio. Thus, composition of Chambourcin grapes at harvest reported in this study were similar to those found by others.

In each year, wines were fermented for about five months at 15°C, bottled in January, and stored at 15°C. For 2018 and 2019 Chambourcin wines, the impact of inactivated yeast application to grapevines (Spray treatment) on wine composition, anthocyanin, and color attributes was evaluated at 0-months storage at 15°C. For 2018 wines, composition, anthocyanin, and color attributes were evaluated during storage (0, 6, and 12 months at 15°C). Aroma attributes of the 2018 wines were evaluated at 3-months storage at 15°C, and sensory attributes were evaluated at 6-8-months storage at 15°C. “Sprayed wines” refer to wines produced from sprayed grapevines, and “control wines” refer to wines produced from grapevines that were not sprayed.

Analysis of composition, anthocyanin, and color attributes at 0-months storage (2018 and 2019)

In both years, Chambourcin wines had acceptable compositions within the typical ranges for a dry, red table wine. Sprayed wines had higher tartaric acid and lower citric and lactic acid concentrations than control wines in both years. Chambourcin wines had a complex mixture of

monoglucoside and diglucoside anthocyanins and their coumaroyl derivatives typical of wines produced from French-American hybrid grapes. Malvidin-3,5-diglucoside was the predominant anthocyanin in both years. The effect of inactivated yeast foliar application on the anthocyanin content and color of Chambourcin wines varied between 2018 and 2019.

Composition. The 2018 and 2019 Chambourcin wines were analyzed at 0-months storage at 15°C for pH, TA, glycerol, ethanol, total residual sugars, tartaric acid, malic acid, citric acid, succinic acid, lactic acid, and total organic acids. Regardless of Spray treatment, the wines had acceptable minimum and maximum composition values at 0-months storage in both years. The 2018 wines had 3.4 pH, 0.7% TA, 12-13 g/L glycerol, 11-12% (v/v) ethanol, 51-53 mg/100 mL total residual sugars, 84-98 mg/100 mL tartaric acid, 35-52 mg/100 mL malic acid, 49-54 mg/100 mL citric acid, 342-384 mg/100 mL succinic acid, 460-523 mg/100 mL lactic acid, and 985-1,098 mg/100 mL total organic acids (Table 4). The 2019 wines had 3.5 pH, 0.7% TA, 13 g/L glycerol, 12% (v/v) ethanol, 66-75 mg/100 mL total residual sugars, 296-314 mg/100 mL tartaric acid, 19-27 mg/100 mL malic acid, 29-36 mg/100 mL citric acid, 418-435 mg/100 mL succinic acid, 472-512 mg/100 mL lactic acid, and 1,269-1,290 mg/100 mL total organic acids.

In a general comparison of the values from 2018 and 2019, the 2019 wines were slightly more acidic than the 2018 wines in terms of organic acid concentrations. However, the pH and TA values were similar in both years and were within the 3.2-3.8 pH and 0.5-0.8% TA ranges for Chambourcin wine reported in the literature (Homich et al. 2016, Prajitna et al. 2007, Sommer and Cohen 2018, Zhu et al. 2012). Glycerol and ethanol levels were similar in both years, but the total residual sugars were slightly higher in 2019. However, the total residual sugars (fructose) in both years were similar to the 60 mg/100 mL residual sugars in Chambourcin wine found by Zhu et al. (2012). Lactic acid was the most prevalent organic acid in both years, and malic acid was

the least prevalent. This was because wines were inoculated with lactic acid bacteria, which converted malic acid to lactic acid to decrease perceived acidity, in a process known as malolactic fermentation (Boulton 1980). In both years, the Spray main effect was significant for tartaric acid, citric acid, and lactic acid.

2018 Wines. There was no effect of Spray treatment on pH, TA, glycerol, ethanol, total residual sugars, or malic acid at 0-months storage in 2018. The glycerol concentrations in Chambourcin wines were slightly higher than the average range for dry, red table wine. Glycerol is typically found at concentrations of 7-10 g/L in dry wine, but levels over 20 g/L are not uncommon in botrytized late-harvest wines (Liu and Davis 1994, Sarrazin et al. 2007). The ethanol content was within the typical range of 9-13% for dry table wines (Waterhouse et al. 2016). There was no glucose detected in Chambourcin wines, likely because yeast preferentially ferment glucose, decreasing its concentration throughout fermentation. Total residual sugar (fructose only) concentrations were within the range of 20-400 mg/100 mL fructose in dry, red table wines reported by Liu and Davis (1994) and were below the sensory detection threshold for fructose of 180-240 mg/100 mL in wine (Hufnagel and Hofmann 2008, Noble and Bursick 1984). Therefore, Chambourcin wines did not have a perceptible sweetness.

The Spray main effect was significant for tartaric acid, citric acid, succinic acid, lactic acid, and total organic acids. Sprayed wine (98.37 mg/100 mL) had a higher tartaric acid concentration than control wine (84.16 mg/100 mL), but control wine had higher concentrations of citric acid (53.77 mg/100 mL), succinic acid (384.18 mg/100 mL), lactic acid (523.47 mg/100 mL), and total organic acids (1,097.58 mg/100 mL) than sprayed wines (49.20, 342.18, 459.93, and 984.80 mg/100 mL, respectively).

2019 Wines. There was no effect of Spray treatment on pH, TA, glycerol, ethanol, malic acid, succinic acid, or total organic acids at 0-months storage in 2019. Sprayed wines (74.86 mg/100 mL) had higher total residual sugars than control wines (65.65 mg/100 mL), but residual sugar concentrations of both wines were below the 180-240 mg/100 mL detection threshold (Hufnagel and Hofmann 2008, Noble and Bursick 1984). Similar to 2018, sprayed wines had higher tartaric acid (313.76 mg/100 mL) and lower citric acid (29.33 mg/100 mL) and lactic acid (471.85 mg/100 mL) than control wines (296.32, 36.17, and 512.36 mg/100 mL, respectively).

Anthocyanins. The 2018 and 2019 Chambourcin wines were analyzed at 0-months storage at 15°C for individual and total anthocyanin compounds. Anthocyanins identified in wines included delphinidin-3,5-diglucoside, cyanidin-3,5-diglucoside, petunidin-3,5-diglucoside, delphinidin-3-glucoside, peonidin-3,5-diglucoside, malvidin-3,5-diglucoside, petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, delphinidin-3-(6-*p*-coumaroyl)-5-diglucoside, petunidin-3-(6-*p*-coumaroyl)-5-diglucoside, malvidin-3-(6-*p*-coumaroyl)-5-diglucoside, cyanidin-3-(6-*p*-coumaroyl)-glucoside, and malvidin-3-(6-*p*-coumaroyl)-glucoside (Figure 5). This complex mixture of monoglucoside and diglucoside anthocyanins and their coumaroyl-derivatives is typical of hybrid wine grapes (Spayd et al. 2015, Wu and Prior 2005). Zhu et al. (2012) determined the anthocyanin profile of Chambourcin wines and found only malvidin and petunidin monoglucosides and diglucosides, with malvidin-3,5-diglucoside present in the greatest amount. These results do not coincide with the wider range of anthocyanins identified in Chambourcin grapes in the present study. However, complex wine chemistry, such as the formation of polymeric pigments and anthocyanin-phenolic complexes, could explain this discrepancy. Most studies on Chambourcin grape/wine anthocyanins have used the pH-

differential method (Giusti and Wrolstad 2001) to determine total monomeric anthocyanins, rather than classifying and quantifying individual anthocyanins.

In both 2018 and 2019, malvidin-3-glucoside and malvidin-, delphinidin-, and petunidin-3,5-diglucoside made up approximately 75% of total anthocyanins at 0-months storage, and thus only these four individual compounds, along with total anthocyanins, were discussed in this study. Malvidin-3,5-diglucoside was the predominant anthocyanin in both years, making up approximately 40% of total anthocyanins. At 0-months storage, 2018 Chambourcin wines had 20-21 mg/100 mL malvidin-3-glucoside, 44 mg/100 mL malvidin-3,5-diglucoside, 11-12 mg/100 mL petunidin-3,5-diglucoside, 7-8 mg/100 mL delphinidin-3,5-diglucoside, and 111-112 mg/100 mL total anthocyanins. In 2019, Chambourcin wines had 13-16 mg/100 mL malvidin-3-glucoside, 37-41 mg/100 mL malvidin-3,5-diglucoside, 9-11 mg/100 mL petunidin-3,5-diglucoside, 6 mg/100 mL delphinidin-3,5-diglucoside, and 83-96 mg/100 mL total anthocyanins.

In a general comparison of the values from 2018 and 2019, 2018 Chambourcin wines had higher total and individual anthocyanins than 2019 wines. Chambourcin grape musts in 2018 had higher soluble solids than 2019 musts (Table 1), indicating that grapes were riper at harvest. This could explain the 25% greater total anthocyanins at 0-months storage in 2018. Total anthocyanin concentrations for Chambourcin wines in previous studies ranged from 9-114 mg/100 mL (Prajitna et al. 2007, Sánchez-Moreno et al. 2003, Sommer and Cohen 2018, Spayd et al. 2015, Zhu et al. 2012), and total anthocyanin concentrations for Chambourcin wines at 0 months storage in the current study were 83-112 mg/100 mL for both years. The wide range of values reported in the literature is likely because a majority of those studies used commercial wines, which had been aged for some amount of time. Therefore, anthocyanins had potentially formed

copigment or acylated complexes with other wine components, stabilizing the color but decreasing the quantifiable monomeric anthocyanin concentration (Ballinger et al. 1973, Nagel and Wulf 1979, Scudamore-Smith et al. 1990).

2018 Wines. There was no effect of Spray treatment on anthocyanin attributes at 0-months storage in 2018. Therefore, inactivated yeast foliar application did not impact anthocyanin content of Chambourcin wines at 0-months storage in 2018.

2019 Wines. The Spray main effect was significant for all anthocyanin attributes at 0-months storage in 2019. The sprayed wine had higher concentrations of malvidin-3-glucoside (15.80 mg/100 mL), malvidin-3,5-diglucoside (40.79 mg/100 mL), petunidin-3,5-diglucoside (10.68 mg/100 mL), delphinidin-3,5-diglucoside (6.05 mg/100 mL), and total anthocyanins (96.20 mg/100 mL) than the control wine (12.91, 36.94, 9.35, 5.53, and 83.25 mg/100 mL, respectively). This could mean that wines produced from Chambourcin grapevines treated with an inactivated yeast would have a more intense red color than wines produced from untreated vines. As anthocyanins in wine come from the grapes, this finding is consistent with the results of Giacosa et al. (2019) and Villangó et al. (2015). These studies determined that specific inactivated yeast application increased the anthocyanin content of red-wine grapes.

Color. The 2018 and 2019 Chambourcin wines were analyzed at 0-months storage at 15°C for L*, hue angle, chroma, red color, and color density. The 2018 wines had 7.6-7.8 L*, 360° hue angle, 36 chroma, 4.4-4.7 red color, and 7.0-7.3 color density. The 2019 wines had 7.2-8.4 L*, 360° hue angle, 19-35 chroma, 5.1-5.6 red color, and 8.0-8.9 color density.

In a general comparison of the values from 2018 and 2019, wines from both years had a hue angle of pure red (360°), but 2019 wines had higher red color and color density than 2018

wines. The red color measurements across both years were similar to the range of 3-6 found by Auw et al. (1996) for Chambourcin wines.

2018 Wines. There was no effect of Spray treatment on color attributes at 0-months storage in 2018. Therefore, foliar application of an inactivated yeast did not impact color attributes of Chambourcin wines at 0-months storage in 2018.

2019 Wines. There was no effect of Spray treatment on L*, red color, or color density at 0-months storage in 2019. The Spray main effect was significant for hue angle and chroma. Sprayed wines (360.12°) had a lower hue angle than control wines (360.31°), and therefore a hue closer to that of pure red (360°). Control wines (35.21) had a higher chroma than sprayed wines (19.29). This meant that the color of control wines was more saturated than that of sprayed wines. As red wines age, anthocyanins form polymeric pigments with other phenolic compounds that can shift the color from pure red to more brick- or orange-red (Cheynier et al. 2006, 2000, He et al. 2012). Anthocyanins begin complexing with other phenolics to form these stable pigments as soon as they are extracted into the must during maceration (Romero-Cascales et al. 2005), and this process continues through aging. Because sprayed wines had higher individual and total anthocyanin levels than control wines at 0-months storage in 2019, the lower chroma of sprayed wines could be due to this color shift.

Analysis of composition, anthocyanin, and color attributes during storage (2018)

The composition of Chambourcin wines remained commercially acceptable during storage (0, 6, and 12 months at 15°C). Monomeric anthocyanins decreased during storage, likely due to the formation of stable polymeric pigment complexes. The color of wines became darker and less pure-red during storage, likely due to a color shift from pure red to more orange- or brick-red that is typical of well-aged wines. Sprayed wines had higher red color than control

wines during storage, but other effects of Spray treatment on composition, anthocyanin, or color attributes during storage were not seen.

Composition. The 2018 Chambourcin wines were analyzed at 0-, 6-, and 12-months storage at 15°C for pH, TA, glycerol, ethanol, total residual sugars, and total organic acids. The Storage main effect was significant for pH (Table 5). The pH of Chambourcin wines increased from 3.41 to 3.53 from month 0 to month 6. However, the pH decreased to 3.36 at month 12, and month 12 had the lowest pH value of all storage times. Despite these fluctuations, the pH remained within acceptable ranges during storage. The Spray x Storage interaction was significant for TA, glycerol, ethanol, and total residual sugars. The TA of Chambourcin wines remained within typical ranges of 0.5-0.8% TA for a dry red wine (Waterhouse et al. 2016) over 12-months storage (Figure 6). Chambourcin wines from both Spray treatments had the highest TA at 0-months storage (0.72%) and the sprayed wine at 12-months storage had the lowest TA (0.64%). The control wine at 12-months storage (0.70%) had a higher TA than the sprayed wine (0.64%) at 12-months storage, but no differences between Spray treatments were seen at other storage times. In general, the glycerol content of wines remained stable during storage (Figure 7). The control wine (12.86 g/L) at 6 months storage had a higher glycerol concentration than the sprayed wine (11.95 g/L) at 6 months storage, but differences between Spray treatments were not seen at other storage times. The ethanol content of wines varied slightly during storage but remained mostly stable, and was within the typical range of 9-13% for a dry table wine (Waterhouse et al. 2016). Sprayed wine at 12-months storage (12.27%) had the highest ethanol content, and control wine at 0-months storage (11.21%) had the lowest. Control wine at 6-months storage (12.07%) had a higher ethanol content than sprayed wine at 6-months storage (11.51%), but differences between Spray treatments were not seen at other storage times. Total

residual sugar levels fluctuated slightly over 12-months storage (Figure 8). Control wine at 6-months storage (58.09 mg/100 mL) had a higher total residual sugar content than all the other wines, including the sprayed wine at 6-months storage (52.41 mg/100 mL). There were no differences in total residual sugar concentration between Spray treatments at other storage times. Total residual sugar (fructose) concentrations in Chambourcin wines at all storage times were within the typical range of 20-400 mg/100 mL fructose in dry red wine reported by Liu and Davis (1994) and were below the sensory threshold for fructose of 180-240 mg/100 mL in wine (Hufnagel and Hofmann 2008, Noble and Bursick 1984).

The Spray and Storage main effects were significant for total organic acids. Control wines (1,090.12 mg/100 mL) had higher total organic acids than sprayed wines (978.37 mg/100 mL) across all storage times. Wines at 6-months storage (1,050.57 mg/100 mL) had the highest total organic acid concentration, followed by 0-months storage (1,041.19 mg/100 mL), and 12-months storage (1,010.98 mg/100 mL). Individual organic acids were considered during storage, but followed the same pattern as total organic acids.

Anthocyanins. The 2018 Chambourcin wines were analyzed at 0-, 6-, and 12-months storage at 15°C for individual and total anthocyanins. The Storage main effect was significant for all anthocyanin attributes (Table 6). Malvidin-3-glucoside (20.35 mg/100 mL), malvidin-3,5-diglucoside (43.73 mg/100 mL), petunidin-3,5-diglucoside (11.57 mg/100 mL), delphinidin-3,5-diglucoside (7.62 mg/100 mL), and total anthocyanins (111.33 mg/100 mL) were the highest at 0-months storage, followed by 6-months storage (11.73, 32.47, 7.71, 5.15, and 70.20 mg/100 mL, respectively), and 12-months storage (4.75, 17.32, 4.62, 2.79, and 38.53 mg/100 mL, respectively). A 65% decrease in total anthocyanins was observed from 0-months storage to 12-months storage. This was likely due to the complexation of anthocyanins with other wine

components to form stabilized complexes that preserve wine color but decrease quantifiable monomeric anthocyanin levels (Ballinger et al. 1973, Nagel and Wulf 1979, Scudamore-Smith et al. 1990).

The Spray main effect was significant for petunidin-3,5-diglucoside and delphinidin-3,5-diglucoside. Control wines had higher concentrations of petunidin-3,5-diglucoside (8.12 mg/100 mL) and delphinidin-3,5-diglucoside (5.35 mg/100 mL) than sprayed wines (7.82 and 5.03 mg/100 mL, respectively). However, as the differences in concentration were small and petunidin- and delphinidin-3,5-diglucoside only made up 11% and 7%, respectively, of total anthocyanins across all Spray and Storage treatments, it is unlikely that these difference would affect the visual color of the wines.

Color. The 2018 Chambourcin wines were analyzed at 0-, 6-, and 12-months storage at 15°C for L*, hue angle, chroma, red color, and color density. The Spray x Storage interaction was significant for L* (Table 7). The control (7.77) and sprayed wines (7.63) at 0-months storage had higher L* values (lighter color) than the control and sprayed wines at 6-months storage (6.27 and 6.62, respectively) and 12-months storage (6.28 and 5.83, respectively) (Figure 9). This meant that, regardless of Spray treatment, Chambourcin wines became darker during storage. There were no differences in L* between Spray treatments at any of the storage times. The Storage main effect was significant for hue angle and chroma. The hue angle decreased from 0-months storage (360.35°) to 6-months storage (360.31°), but this decrease was so small that it would likely not have resulted in a visible color change. Chroma of wines decreased from 0-months storage (36.09) to 6-months storage (33.27). This represented a decrease in the saturation of the red color of the wine. As red wines age, anthocyanins form polymeric pigments with other phenolic compounds that can transition the color from pure red to more brick- or orange-red

(Cheynier et al. 2006, 2000, He et al. 2012). This color shift could explain the decrease in chroma as Chambourcin wines aged.

The Spray and Storage main effects were significant for red color. Sprayed wines (4.47) had a higher red color than control wines (4.27). This meant that the application of inactivated yeast to Chambourcin grapevines produced wines with a stronger red color. Red color remained stable from 0-months storage (4.55) to 6-months storage (4.46) but decreased at 12-months storage (4.10). Similar to what was seen with the chroma measurements, this could mean that a shift in color occurred from a purer red to a brick- or orange-red characteristic of well-aged red wines. The Spray x Storage interaction was significant for color density. In general, there was no decrease in color density during storage (Figure 10). Sprayed wine at 0-months storage (7.31) had the highest color density and control wine at 12-months storage (6.66) had the lowest color density. There was no difference between the sprayed and control wine for color density at any of the storage times.

Analysis of aroma attributes (2018)

The aroma attributes of 2018 Chambourcin wines were analyzed at 3-months storage at 15°C. GC-MS analysis was used to identify the volatile aroma compounds in Chambourcin wines, and GC-O analysis was used to determine which of these compounds were odor-active. Of the 56 volatile compounds identified in Chambourcin wines, 10 were odor-active. These compounds included methyl hexanoate (vegetal, green, and roasted aroma), ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, isoamyl acetate, diethyl succinate, and 2-phenylethanol (red fruit, apple, grape-like, and fermented aromas), and isovaleric acid and methionol (cheesy, sweaty, and mushroom aromas). The ethyl esters were the largest class of odor-active compounds and provided the most distinction among Spray treatments. Sprayed

wines were associated with higher amounts of ethyl esters, and therefore these compounds were chosen for quantitation. The concentrations of ethyl esters in Chambourcin wines were consistent with previously-reported values for wines. The sprayed wines contained higher amounts of ethyl butanoate, hexanoate, and octanoate at 3-months storage at 15°C. Therefore, Chambourcin wines from inactivated yeast-treated grapevines could have more impactful red- and dark-fruit aromas.

Determination of odor-active compounds. In 2018, Chambourcin wines were analyzed at 3-months storage at 15°C for odor-active volatile compounds. There were 56 volatile compounds positively identified in Chambourcin wines by GC-MS (data not shown). Initial exploration of GC-MS volatile aroma chromatograms showed that sprayed and control wines had similar chromatogram peaks, but peak areas differed (data not shown). Therefore, odor-active compound analysis was only done with sprayed wines to minimize panelist fatigue. Five trained panelists evaluated the odor-active compounds in Chambourcin wine using a GC-O instrument equipped with an olfactory detection port. Panelists indicated when they detected an odor and described the odor if possible. Volatile compounds were extracted from varying amounts of sample (50-500 μL) to determine which compounds were the most odor-active even at low concentrations.

There were a total of 10 odor-active compounds identified in Chambourcin wines (Table 8). The largest class of odor-active compounds was the ethyl esters. Ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate were detected by a majority of panelists (NIF > 60%) in the 500 and 100 μL samples. Ethyl butanoate, hexanoate, and decanoate were detected by a majority of panelists in the 50 μL sample, and ethyl hexanoate was detected by a majority of panelists in the 10 μL sample. Therefore, ethyl hexanoate (apple, fresh, artificial, and red fruit aroma) was likely the most impactful of the ethyl esters in Chambourcin wine. The ethyl esters were described as having red fruit, fermented fruit, and grape-like aromas. Delaquis et al. (2000)

identified these same ethyl esters as being major aroma compounds in wines produced from Chancellor grapes (Seibel 5163 x Seibel 880), a red wine grape interspecific hybrid that is one of the parents of Chambourcin (Robinson et al. 2012). Methyl hexanoate, a methyl ester, was odor-active at all tested sample levels and was perceived as having vegetal, bread dough, and green aromas. Isoamyl acetate (acetate ester), diethyl succinate (diethyl ester), and 2-phenylethanol (primary alcohol) were also among the fruity-smelling compounds detected in Chambourcin wines. Isoamyl acetate (banana, pear, apple, artificial, and ripe aroma) and 2-phenylethanol (rose, honey, fermented, and wine-like aroma) were detected by a majority of panelists at all tested sample levels and diethyl succinate (fruity, flowery, spicy, and roasted aroma) was detected by a majority of panelists in the 500 μL sample only. Isovaleric acid (cheese, sweat, and vomit aroma) was detected by a majority of panelists in all samples, and methionol (mushroom, fatty, and musty aroma) was detected by a majority of panelists in the 500, 100, and 50 μL samples.

In order to determine which odor-active compounds provided the best distinction between sprayed and control Chambourcin wines, a PCA was done using the TIC areas of the 10 odor-active compounds from GC-MS analysis of sprayed and control wines. Two components explained over 80% of the variation in the data (Figure 11). The ethyl esters (ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate) and methyl hexanoate had positive loadings on PC1, and all other compounds (isoamyl acetate, diethyl succinate, 2-phenylethanol, isovaleric acid, and methionol) had negative loadings on PC1. Therefore, PC1 represented high amounts of methyl- and ethyl-esters. All compounds had positive loadings on PC2, and therefore PC2 represented high levels of odor-active compounds in general. The sprayed wines had a positive loadings on PC1 and PC2, and the control wines had negative loadings on both

components. Therefore, it is possible that inactivated yeast application produced wines with higher amounts of fruity-smelling ester compounds and higher overall aroma impact. Ethyl esters were selected for quantitation in Chambourcin wines, as this class of compounds appeared to provide the best distinction between Spray treatments based on GC-MS peak area.

Quantitation of ethyl esters. Ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate were quantified in 2018 Chambourcin wines at 3-months storage at 15°C. The data on ethyl esters in this section will be discussed in text (data not shown). Wines contained 0.6-0.7 mg/L ethyl butanoate, 0.6-0.7 mg/L ethyl hexanoate, 0.4-0.6 mg/L ethyl octanoate, and 0.2 mg/L ethyl decanoate. The concentrations of ethyl esters in the present study were close to the values reported by Benkwitz et al. (2012) and Ferreira et al. (2000), who found 0.1-0.5 mg/L ethyl butanoate, 0.2-1.5 mg/L ethyl hexanoate, 0.1-2.5 mg/L ethyl octanoate, and 0.01-0.9 mg/L ethyl decanoate in Spanish red wines and New Zealand Sauvignon blanc wines (*V. vinifera*). In addition, the concentrations of ethyl butanoate, hexanoate, and octanoate in Chambourcin wines at 3-months storage were above the threshold values of 0.02 mg/L, 0.01 mg/L, and 0.005 mg/L, respectively, reported for these compounds in an artificial wine matrix (Ferreira et al. 2002, Juan et al. 2012). The concentration of ethyl decanoate was at the threshold value of 0.2 mg/L. Slegers et al. (2015) determined the ethyl ester content of five red wines produced from interspecific hybrid grapes. Frontenac (Landot Noir x *V. riparia*), Marquette (MN-1094 x Ravat 262), Maréchal Foch (Millardet et Grasset 101-14 OP x Goldriesling), Sabrevois (Elmer Swenson 283 x Elmer Swenson 193), and St. Croix (Elmer Swenson 283 x Elmer Swenson 193) contained 0.05-0.3 mg/L ethyl butanoate, 0.2-0.8 mg/L ethyl hexanoate, 0.6-2.4 mg/L ethyl octanoate, and 0.06-0.4 mg/L ethyl decanoate. Wines produced from Chambourcin, also an interspecific hybrid, in the present study contained similar concentrations.

The Spray main effect was significant for ethyl butanoate, ethyl hexanoate, and ethyl octanoate. The sprayed wines had higher concentrations of ethyl butanoate (0.73 mg/L), ethyl hexanoate (0.74 mg/L), and ethyl octanoate (0.55 mg/L) than control wines (0.61, 0.61, and 0.39 mg/L, respectively). This was especially significant, as these esters were present at suprathreshold concentrations in Chambourcin wines, meaning that they could potentially give sprayed wines higher perceivable fruit aromas. There was no effect of Spray on ethyl decanoate. The concentration of ethyl esters in wines is influenced by must composition, oxygen availability, temperature, and yeast strain (Waterhouse et al. 2016). Therefore, it is possible that application of an inactivated yeast to Chambourcin grapevines yielded wines with more ideal must composition for ester production during fermentation. Šuklje et al. (2016) evaluated the application of inactivated yeast to Sauvignon Blanc grapes grown in South Africa and analyzed the volatile aroma compound profiles and sensory attributes of sprayed and control wines. While sprayed wines 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 thresholds.

In most GC-O studies on red wines, ethyl butanoate, ethyl hexanoate, and ethyl octanoate are listed as some of the most potent compounds. While the removal of these individual compounds generally does not have a noticeable impact on wine aroma, in combination they appear to be responsible for the red- and dark-fruit aromas of wines (Lytra et al. 2012). Therefore, it is possible that Chambourcin wines from grapevines treated with an inactivated yeast will have more impactful red- and dark-fruit aromas.

Sensory attributes analysis (2018)

Descriptive sensory evaluation and an industry sensory panel were conducted for Chambourcin wines at 6-8-months storage at 15°C. Descriptive panelists developed a lexicon for Chambourcin wine appearance (n = 1), aroma (n = 19), aromatic (n = 19), basic taste (n = 2), and mouthfeel (n = 2) attributes using Chambourcin wines produced from grapes grown in Arkansas. Chambourcin wines had dark- and red-fruit and woody aromas and aromatics. Panelists in both the descriptive and industry sensory panels were unable to consistently identify differences in sensory attributes between sprayed and control wines. The descriptive panel rated sprayed wines as having a higher red color than control wines, and sprayed wines had higher mouthfeel liking ratings in the industry sensory panel. However, these differences were only significant at the $p < 0.10$ level.

Descriptive sensory evaluation. The appearance, aroma, aromatics, basic tastes, and mouthfeel of 2018 Chambourcin wines were evaluated by a descriptive sensory panel at 6-months storage at 15°C. During orientation and training, 11 trained panelists created a descriptive sensory lexicon for appearance and aroma attributes and nine trained panelists created a descriptive sensory lexicon for aromatics, basic taste, and mouthfeel attributes using the Chambourcin wines from this study (Table 2). The panelists used the lexicon to evaluate the sprayed and control Chambourcin wines in duplicate using a scale where 1 was less of an attribute and 15 was more of an attribute. There was one appearance attribute, 19 aroma attributes, 19 aromatics attributes, two basic taste attributes, and two mouthfeel attributes.

The Panelist main effect was significant for a majority of descriptive sensory attributes, indicating that panelists varied among themselves in their ratings of the same wines (data not shown). This panelist-to-panelist difference is commonly reported in the literature, however, and

can be attributed to individual physiological and scoring differences (Delaquis et al. 2000, Guinard and Cliff 1987, Sivertsen and Risvik 1994). The Panelist x Spray interaction was only significant for berry aroma, floral aroma, and acetone aromatics (data not shown). This meant that, in general, panelists were consistent in their ratings for the replicates of each wine and indicated that the training of the descriptive panel was adequate (Biasoto et al. 2014).

There were no differences among Spray treatments for any of the attributes evaluated by the descriptive sensory panel at the $p < 0.05$ significant level. However, there were some attributes significant at the $p < 0.10$ level, including red color, floral aroma, and acetone aromatics. These differences will be discussed as they represent potential trends in the data.

The panelists evaluated the appearance (red color) of the surface color of Chambourcin wine in a glass test-tube tilted at an angle against a white background. Red color was rated 7.8-8.2 for all wines on the 15-point scale (Figure 12a). The sprayed wines were rated with a higher red color (8.2) than control wines (7.8) ($p = 0.0739$). This was consistent with the higher spectrophotometric red color seen in 2018 sprayed wines. The aroma attributes included ethanol, overall fruit, black currant, berry, dried fruit, citrus fruit, overall woody, smokey, oak, floral, canned vegetables, bell pepper, grassy, earthy, black pepper, sulfur, tobacco, acetone, and vinegar. The highest-rated aromas (> 2.5) were ethanol, overall fruit, overall woody, black currant, dried fruit, and berry. This indicated that Chambourcin wines had dark- and red-fruit and fresh-cut wood-like aroma notes. This was consistent with the supra-threshold levels of dark- and red-fruit aroma ethyl esters identified as odor-active in Chambourcin wines. Control wines (1.4) had higher floral aromas than sprayed wines (1.1) ($p = 0.0534$), but panelists did not detect differences between Spray treatments for other aroma attributes. Citrus, canned vegetable, bell

pepper, grassy, earthy, smokey, tobacco, and sulfur aromas were the lowest-rated aromas (≤ 0.5), indicating that Chambourcin wines had very low intensity for these aromas.

The aromatic attributes evaluated by panelists included ethanol, overall fruit, black currant, berry, dried fruit, citrus fruit, overall woody, smokey, oak, floral, canned vegetables, bell pepper, grassy, earthy, black pepper, sulfur, tobacco, acetone, and vinegar. Similar to the aroma evaluation, ethanol, overall fruit, overall woody, oak, dried fruit, black currant, and berry aromatics had intensity ratings > 2 (Figure 12b). This indicated that Chambourcin wines had dark- and red-fruit and woody aromatics and aroma notes. Reynolds et al. (2004) found that Chancellor wines (a parent of Chambourcin) also had berry, black currant, and earthy/woody orthonasal and retronasal aromas. Control wines (2.3) had a higher acetone aromatic note than sprayed wines (1.9) ($p = 0.0833$). This could indicate that control wines had higher concentrations of ethyl acetate and/or acetic acid. These compounds are responsible for the perception of volatile acidity, one of the most common wine faults characterized by nail polish remover or vinegar off-aromas/aromatics (Fugelsang and Edwards 2007). However, there was no perceived difference in the vinegar aromatic intensity between sprayed and control wines, and panelists did not detect differences between sprayed and control wines for other aromatic attributes. Citrus, floral, canned vegetable, bell pepper, grassy, earthy, smokey, tobacco, and sulfur aromatics were the lowest-rated (<1), indicating that Chambourcin wines had very low intensities for these aromas.

The basic taste attributes evaluated by panelists included sourness (5.8-6.3) and bitterness (3.2-3.4). It was of note that no sweetness was detected in Chambourcin wines, and this coincided with the sub-detection-threshold concentrations of total residual sugars found in wines. Panelists did not detect differences between sprayed and control wines for basic taste attributes.

The mouthfeel attributes evaluated by panelists included astringency and length of finish, where length of finish was the time that aromatics lingered in the mouth after swallowing (Table 2). Chambourcin wines had an astringency of 9.9-10.2. The length of finish of Chambourcin wines was 9.7-10.6, which coincided with aromatics lingering in the mouth 9.7-10.6 seconds after swallowing. Panelists did not detect differences between sprayed and control wines for mouthfeel attributes.

Industry sensory panel. The liking and intensity of 2018 Chambourcin wine color, aroma, flavor, mouthfeel, and overall impression were evaluated by an industry panel at 6-8 months storage at 15°C. There were a total of 106 panelists from various locations across North America (Arkansas, California, New Mexico, Pennsylvania, Texas, and Canada) and Europe (Austria and Spain). All panelists were experienced in wine tasting and were from the grape/wine industry or related academia. Liking of Chambourcin wine color, aroma, flavor, mouthfeel, and overall impression were evaluated using a nine-point hedonic scale (Figure 3). Similar to the descriptive sensory analysis, no hedonic-scaled attributes in the industry sensory panel were significant at the $p < 0.05$ level. However, the Spray main effect was significant as $p < 0.01$ for mouthfeel. Acceptance of wine color, aroma, flavor, and mouthfeel intensities were evaluated using a JAR scale. After evaluating both wines, panelists were instructed to indicate which wine they preferred.

On average, the color of wines was scored “like moderately”, the aroma was scored “like slightly”, and the flavor, mouthfeel, and overall impression was scored “neither like nor dislike” (data not shown). This indicated that panelist reactions to Chambourcin wines were generally neutral to positive. A possible explanation for the lower-than-optimal ratings for Chambourcin wines is that the wines were not finished for commercial sale. Chambourcin wines in this study

were fairly sour, and wines produced from Chambourcin grapes are known for their high acid retention and sourness (Homich et al. 2016). To reduce the perceived acidity of Chambourcin wines, winemakers could add small amounts of sugar prior to bottling to balance sourness and mouthfeel. Industry panelists in this study were told that the wines were not commercially finished and were instructed to evaluate wines as if they were preliminary tank samples, focusing less on the sour taste and more on the aroma and aromatics. However, many panelists remarked that they disliked the wines because they were too sour, likely skewing the results towards the lower end of the scale. Sprayed wines were scored as having a more pleasant mouthfeel than control wines ($p = 0.0910$; Figure 13). Therefore, it is possible that the application of an inactivated yeast to Chambourcin grapevines produced wines with better mouthfeel. The perception of mouthfeel in wine can be influenced by several factors, including condensed tannin, ethanol, residual sugar, and glycerol concentrations (Hufnagel and Hofmann 2008, Noble and Bursick 1984, Peleg et al. 1999, Robichaud and Noble 1990).

For both Spray treatments, a majority of panelists scored the intensity of Chambourcin wine color and aroma as being JAR (Table 9). Seventy-five percent of panelists rated the color intensity of control wine JAR, and 76% rated the color intensity of sprayed wine JAR. For control wine, 70% of panelists rated aroma intensity JAR and 16% rated it too low, whereas 57% rated the aroma intensity of sprayed wine JAR and 29% rated it too low. This indicated that panelists thought the aroma intensity of the control wine was more appropriate and that the aroma intensity of sprayed wine was too low for a Chambourcin wine. For flavor intensity, 37-43% of panelists rated Chambourcin wines JAR, 36-38% rated wines too low, and 21-25% rated wines too high. Therefore, the perception of flavor intensity was roughly split between too low and JAR for Chambourcin wines. There did not appear to be major differences between sprayed

and control wines for flavor intensity. For mouthfeel intensity, 40% of panelists rated control wine JAR and 49% rated sprayed wine JAR. This was consistent with the findings of the hedonic-scaled questions, where sprayed wines had significantly higher ratings for mouthfeel liking than control wines. Thirty-three percent of panelists rated the mouthfeel of control wine as too weak, and 26% rated the mouthfeel of sprayed wine as too weak. Therefore, it was possible that the lower hedonic scores for control wine mouthfeel were because the mouthfeel was weaker.

Industry sensory panelists did not have a preference for either the sprayed or control wine ($\chi^2 = 0.25$; data not shown). Panelists' preference of sprayed or control Chambourcin wines varied depending on the test location (Figure 14). Panelists in Canada (60%), New Mexico (62%), and Texas (73%) preferred the sprayed wines, whereas panelists in Arkansas (73%), Austria (60%), California (63%), and Pennsylvania (60%) preferred the control wines. Panelists in Spain had equal preferences for sprayed and control wines. Therefore, the results of the industry sensory panel did not show that application of an inactivated yeast improved the sensory attributes of Chambourcin wine, other than a slightly higher liking of the mouthfeel.

Conclusions

In both 2018 and 2019, Chambourcin wines had compositions at bottling within typical ranges for a dry red table wines, remaining mostly stable during one year of storage at 15°C. Sprayed wines had higher tartaric acid and lower citric and lactic acids than control wines at 0-months storage in both years.

A mixture of monoglucoside and diglucoside anthocyanins and their coumaroyl derivatives, typical of hybrid grapes and wine, was identified in Chambourcin wines. Malvidin-

3,5-diglucoside was the predominant anthocyanin. Wines from 2018 had higher total and individual anthocyanins than 2019 wines, but 2019 wines had higher red color and color density. There was no difference in total anthocyanins between Spray treatments in 2018, but sprayed wines had higher levels of individual and total anthocyanins than control wines in 2019. Sprayed wines had a higher red color than control wines in 2018, but less pure red chroma in 2019. This could signify a shift in color due to the formation of stable polymeric pigments. Anthocyanins decreased during storage, and the red hue of the wine became less pure, possibly indicating a shift to the brick- or orange-red colors characteristic of well-aged red wine.

Of the odor-active compounds identified in Chambourcin wines, the ethyl esters (dark- and red-fruit aromas) were some of the most impactful aromas. Sprayed wines had higher concentrations of ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate than control wines at 3-months storage. This indicated that inactivated yeast application could increase the dark- and red-fruit aromas of Chambourcin wines. The fruity character of Chambourcin wines was confirmed by sensory analysis, where overall fruit, dried fruit, berry, and black currant aroma and aromatic notes were among the highest-rated in descriptive analysis. Sprayed wines were perceived as having a more intense red color, less acetone off-flavors, and better mouthfeel than control wines. However, panelists in both the descriptive and industry sensory panels were unable to consistently identify differences in sensory attributes between sprayed and control wines.

This is the first data on the use of a specific inactivated yeast on Chambourcin grapevines, but it shows potential for wines with higher anthocyanins, deeper red color, higher amounts of fruity, fresh ester aromas, and improved sensory attributes. However, results were inconsistent among years.

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Tables

Table 1. Initial composition of Chambourcin grape musts from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[a] (2018 and 2019).

Harvest date	Spray treatment	Soluble solids (%)	pH	Titratable acidity (%)
27 August 2018	Control	20.4	3.4	1.0
	Sprayed	20.2	3.3	1.0
29 August 2019	Control	18.5	3.6	0.9
	Sprayed	19.0	3.5	0.9

[a] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

Table 2. Lexicon developed for Arkansas-grown Chambourcin wine appearance, aroma, aromatics, basic taste, and mouthfeel attributes by a trained descriptive sensory panel with 9-11 panelists.

Term	Definition	Technique	Reference
Appearance (wine tilted in glass tube against white background)			
Color- red	Surface color of the red wine		Royal Horticultural Society color chart greyed-purple group 187D=4 and 187B=10[a]
Aroma			
Ethanol	Pungent aroma of ethanol	Solutions of ethanol in water	10%=5 and 20%=10
Overall fruit	General fruit-like aroma	General fruit	Universal scale[b]
Dried fruit	Aroma of dried fruit	Raisins or prunes	Universal scale
Berry	Aroma of berries	Strawberry or raspberry	Universal scale
Black currant	Aroma of black currant	Black currant	Universal scale
Citrus	Aroma of citrus fruits	Orange or lemon	Universal scale
Floral	Aroma of flowers	Floral	Universal scale
Canned vegetable	Aroma of cooked/canned vegetables	Canned corn or asparagus	Universal scale
Bell pepper	Aroma of green bell peppers	Green bell pepper	Universal scale
Grassy	Aroma of fresh-cut vegetation	Fresh-cut grass	Universal scale
Earthy	Aroma of damp soil or wet foliage	Damp potting soil	Universal scale
Black pepper	Aroma of black pepper	Ground black pepper	Universal scale
Overall woody	Aroma of dry fresh-cut wood	Balsamic or bark-like	Universal scale
Smokey	Aroma of smoke from burning wood	Wood smoke	Universal scale
Oak	Aroma of oak wood	Toasted oak chips	Universal scale
Tobacco	Aroma of fresh, unburned tobacco	Dried pipe tobacco	Universal scale
Sulfur	Sulfur-like aroma of alliums	Onions or garlic	Universal scale
Acetone	Aroma of ketones, specifically acetone	Nail polish remover	Universal scale
Vinegar	Aroma of vinegar	White vinegar	Universal scale
Aromatics			
Ethanol	Pungent aromatic note of ethanol	Solutions of ethanol in water	10%=5 and 20%=10
Overall fruit	General fruit-like aroma	General fruit	Universal scale
Dried fruit	Aromatic note of dried fruit	Raisins or prunes	Universal scale
Berry	Aromatic note of berries	Strawberry or raspberry	Universal scale
Black currant	Aromatic note of black currant	Black currant	Universal scale
Citrus	Aromatic note of citrus fruits	Orange or lemon	Universal scale
Floral	Aromatic note of flowers	Floral	Universal scale

Table 2 (Cont.)

Term	Definition	Technique	Reference
Canned vegetable	Aromatic note of cooked/canned vegetables	Canned corn or asparagus	Universal scale
Bell pepper	Aromatic note of green bell peppers	Green bell pepper	Universal scale
Grassy	Aromatic note of fresh-cut vegetation	Fresh-cut grass	Universal scale
Earthy	Aromatic note of damp soil or wet foliage	Damp potting soil	Universal scale
Black pepper	Aromatic note of black pepper	Ground black pepper	Universal scale
Overall woody	Aromatic note of dry fresh-cut wood	Balsamic or bark-like	Universal scale
Smokey	Aromatic note of smoke from burning wood	Wood smoke	Universal scale
Oak	Aromatic note of oak wood	Toasted oak chips	Universal scale
Tobacco	Aromatic note of fresh, unburned tobacco	Dried pipe tobacco	Universal scale
Sulfur	Sulfur-like aromatic note of alliums	Onions or garlic	Universal scale
Acetone	Aromatic note of ketones, specifically acetone	Nail polish remover	Universal scale
Vinegar	Aromatic note of vinegar	White vinegar	Universal scale
Basic tastes			
Sour	Basic taste, perceived on the tongue, stimulated by acids, such as citric acid	Solutions of citric acid in water	0.05%=2.0, 0.08%=5.0, 0.15%=10.0, and 0.20%=15.0
Bitter	Basic taste, perceived on the tongue, stimulated by substances such as quinine, caffeine, and certain other alkaloids	Solutions of caffeine in water	0.05%=2.0, 0.08%=5.0, 0.15%=10.0, 0.20%=15.0
Mouthfeel			
Astringency	Chemical feeling factor on the tongue or other skin surfaces of the mouth described as puckering or drying	Grape juice	Grape juice=10.0
Finish	Length of time that aromatics linger in the mouth after swallowing	Length of finish	0 seconds, 7.5 seconds, 15 seconds

[a] Intensities based on standardized colors presented in the Royal Horticultural Society (RHS) color chart (RHS Colour Chart 2007).
[b] Intensities based on universal scale (saltine cracker=3.0; applesauce=7.0; orange juice=10.0; grape juice=14.0; Big Red Gum®=15.0).

Table 3. Supplies included in kits for industry sensory panel evaluation of wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[a].

Supplies
1 x 750-mL bottle of each wine (sprayed and control wines)
Paperwork
Informed consent sheet for panelist signatures
Instructions for panel leader
Paper ballots, pre-labeled with sample codes indicating sample order
Envelope with pre-paid postage for mailing completed ballots back to UA System Department of Food Science
Miscellaneous supplies
Pour spouts for wine bottles
Plastic cups marked with 30-mL line for filling wine glasses
Food-grade plastic discs to cover wine glasses and prevent aroma dissipation
Plastic water cups for cleansing palate between samples
Unsalted crackers for cleansing palate between samples
Plastic expectorant cups
Marker to label wine glasses with sample code
Pens for completing ballots

[a] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

Table 4. Main effect from ANOVA for Spray on wine composition, anthocyanin, and color attributes at 0 months storage at 15°C for wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[a] (2018 and 2019).

	2018			2019		
Attribute	**Control**	**Sprayed**	*P value*	**Control**	**Sprayed**	*P value*
Composition						
pH	3.39 a[b]	3.43 a	*0.0794*	3.46 a	3.46 a	*0.9797*
Titratable acidity (%)	0.72 a	0.72 a	*0.8078*	0.72 a	0.71 a	*0.4814*
Glycerol (g/L)	12.50 a	12.43 a	*0.6588*	12.98 a	12.58 a	*0.4709*
Ethanol (% v/v)	11.21 a	11.59 a	*0.0981*	12.29 a	12.34 a	*0.3575*
Total residual sugars (mg/100 mL)	52.84 a	50.63 a	*0.1366*	65.65 b	74.86 a	***0.0117***
Tartaric acid (mg/100 mL)	84.16 b	98.37 a	***0.0001***	296.32 b	313.76 a	***0.0158***
Malic acid (mg/100 mL)	51.99 a	35.11 a	*0.2923*	26.66 a	19.29 a	*0.7293*
Citric acid (mg/100 mL)	53.77 a	49.20 b	***0.0045***	36.17 a	29.33 b	***0.0004***
Succinic acid (mg/100 mL)	384.18 a	342.18 b	***0.0055***	418.17 a	434.59 a	*0.0686*
Lactic acid (mg/100 mL)	523.47 a	459.93 b	***0.0010***	512.36 a	471.85 b	***0.0002***
Total organic acids (mg/100 mL)	1097.58 a	984.80 b	***0.0017***	1289.69 a	1268.81 a	*0.4159*
Anthocyanins						
Malvidin-3-glucoside (mg/100 mL)	20.06 a	20.64 a	*0.3500*	12.91 b	15.80 a	***0.0113***
Malvidin-3,5-diglucoside (mg/100 mL)	43.68 a	43.78 a	*0.8800*	36.94 b	40.79 a	***0.0009***
Petunidin-3,5-diglucoside (mg/100 mL)	11.84 a	11.30 a	*0.0559*	9.35 b	10.68 a	***<0.0001***
Delphinidin-3,5-diglucoside (mg/100 mL)	7.85 a	7.39 a	*0.1652*	5.53 b	6.05 a	***0.0322***
Total anthocyanins (mg/100 mL)	111.52 a	111.14 a	*0.8529*	83.25 b	96.20 a	***0.0014***
Color						
L*	7.77 a	7.63 a	*0.5272*	7.24 a	8.43 a	*0.2961*
Hue angle (°)[c]	360.35 a	360.34 a	*0.5663*	360.31 a	360.12 b	***0.0071***
Chroma	35.96 a	36.23 a	*0.6278*	35.21 a	19.29 b	***0.0008***
Red color[d]	4.40 a	4.69 a	*0.0642*	5.06 a	5.64 a	*0.2424*
Color density[e]	7.03 a	7.31 a	*0.1689*	8.01 a	8.86 a	*0.2191*

[a] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

[b] Means with different letters for each attribute within years are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

[c] Hue angles <90° were subjected to a 360° compensation to account for discrepancies between red samples near 0° and those near 360°.

[d] Red color was calculated as absorbance of wine at 520 nm.

[e] Color density was calculated as absorbance 520 nm + absorbance 420 nm.

Table 5. Main and interaction effects from ANOVA for Spray and Storage (0, 6, and 12 months at 15°C) on wine composition attributes for wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[a] (2018).

Effects	**pH**	**Titratable acidity (%)**	**Glycerol (g/L)**	**Ethanol (% v/v)**	**Total residual sugars (mg/100 mL)**	**Total organic acids (mg/100 mL)**
Spray						
Control	3.43 a[b]	0.71 a	12.64 a	11.70 a	52.41 a	1090.12 a
Sprayed	3.44 a	0.68 b	12.42 b	11.79 a	50.49 b	978.37 b
P value	*0.5257*	***0.0265***	***0.0333***	*0.3218*	***0.0239***	***<0.0001***
Storage						
Month 0	3.41 b	0.72 a	12.46 ab	11.40 b	51.74 b	1041.19 ab
Month 6	3.53 a	0.69 ab	12.41 b	11.79 a	55.25 a	1050.57 a
Month 12	3.36 c	0.67 b	12.72 a	12.05 a	47.37 c	1010.98 b
P value	***<0.0001***	***0.0027***	***0.0277***	***<0.0001***	***<0.0001***	***0.0375***
Spray x Storage (P value)	*0.1392*	***0.0302***	***0.0001***	***0.0002***	***0.0026***	*0.5904*

[a] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

[b] Means with different letters for each attribute within effects are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

Table 6. Main and interaction effects from ANOVA for Spray and Storage (0, 6, and 12 months at 15°C) on wine anthocyanin attributes for wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[a] (2018).

Effects	**Malvidin-3-glucoside (mg/100 mL)**	**Malvidin-3,5-diglucoside (mg/100 mL)**	**Petunidin-3,5-diglucoside (mg/100 mL)**	**Delphinidin-3,5-diglucoside (mg/100 mL)**	**Total anthocyanins (mg/100 mL)**
Spray					
Control	12.44 a	31.39 a	8.12 a	5.35 a	73.62 a
Sprayed	12.12 a	30.95 a	7.82 b	5.02 b	73.09 a
P value	*0.1565*	*0.5948*	***0.0066***	***0.0076***	*0.6066*
Storage					
Month 0	20.35 a	43.73 a	11.57 a	7.62 a	111.33 a
Month 6	11.73 b	32.47 b	7.71 b	5.15 b	70.20 b
Month 12	4.75 c	17.32 c	4.62 c	2.79 c	38.53 c
P value	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
Spray x Storage (P value)	*0.6504*	*0.6937*	*0.2629*	*0.3798*	*0.7577*

[a] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

[b] Means with different letters for each attribute within effects are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

Table 7. Main and interaction effects from ANOVA for Spray and Storage (0, 6, and 12 months at 15°C) on wine color attributes for wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[a] (2018).

Effects	L*	Hue angle (°)[b]	Chroma	Red color[c]	Color density[d]
Spray					
Control	6.77 a	360.32 a	34.21 a	4.27 b	6.97 b
Sprayed	6.69 a	360.32 a	34.05 a	4.47 a	7.14 a
P value	*0.5211*	*0.6356*	*0.6403*	***0.0013***	***0.0342***
Storage					
Month 0	7.70 a	360.35 a	36.09 a	4.55 a	7.17 a
Month 6	6.44 b	360.31 b	33.27 b	4.46 a	7.15 a
Month 12	6.06 c	360.30 b	33.02 b	4.10 b	6.83 b
P value	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***0.0024***
Spray x Storage (P value)	***0.0342***	*0.1848*	*0.0768*	*0.0893*	***0.0478***

[a] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

[b] Hue angles <90° were subjected to a 360° compensation to account for discrepancies between red samples near 0° and those near 360°.

[c] Red color was calculated as absorbance of wine at 520 nm.

[d] Color density was calculated as absorbance 520 nm + absorbance 420 nm.

[e] Means with different letters for each attribute within effects are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

Table 8. Compound class, nasal impact factors[a], and odor descriptors[b] for odor-active compounds identified by gas chromatography-olfactometry (GC-O) and GC-mass spectrometry (GC-MS) in wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[c] at 3 months storage at 15°C (2018).

		Nasal impact factor (%)				
Compound class	**Compound**	**500 µL**	**100 µL**	**50 µL**	**10 µL**	**Odor descriptors**
Methyl ester	Methyl hexanoate	100	100	100	100	Vegetal, bread dough, leaves, green
Ethyl ester	Ethyl butanoate	100	60	80	40	Red fruit, strawberry, artificial, bubblegum
	Ethyl hexanoate	100	80	60	80	Apple, fresh, artificial, red fruit
	Ethyl octanoate	60	60	40	20	Wine, fermented fruit, grape, caramel
	Ethyl decanoate	100	100	80	0	Grape juice, wine, red fruit, cherries
Acetate ester	Isoamyl acetate	60	80	60	80	Banana, pear, apple, artificial, ripe
Diethyl ester	Diethyl succinate	60	20	20	20	Fruity, flowery, spicy, roasted
Primary alcohol	2-Phenylethanol	80	80	100	100	Rose, honey, fermented, wine
Fatty acid	Isovaleric acid	80	100	100	100	Cheese, sweat, vomit
Alkyl sulfide	Methionol	80	60	80	40	Mushrooms, fatty, musty

[a] Nasal impact factors were calculated as a percentage of the panelists that detected the volatile compound in each sample. Nasal impact factors > 60% were considered a positive identification.

[b] Odor descriptors were determined based on panelists' descriptions odors perceived at the olfactory detection port.

[c] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

Table 9. Percent (%) of responses for industry sensory panel analysis of color, aroma, flavor, and mouthfeel intensity using a collapsed five-point just-about-right (JAR) scale[a] at 6-8 months storage at 15°C for wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[b] (2018).

Wine	Color intensity			Aroma intensity			Flavor intensity			Mouthfeel intensity		
	Too light	JAR	Too dark	Too low	JAR	Too much	Too low	JAR	Too much	Too weak	JAR	Too strong
Control	16	75	9	16	70	14	38	37	25	33	40	27
Sprayed	20	76	4	29	57	14	36	43	21	26	49	25

[a] Wines were evaluated by 106 industry panelists. The five-point JAR scale (1=much too low; 2= too low; 3= just about right; 4=too much; 5=much too much) was collapsed to Too low, JAR, and Too much.
[b] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

Figures

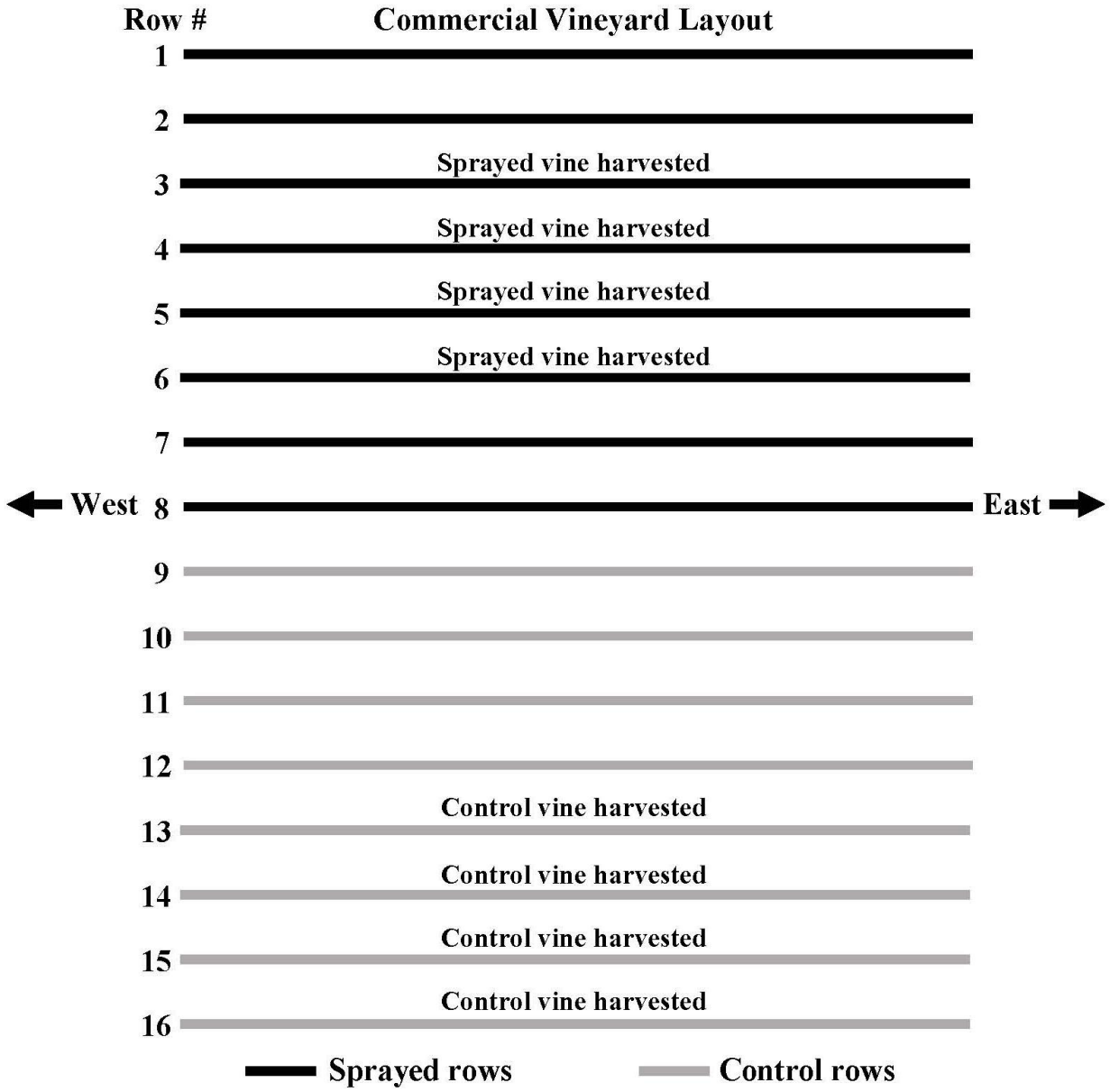

Figure 1. Vineyard layout of Chambourcin grapevine rows[a] harvested from a commercial vineyard in Hindsville, Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[b] (2018 and 2019).
[a] Rows were approximately 200-m long with east-west orientation.
[b] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

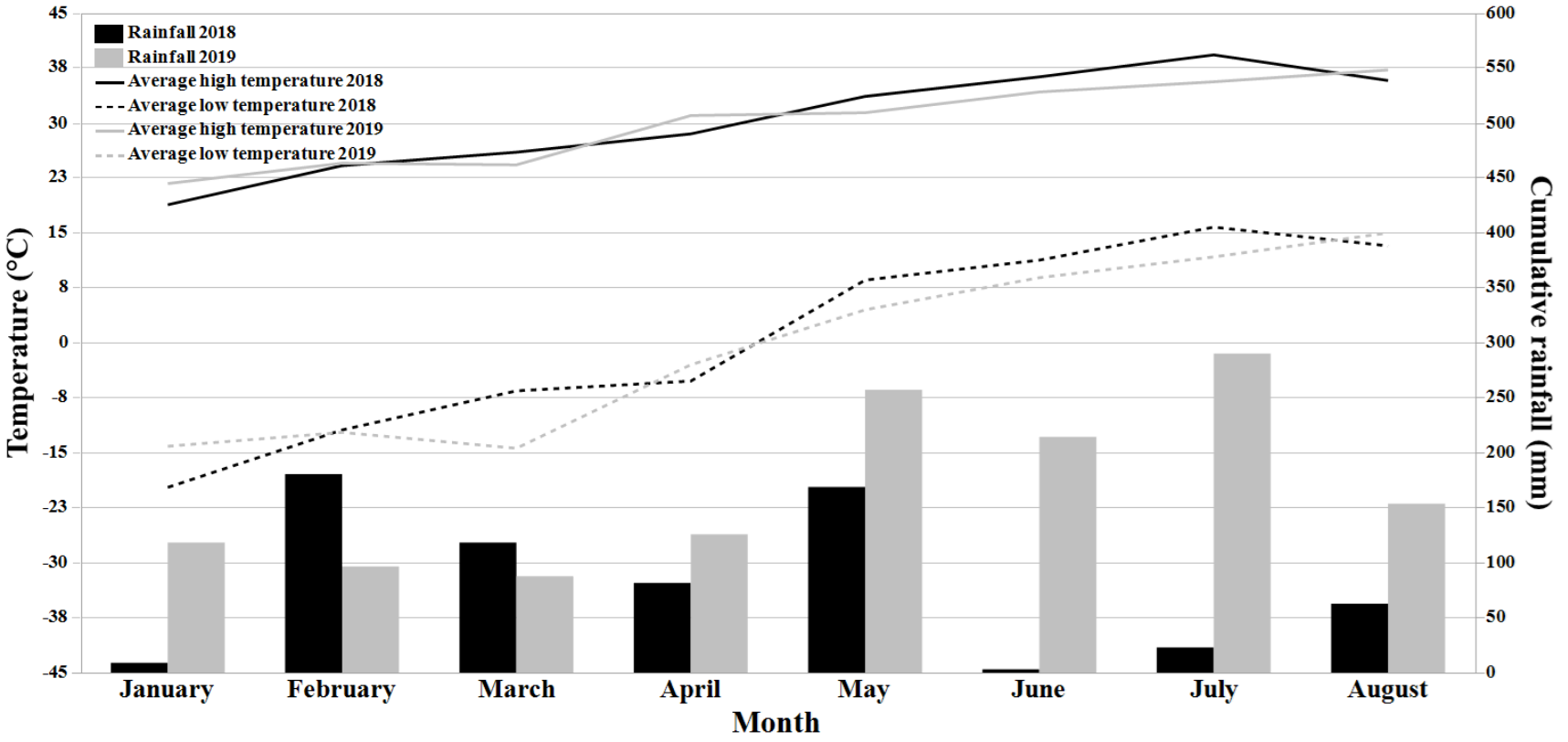

Figure 2. Average monthly high and low temperatures and cumulative rainfall[a] from January-August 2018 and 2019 near Hindsville, AR.

[a] Data was gathered from a weather station in Huntsville, AR (https://www.wunderground.com/dashboard/pws/K).

To:	Renee Terrell Threlfall FDSC B-3
From:	Douglas James Adams, Chair IRB Committee
Date:	04/09/2019
Action:	**Exemption Granted**
Action Date:	04/09/2019
Protocol #:	1903181159
Study Title:	Identify wine attributes from application of inactive dry yeast in a commercial French-American hybrid vineyard

The above-referenced protocol has been determined to be exempt.

If you wish to make any modifications in the approved protocol that may affect the level of risk to your participants, you must seek approval prior to implementing those changes. All modifications must provide sufficient detail to assess the impact of the change.

If you have any questions or need any assistance from the IRB, please contact the IRB Coordinator at 109 MLKG Building, 5-2208, or irb@uark.edu.

cc: Sarah Mayfield, Key Personnel

Figure 3. University of Arkansas Institutional Review Board (IRB) protocol approval notice for sensory analysis of wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[a].
[a] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

Sample **542**

COLOR: Tilt the glass and observe the **COLOR** of the wine against a white background.

Which statement best describes your impression of the **COLOR** of this wine? (check one)

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

Considering the **COLOR** of the wine, which statement best describes your impression of this wine? (check one)

Much too Light	Too Light	Just About Right	Too Dark	Much too Dark
☐	☐	☐	☐	☐

What do you ***LIKE*** about the color of the wine?
What do you ***DISLIKE*** about the color of the wine?

AROMA: Swirl the wine in the glass with the cover on the glass, remove cover and smell the **AROMA** of the wine.

Which statement best describes your impression of the **AROMA** of this wine? (check one)

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

Considering the intensity of the **AROMA** of the wine, which statement best describes your impression of this wine? (check one)

Much too low	Too Low	Just About Right	Too Much	Much too Much
☐	☐	☐	☐	☐

What do you ***LIKE*** about the aroma of the wine?
What do you ***DISLIKE*** about the aroma of the wine?

FLAVOR: Taste the wine and evalute the **FLAVOR** of the wine.

Which statement best describes your impression of the **FLAVOR** of this wine? (check one)

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

Considering the intensity of the **FLAVOR** of the wine, which statement best describes your impression of this wine? (check one)

Much too low	Too Low	Just About Right	Too Much	Much too Much
☐	☐	☐	☐	☐

What do you ***LIKE*** about the flavor of the wine?
What do you ***DISLIKE*** about the flavor of the wine?

MOUTHFEEL: Taste the wine and evalute the **MOUTHFEEL** (dryness, moistness, thickness, thinness, or smoothness felt in the mouth) of the wine.

Which statement best describes your impression of the **MOUTHFEEL** of this wine? (check one)

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

Which statement best describes your impression of the **MOUTHFEEL**of this wine? (check one)

Much too weak	Too Weak	Just About Right	Too Strong	Much too Strong
☐	☐	☐	☐	☐

What do you ***LIKE*** about the mouthfeel the wine?
What do you ***DISLIKE*** about the flavor of the wine?

OVERALL IMPRESSION: All things considered, which statement best describes your **OVERALL IMPRESSION** (including color, aroma, flavor, and mouthfeel) of this wine? (check one)

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

Take a bite of the saltine and sip of the water then proceed to the next page.

Preference

Now that you have evaluated the two wines, which wine did you **PREFER**? (circle one)

542 **803**

Figure 4. Ballot presented to panelists for industry sensory panel evaluation of wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[a].

[a] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

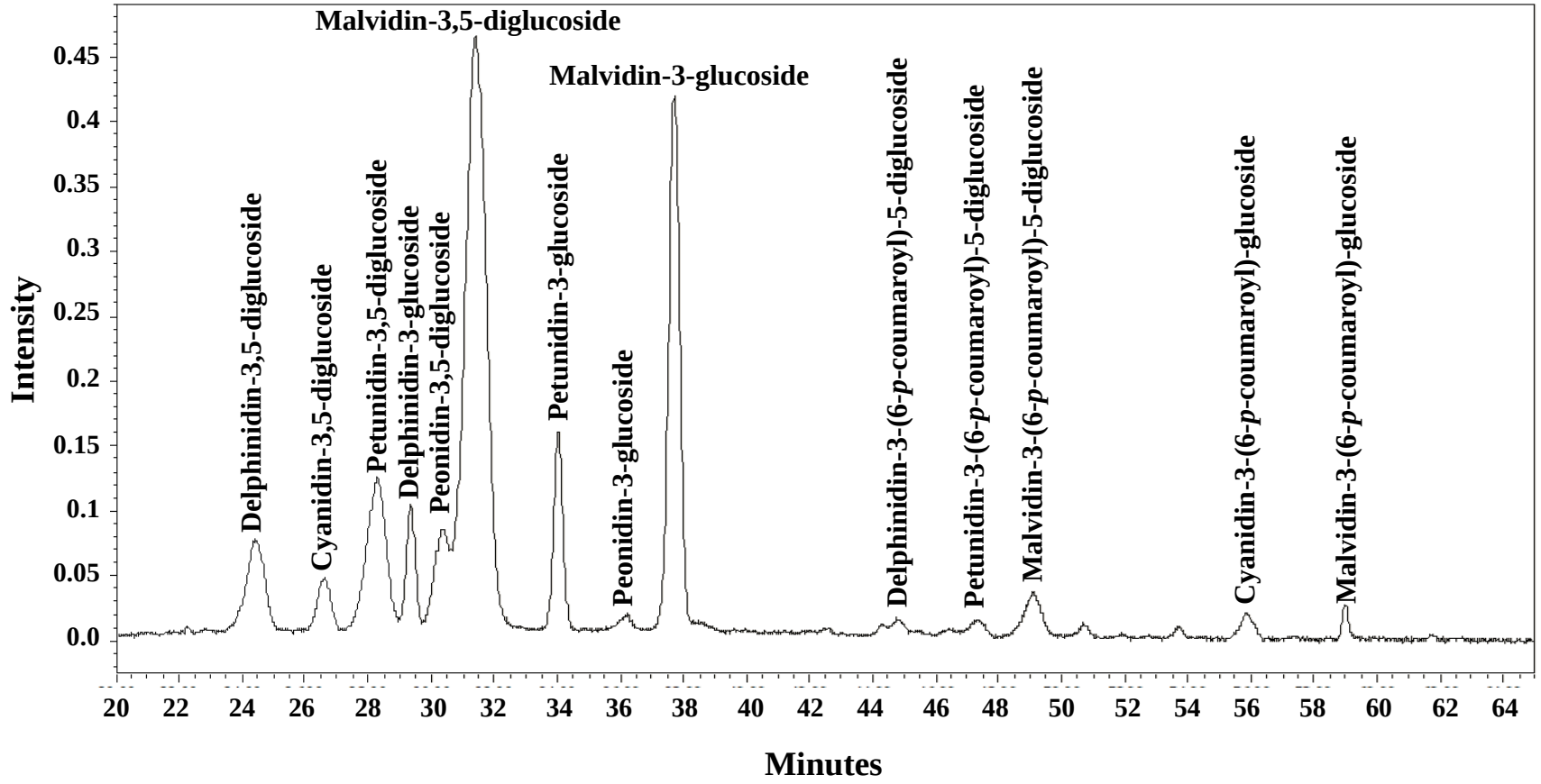

Figure 5. High performance liquid chromatography (HPLC) chromatogram for anthocyanins positively identified in wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[a] (2018).

[a] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

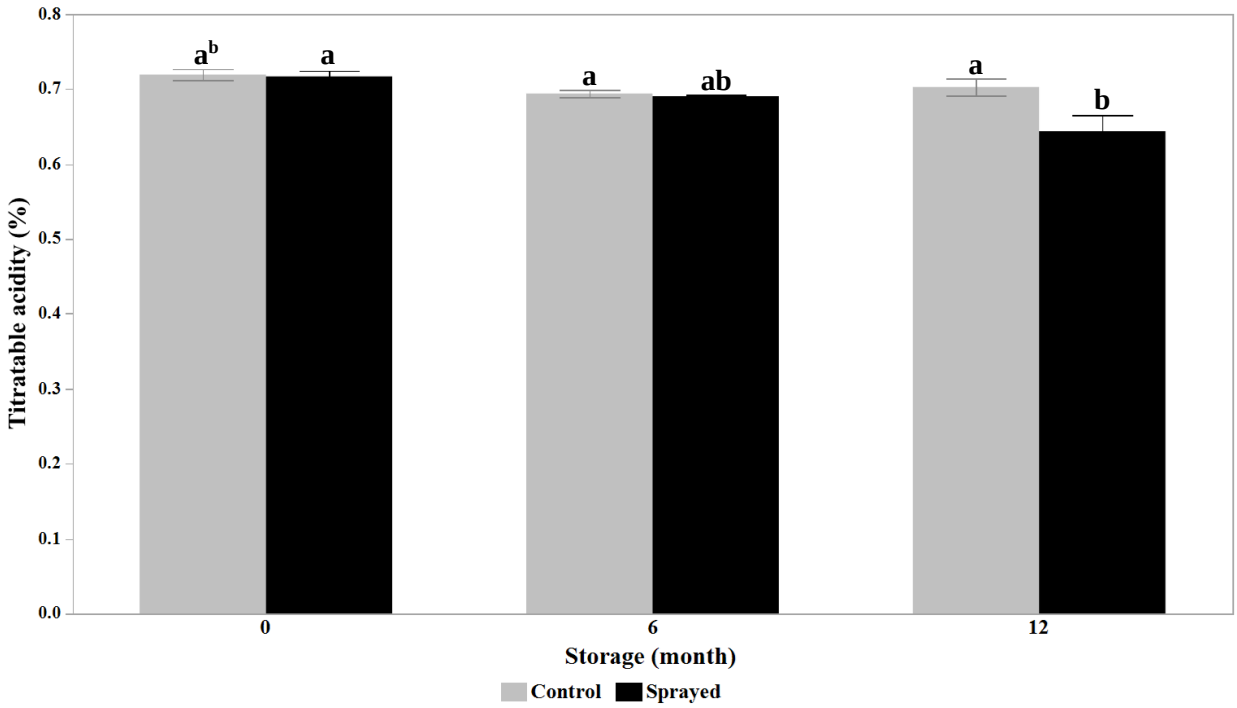

Figure 6. Effect of Spray and Storage on titratable acidity during storage (0, 6, and 12 months at 15°C) of wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[a] (2018).
[a] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.
[b] Means with different letters are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test

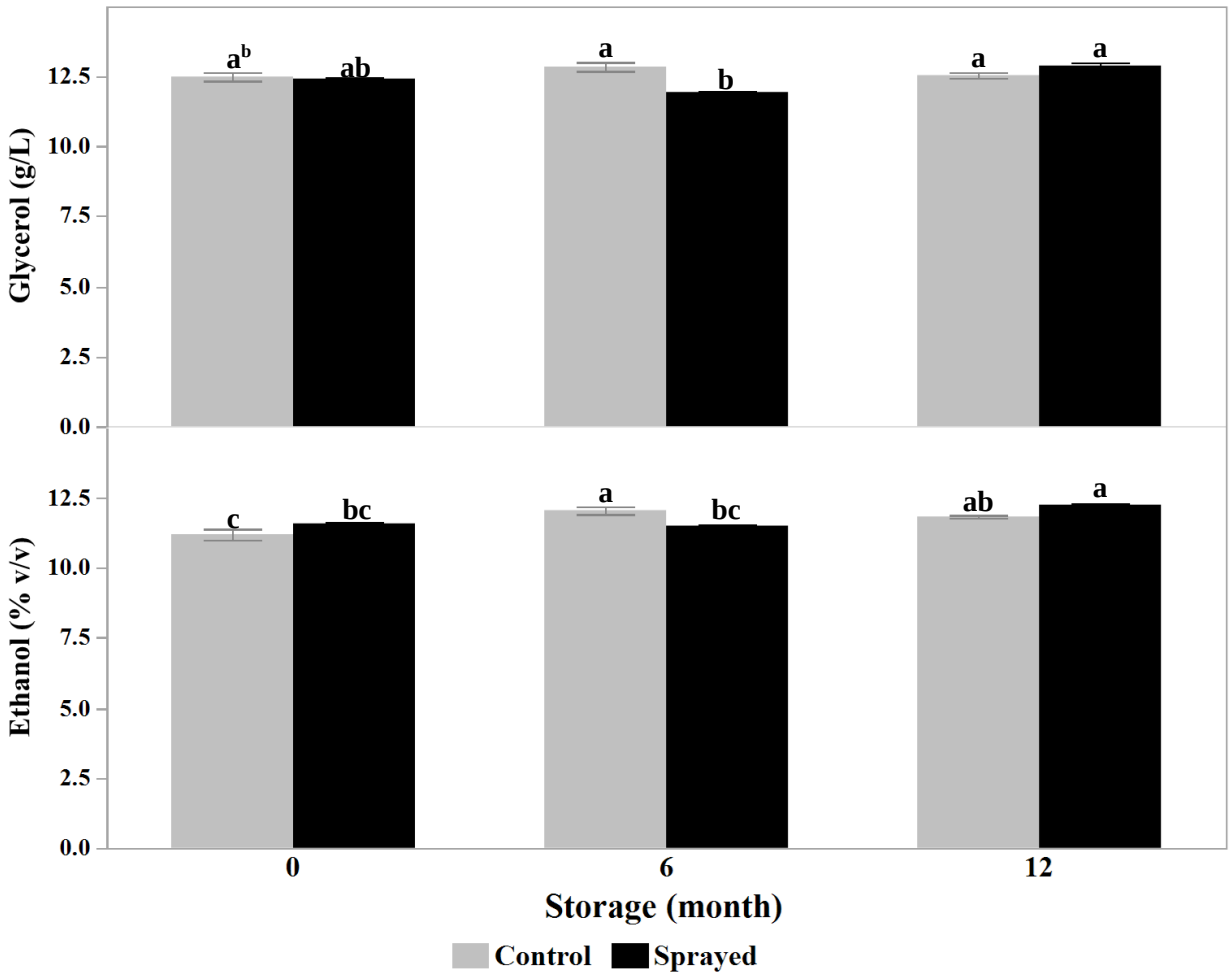

Figure 7. Effect of Spray and Storage on glycerol and ethanol during storage (0, 6, and 12 months at 15°C) of wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[a] (2018).
[a] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.
[b] Means with different letters within each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

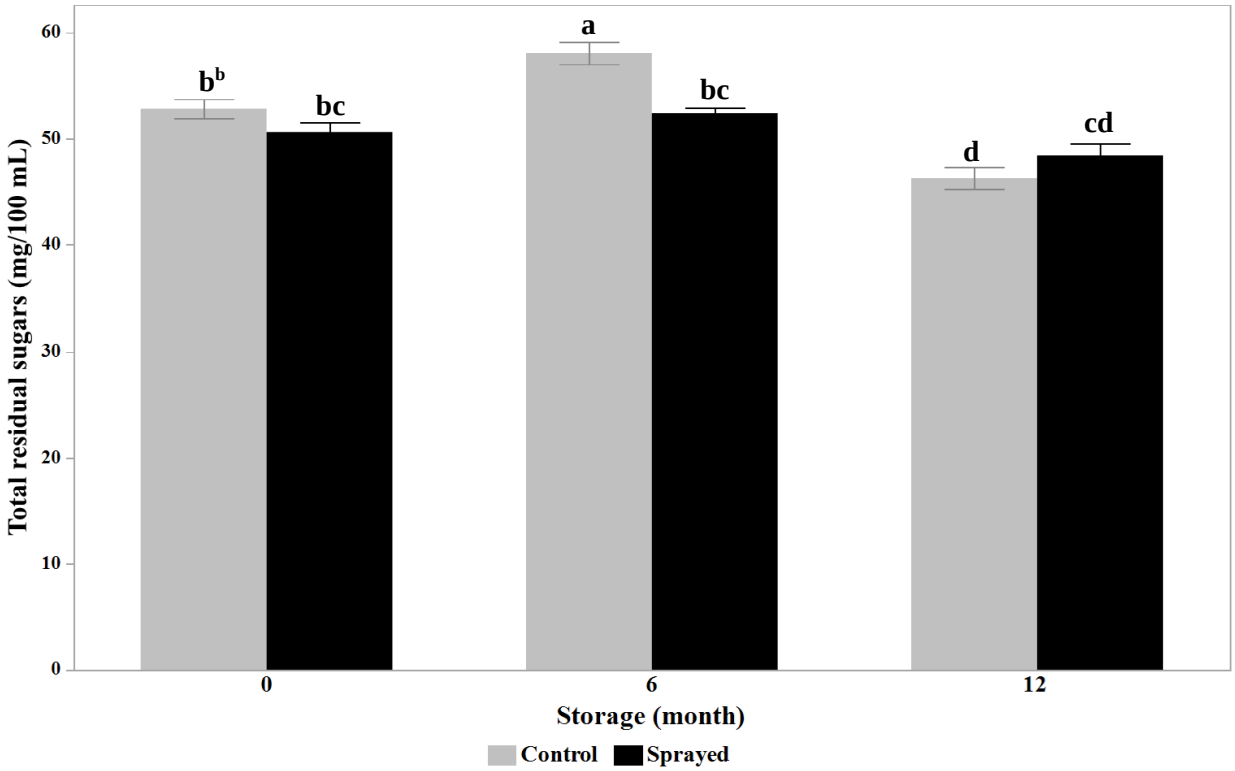

Figure 8. Effect of Spray and Storage on total residual sugars during storage (0, 6, and 12 months at 15°C) of wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[a] (2018).
[a] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.
[b] Means with different letters are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

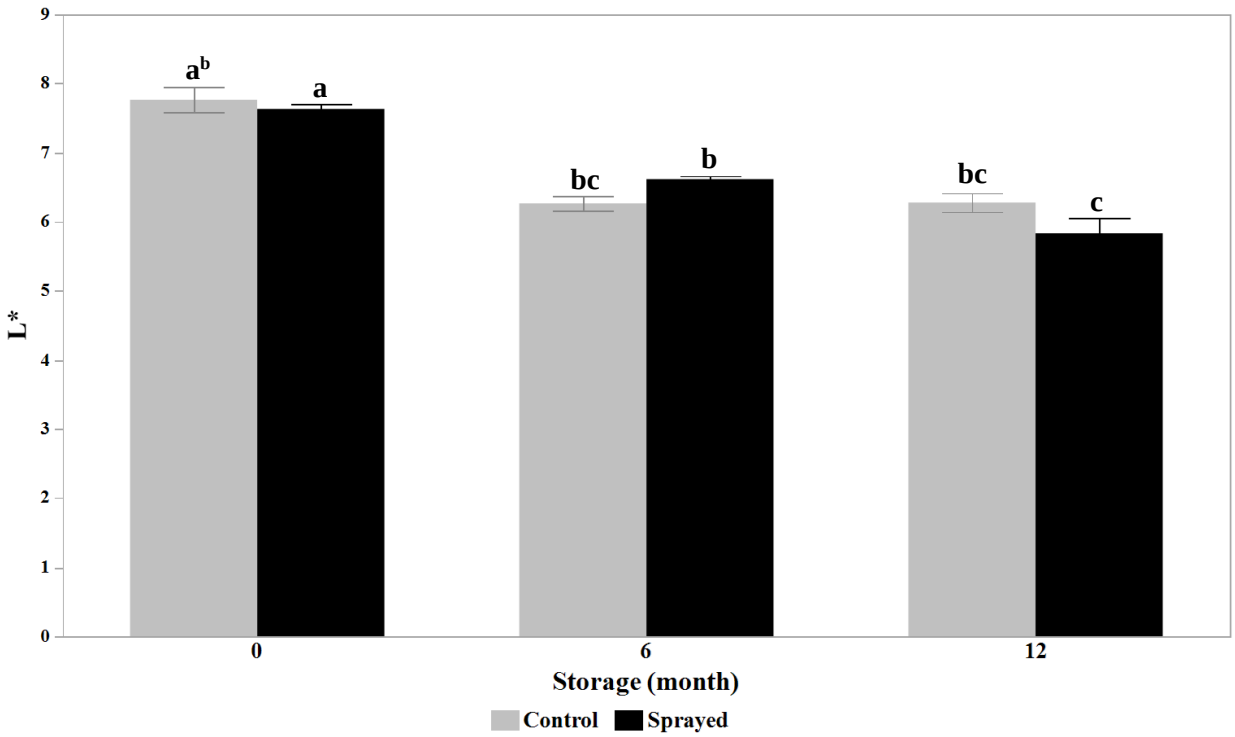

Figure 9. Effect of Spray and Storage on L* during storage (0, 6, and 12 months at 15°C) of wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[a] (2018).
[a] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.
[b] Means with different letters within each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

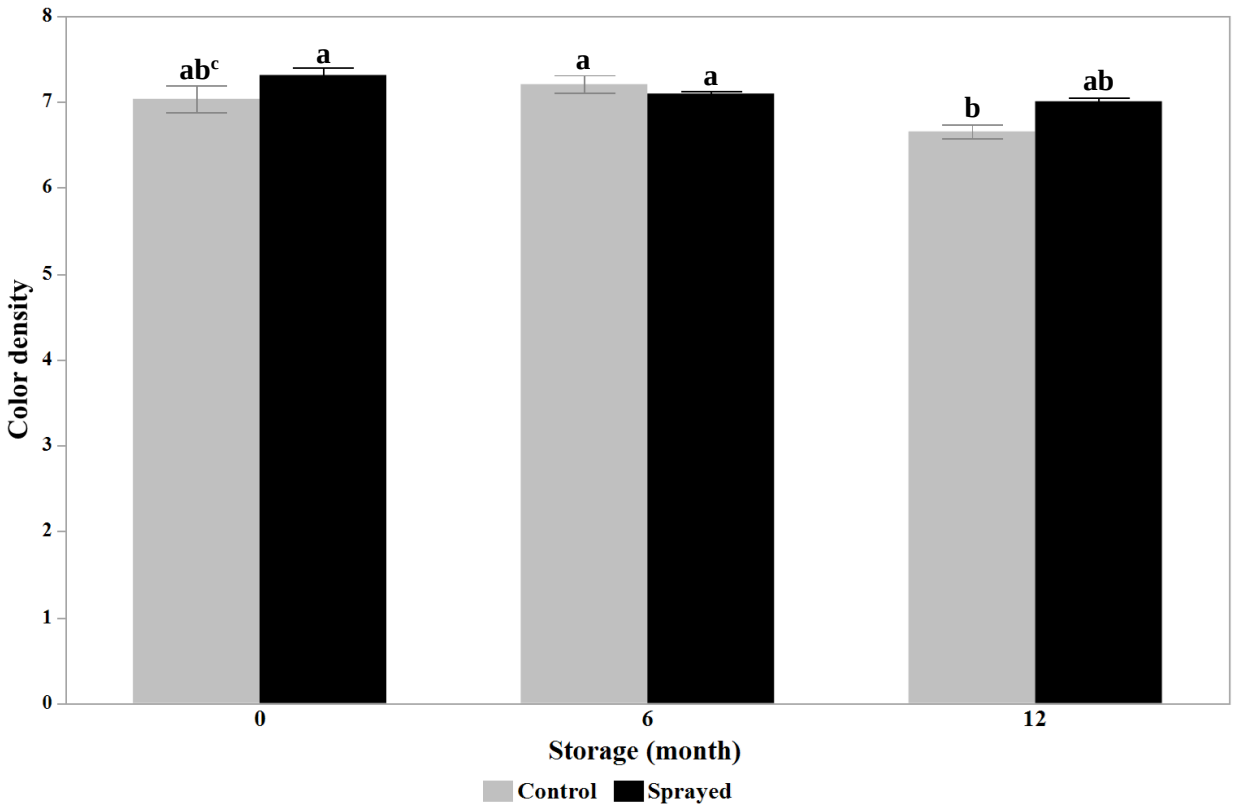

Figure 10. Effect of Spray and Storage on color density[a] during storage (0, 6, and 12 months at 15°C) of wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[b] (2018).
[a] Color density was calculated as absorbance 520 nm + absorbance 420 nm.
[b] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.
[c] Means with different letters within each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

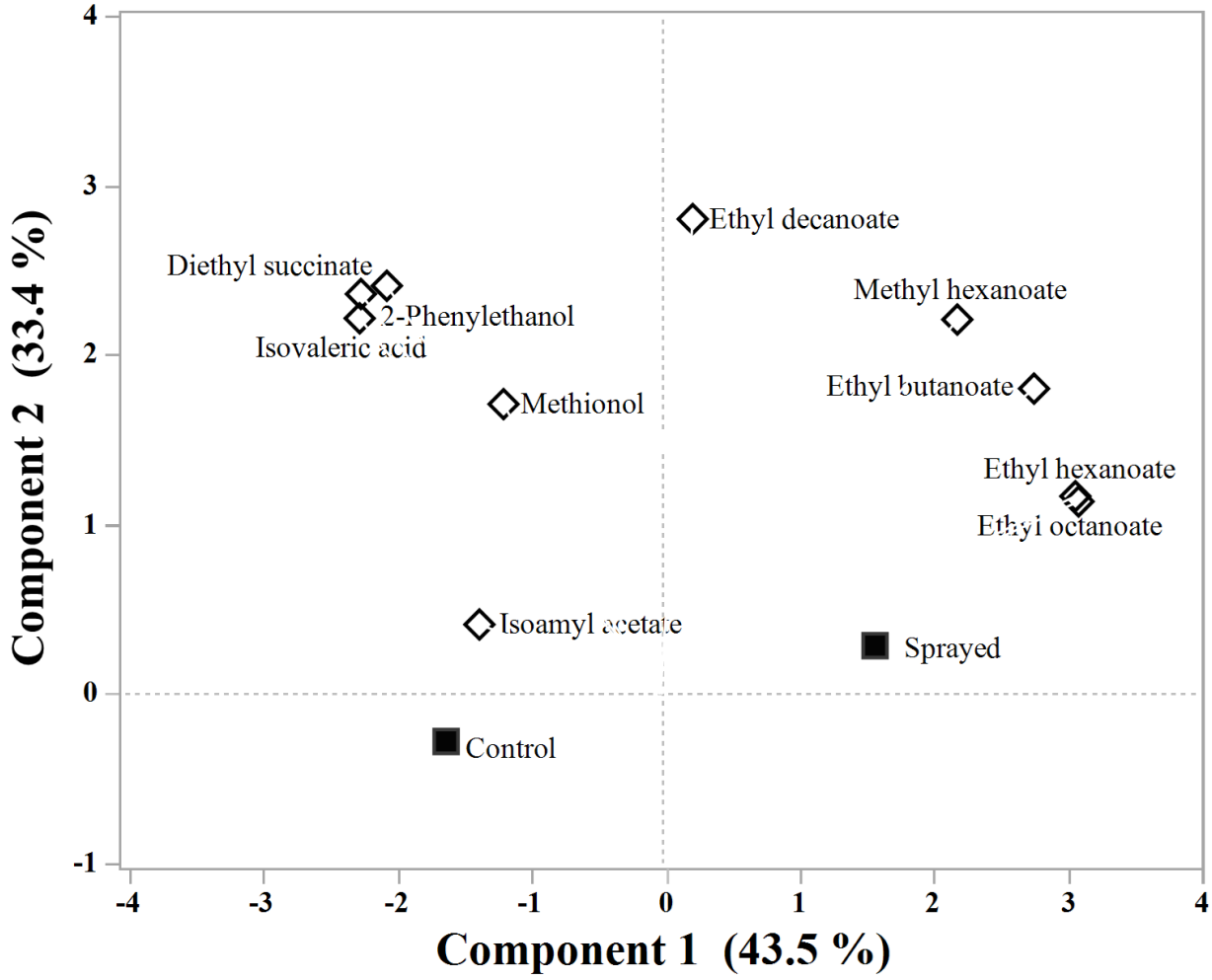

Figure 11. Biplot from principal components analysis on odor-active volatile aroma compounds at 3-months storage at 15°C for wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[a] (2018). [a] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

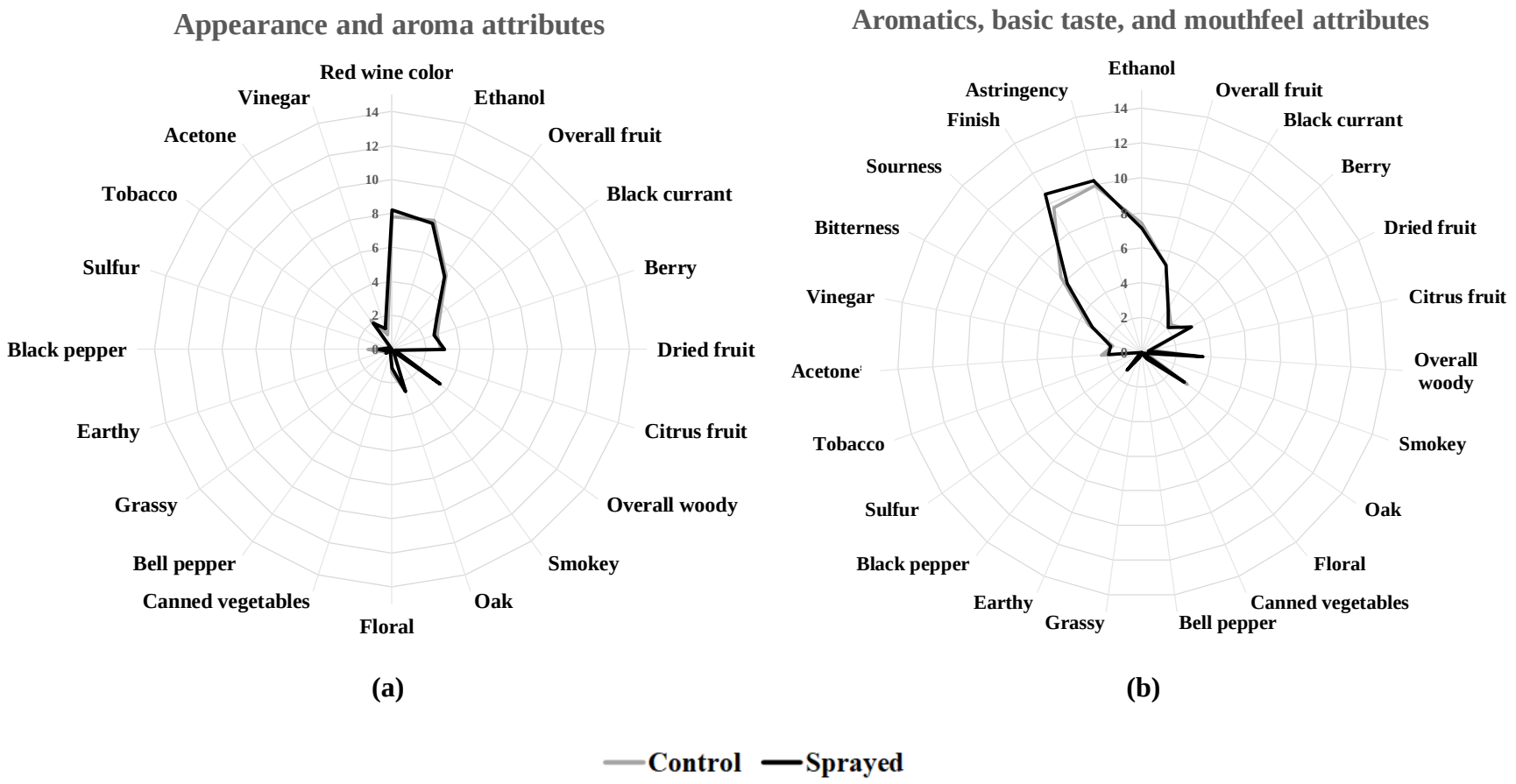

Figure 12. Radar plot for wine appearance (n=1) and aroma (n=19) attributes (9 trained panelists) (a) and aromatic (n=19), basic taste (n=2), and mouthfeel (n=2) attributes (11 trained panelists) (b) from descriptive sensory evaluation with 11 trained panelists at 6 months storage at 15°C for wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[a] (2018).

[a] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

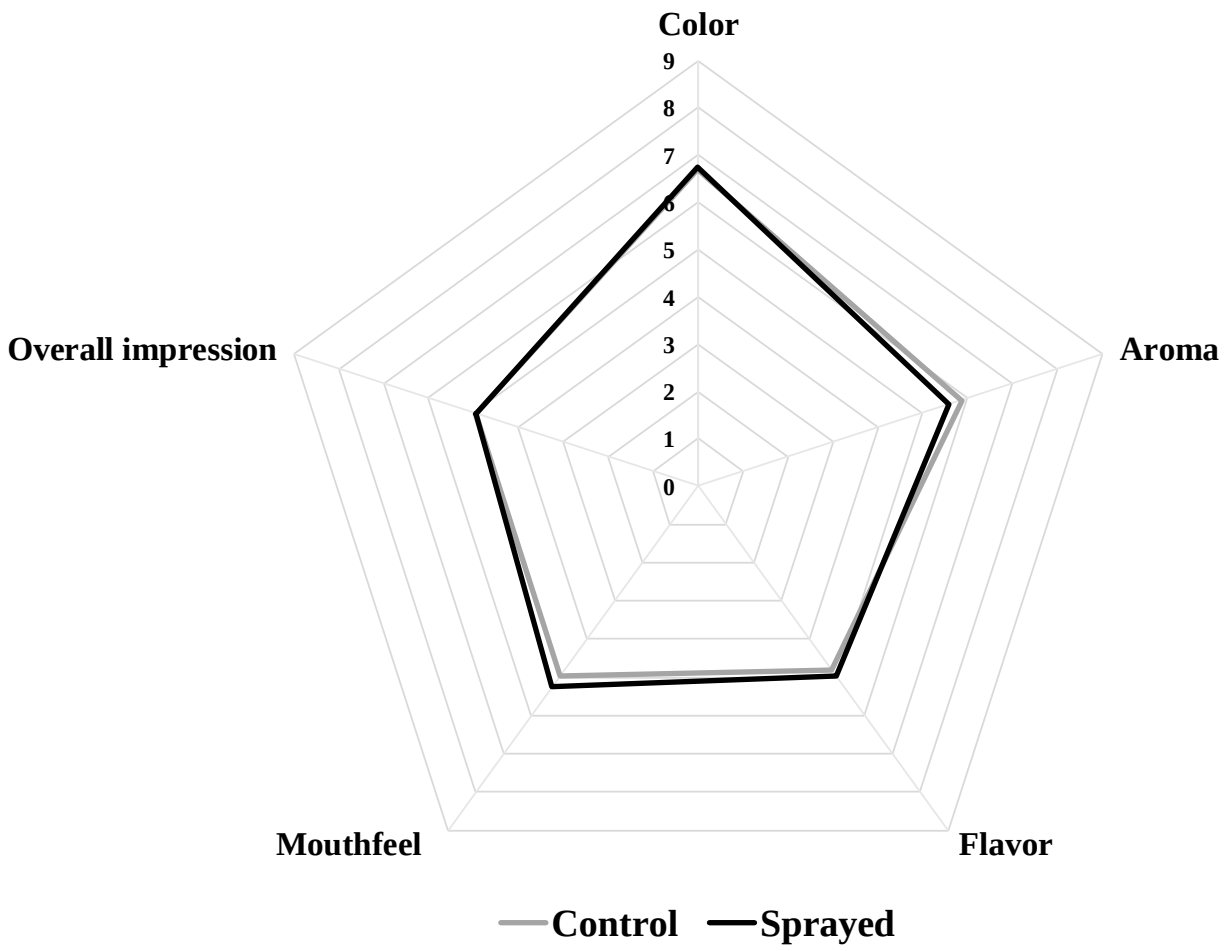

Figure 13. Radar plot for liking[a] of wine attributes from an industry sensory panel (106 panelists) at 6-8 months storage at 15°C for wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[b] (2018).

[a] Nine-point hedonic scale was converted to a numerical scale (1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, and 9=like extremely) for statistical analysis

[b] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

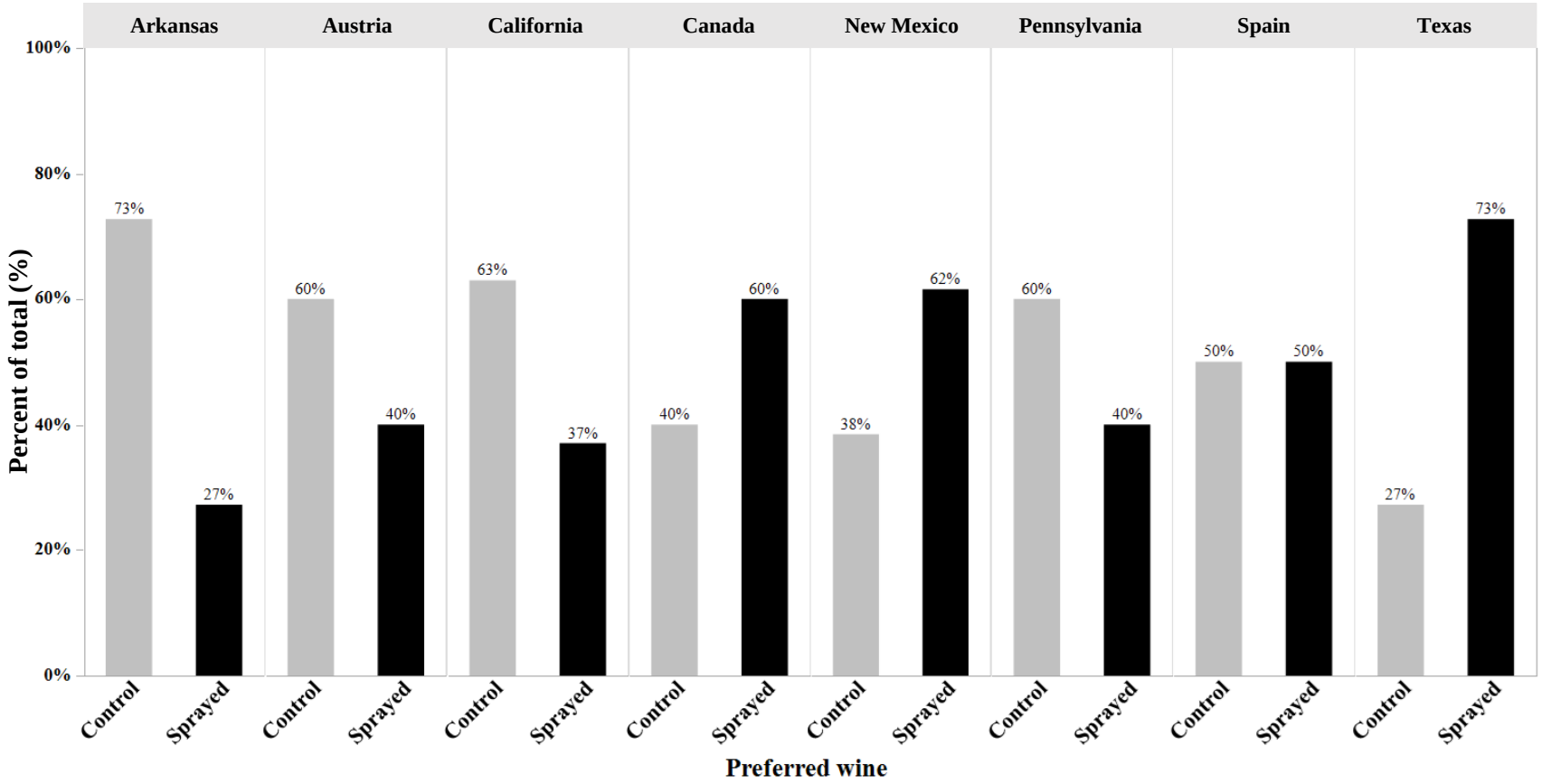

Figure 14. Preference analysis from an industry sensory panel (106 panelists) from eight locations at 6-8 months storage at 15°C for wines produced from Chambourcin grapes from a commercial vineyard in Arkansas unsprayed (control) and sprayed with a specific inactivated yeast[a] (2018).

[a] LalVigne® Mature (Lallemand, Inc., Canada) applied to grapevines at veraison and one week later.

CHAPTER III

Impact of winemaking methods on composition, anthocyanin, color, aroma, and sensory attributes of Noble muscadine wine

Abstract

Grapevines (*Vitis* spp.) are one of the most widely-planted horticultural crops worldwide, and *V. vinifera* is the most commercially-important species of wine grapes. However, *V. vinifera* grapevines are difficult to grow in many regions of the United States, including Arkansas. Muscadine grapes (*V. rotundifolia*) are a species of grapes native to Arkansas and the southeastern United States. Muscadine grapes produce juice and wine with unique fruity and floral characteristics, but these aromas can dissipate. The addition of glycosidic enzymes that release aroma compounds bound to sugars has been shown to increase aroma compounds in muscadine grape juice. Therefore, the objective of this study was to evaluate the effects of skin contact time and glycosidic enzyme addition on the composition, anthocyanin, color, aroma, and sensory attributes of wine from Noble (black-skinned muscadine grapes). Noble grapes were harvested in September 2018 from a private vineyard (Ozark, AR). Wines were produced at the University of Arkansas System Division of Agriculture Department of Food Science in 2018 with different skin contact times (0, 3, and 7 days) and with and without the addition of β-glucosidase (BG) glycosidic enzyme (no BG, BG). The wines were analyzed for composition, anthocyanin, and color attributes during storage (0, 3, and 6 months at 15°C) and for aroma and sensory attributes at 3-months storage. At 0-months storage, wines had compositions within typical ranges for dry table wines (3.1 pH, 0.6-0.7% titratable acidity, and 10.5-11.9% ethanol). Wines with BG enzyme had higher residual glucose concentrations, but enzyme addition did not

affect other composition, anthocyanin, or color attributes. Wines with longer skin contact times had higher titratable acidity and residual sugars and lower ethanol content. The composition of Noble muscadine wines remained stable during storage. Only the diglucoside anthocyanins delphinidin-, malvidin-, petunidin-, peonidin-, and cyanidin-3,5-diglucoside were identified in wines. At 0-months storage, 0-days skin contact wines had lower individual and total anthocyanins (142 mg/100 mL) than wines with 3-days (278 mg/100 mL) or 7-days (290 mg/100 mL) skin contact, and similar patterns were seen at other storage tines. A decrease in total and individual anthocyanin content and color density was observed over 6-months storage, but brown color did not increase. Aroma compounds in Noble muscadine wines included floral alcohols, roasted and caramelized aldehydes, fruity and floral esters, and floral, herbal, and spicy terpenes. Wines with greater skin contact times were associated with herbal and green/unripe aroma compounds, whereas wines with 0-days skin contact were associated with fruity, roasted, caramelized aromas. The fruity, green/unripe, floral, and overall aroma intensities and overall aroma liking of Noble muscadine wines at 3-months storage were evaluated by a consumer panel (68 panelists). Wines without BG enzyme were perceived as having fruitier, more pleasant aromas than wines with BG enzyme. Panelists preferred the aroma of wines with 3-days skin contact. The most commonly-used descriptors for muscadine wine aroma were fruity, floral, earthy, and candy. Wines with higher skin contact times were described as having spicy, dark-fruit aromas typical of red wines, whereas wines with 0-days skin contact were described as having strawberry, candy, and artificial fruity aromas characteristic of muscadine grape juice. Therefore, variations in skin contact time and the addition of a glycosidic enzyme impacted the composition, anthocyanin, color, volatile aroma, and sensory properties of wines produced from Arkansas-grown Noble muscadine grapes.

Acknowledgements

This research was partially funded by the Austrian Marshall Plan Foundation, with support the Graz Technical University Institute of Analytical Chemistry and Food Chemistry (Graz, Austria) and Four Dogs Ranch (Ozark, AR).

Introduction

Grapevines (*Vitis* spp.) are one of the most widely-planted horticultural crops in the world. In the United States, 95% of grape and wine production occurs in California, Washington, New York, Pennsylvania, and Oregon, but production is focused mostly on *V. vinifera*, which is the most popular species of grapevines (Creasy and Creasy 2009, OIV 2000, TTB 2015, USDA NASS 2019). *V. vinifera* grapevines are highly vulnerable to pests, diseases, and extreme temperatures and are difficult to grow in much of the United States, including Arkansas. The high cost of maintaining *V. vinifera* grapevines in non-ideal climates offsets the profit from producing these wines.

Hybrids (a cross of two or more *Vitis* species) and native species, such as *V. rotundifolia*, are better-adapted to surviving stressors that devastate *V. vinifera* grapes (Reisch et al. 2012). Despite the challenges, grape and wine production contribute significantly to the Arkansas economy. In 2010, the Arkansas grape and wine industry was responsible for 1,700 jobs and over $42 million in wages, and wine-related tourism generated $21 million in revenue (Frank 2010).

Grapes grown in Arkansas include mostly hybrids and native species. Muscadine grapes (*V. rotundifolia*) are a species of grapes native to Arkansas and the southeastern United States that produce wines with unique fruity characters (Creasy and Creasy 2009, Sims and Bates 1994). Muscadine grapevines produce large berries (2.5-3.8-cm diameter) with thick, tough

skins, are resistant to Pierce's disease (*Xylella fastidiosa*), fungal pathogens, and the phylloxera insect, and can withstand hot, humid environments that are unfavorable for *V. vinifera* grapevines (Gürbüz et al. 2013, Talcott and Lee 2002, Zhang et al. 2017). Consumption of muscadine grapes and related products has grown in recent years due to the human health benefits associated with muscadine grape consumption, including cancer cell proliferation (Manach et al. 2005, Marshall et al. 2012) and improvement of metabolic responses associated with type-2 diabetes (Banini et al. 2006). These health properties are due to the high antioxidant phenolic content of muscadine grapes (Gris et al. 2013).

Muscadine grapes can be either light-skinned (green or bronze) or dark-skinned (red to almost black) (Ector et al. 1996) and are marketed in fresh and processed forms such as juice, wine, and jelly/jam (Pastrana-Bonilla et al. 2003). There are over 70 cultivars of muscadines available for production (Olien and Hegwood 1990), and a majority of the commercial muscadine crop is used to produce wine (Sims and Morris 1985). Muscadine grapes have been commercially cultivated in Arkansas since the early 1970s (Lanier and Morris 1979). In a 2016 Arkansas grape industry assessment survey conducted by University of Arkansas Department of Horticulture, it was reported that muscadine grapes (*Vitis rotundifolia*) were the most commonly-grown grape variety in the state (Alman 2016), and economic analysis has indicated that muscadine grape production can be profitable for growers in Arkansas (Noguera et al. 2005). Well-known muscadine cultivars for processing include Noble, Scuppernong, Carlos, Magnolia, and Fry. Striegler and Morris (1984) determined that Noble (black-skinned) muscadine grapes grown in Arkansas were excellent for wine production.

Juices and wines produced from muscadine grapes have unique fruity and floral aromas and flavors. Threlfall et al. (2007) found that 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. Lamikanra (1987) determined that higher alcohols and fatty acid ethyl esters were numerically the largest classes of volatile aroma compounds in Noble muscadine wine. The compound 2-Phenylethanol (rose and honey aroma) was determined to be responsible for the characteristic rose aroma of muscadine wines, and Lamikanra et al. (1996) found that 2-phenylethanol was predominantly synthesized during fermentation but was also present in fresh muscadine grape skins. Sims and Bates (1994) evaluated the effect of skin contact time (time that the wine is fermented with the juice, pulp, skins and seeds before pressing) on Noble muscadine wines and found that wines with longer skin contact times had lower general muscadine aroma intensities.

Despite their unique and appealing aromas and flavors, muscadine wines can have high bitterness and astringency due to their phenolic composition, poor color and color stability, and cloudiness/sediment caused by ellagic acid precipitation during storage (Sims et al. 1995, Sims and Morris 1985). The color instability of muscadine wines is due to a low degree of anthocyanin-tannin polymerization. Muscadine grapes and wines contain only diglucoside anthocyanins, which are unable to form stable polymeric pigment complexes like the monoglucoside anthocyanins found in *V. vinifera* grapes and wine (Sims and Morris 1985). Sims and Bates (1994) observed an increase in anthocyanin content with increasing skin contact time for Noble muscadine wines, but also saw that longer skin fermentation times resulted in higher astringency and lower fruity and floral aromas. This study concluded that a balance must be

struck between maximizing color extraction, minimizing astringency, and preserving the typical light, fruity character of muscadine wines.

Muscadine grapes contain significant amounts of glycoside aroma compounds, consisting of a non-sugar component (aglycone) attached to one or more sugar moieties. These "bound" glycoside compounds are non-volatile, and therefore odor-inactive, but are converted to "free" volatile odorants during fermentation and storage when the bond between the sugar and aglycone is cleaved (Hjelmeland and Ebeler 2015, Winterhalter and Skouroumounis 1997). β-glucosidase is an enzyme that frees volatile compound aglycones bound to glucose, and Baek and Cadwallader (1999) evaluated the effects of β-glucosidase addition on muscadine grape juice. High levels of *o*-aminoacetophenone and furaneol, compounds responsible for the foxy (artificial/concord grape) character of muscadine grapes, were found in the bound form. This indicated that addition of β-glucosidase enzyme could increase the foxy character of Muscadine grape juice. However, enzyme addition also increased the concentrations of some unpleasant odor compounds.

Despite use of glycosidic enzymes in the wine industry to improve wine aroma, there have been no studies on glycosidic enzyme addition and muscadine wine aroma. Segurel et al. (2009) determined that exogeneous glycosidic enzyme addition led to a cooked fruit character in Grenache (*V. vinifera*) wines, but that enzyme effects on Syrah (*V. vinifera*) wines were inconsistent and depended on where the grapes were grown. Cabaroglu et al. (2003) evaluated the effects of glycosidic enzyme addition on the volatile aroma profiles and sensory characteristics of Emir (*V. vinifera*) white wine. Wines with added enzyme had higher concentrations of monoterpenes and C_{13}-norisoprenoids and increased honey, lime, and smoky aromas. Rodríguez-Bencomo et al. (2013) found that while β-glucosidase addition slightly

increased the terpene content of dealcoholized Airén (*V. vinifera*) white wines, there was no effect on acid and ester concentrations. In addition, wines with added enzyme had lower tropical and dried fruit aromas in sensory evaluations. Therefore, studies on the effects of β-glucosidase on wine aroma have shown varying results and have focused mostly on *V. vinifera* wines.

There have been several studies examining the attributes and quality of Noble muscadine wines (Gürbüz et al. 2013, Lamikanra 1987, 1997, Lamikanra et al. 1996, Nesbitt et al. 1974, Sims and Bates 1994, Sims et al. 1995, Sims and Morris 1985, 1986, Talcott and Lee 2002, Zhang et al. 2017). However, as muscadine grapes are widely-grown in Arkansas and the southeastern United States, research is still lacking on the effects of winemaking treatments on muscadine wine properties. The objective of this study was to evaluate the effects of skin contact time and glycosidic enzyme addition on the composition, anthocyanin, color, aroma, and sensory attributes of Noble muscadine wines.

Materials and Methods

Grape harvest

Black-skinned Noble muscadine grapes were grown at a private vineyard in Ozark, AR (USDA hardiness zone 7b). The soil type was Linker fine sandy loam (fine-loamy, siliceous, semi active, thermic Typic Hapludult). The grapes were grown on a Geneva Double Curtain trellis system on own-rooted, variable-age vines. Approximately 120 kg of Noble grapes were hand harvested in September 2018. The grapes were taken to the University of Arkansas System (UA System) Food Science Department in Fayetteville, AR and stored at 4°C overnight for wine production the following day.

Wine production

For wine production, Noble grapes were split randomly into six 20-kg batches (0 days, 3 days, and 7 days skin contact, in duplicate). Each batch of grapes was passed twice through a crusher/destemmer, and 30 mg/L sulfur dioxide (SO_2) as potassium metabisulfite (KBMS) was added at crush. The composition of the must (juice, skins, seeds, and pulp after crushing) was evaluated prior to, during, and at the end of fermentation, and adjustments were made to the must to ensure a complete fermentation. The free SO_2 levels of the wine were evaluated and adjusted as needed. Soluble solids (SS), pH, and titratable acidity (TA) of must were evaluated prior to fermentation. The SS (expressed as %) of juice from the must was determined using a Bausch & Lomb Abbe Mark II refractometer (Scientific Instruments, Keene, NH). The pH and TA were measured using a Metrohm 862 Compact Titrosampler (Metrohm AG, Herisau, Switzerland) fitted with a pH meter.

The initial composition of the Noble muscadine grape must was the same for the three skin contact treatments. The must had 16.0% SS, 3.33 pH, and 0.65% TA. Soluble solid levels of the musts were adjusted to 21% using table sugar (sucrose). Musts were inoculated with Lalvin ICV D254® wine yeast (Lallemand, Inc., Montreal, Canada) at a rate of 0.26 g/L estimated juice in the must. Musts were fermented on the skins for zero days, three days, or five days at 15°C. After fermentation on the skins, musts were pressed with a 70-L Enoagricola Rossi Hydropress (Calzolaro, Italy) using three 10-minute press cycles and a pressure of 207 kPa. The wines were collected in 11.4-L glass carboys fitted with fermentation locks filled with SO_2 solution to allow release of carbon dioxide and limit oxygen exposure. Wines were racked (wine removed from the sediment) several times as fermentation at 15°C continued for approximately eight months.

After fermentation completion, the free SO_2 content of wines was determined using the aeration-oxidation method (Iland et al. 1993) and adjusted to 60 mg/L.

Each duplicate skin contact treatment (0, 3, or 7 days) was split into two 3.8-L glass jars for Enzyme treatment, one with β-glucosidase enzyme (BG) and one without (no BG). Scottzyme® BG enzyme (Scott Laboratories, Petaluma, CA, USA) was added at the manufacturer's recommended rate of 0.05 g/L for the Enzyme treatment. Wines were bottled into 125-mL and 375-mL glass bottles, sealed with plastisol-lined lug caps and screw caps, and stored at 15°C until analysis (0, 3, and 6 months storage). The ethanol content of all wines was 10.5-12.1% (v/v) at bottling, measured by high performance liquid chromatography (HPLC) (Walker et al. 2003). The Noble muscadine wines were analyzed during storage (0, 3, and 6 months at 15°C) for composition, anthocyanin, and color attributes and at 3-months storage for volatile aroma and sensory attributes. Wines were stored at 15°C for one week prior to the first analysis (month 0).

Composition attributes analysis

The composition attributes analysis of the wines included pH, TA, glycerol, ethanol, residual sugars, and organic acids. Analysis was done on each wine sample (Skin Contact and Enzyme treatment) during storage (0, 3, and 6 months at 15°C), and samples were measured in analytical duplicates.

pH. The pH of wines was measured using a Metrohm 862 Compact Titrosampler (Metrohm AG, Herisau, Switzerland) fitted with a pH meter. The probe was left in the samples for two minutes to equilibrate before recording the pH value. Wine was degassed prior to analysis.

Titratable acidity (TA). The TA of wines were expressed as % w/v (g/100 mL) tartaric acid and measured using a Metrohm 862 Compact Titrosampler. Six grams of sample was added to 50 mL

degassed, deionized water and titrated with 0.1 N sodium hydroxide to an endpoint of pH 8.2. Wine was degassed prior to analysis.

Glycerol, ethanol, residual sugars, and organic acids. The glycerol, ethanol, residual sugars, and organic acids in wines were identified and quantified according to the HPLC procedure of Walker et al. (2003). Samples were passed through a 0.45 μm polytetrafluoroethylene (PTFE) syringe filter (Varian, Inc., Palo Alto, CA, USA) before injection onto an HPLC system consisting of a Waters 515 HPLC pump, a Waters 717 plus autosampler, and a Waters 410 differential refractometer detector connected in series with a Waters 996 photodiode array (PDA) detector (Water Corporation, Milford, MA, USA). Analytes were separated with a Bio-Rad HPLC Organic Acids Analysis Aminex HPX-87H ion exclusion column (300 x 7.8 mm) connected in series with a Bio-Rad HPLC column for fermentation monitoring (150 x 7.8 mm; Bio-Rad Laboratories, Hercules, CA, USA). A Bio-Rad Micro-Guard Cation-H refill cartridge (30 x 4.5 mm) was used as a guard column. Columns were maintained at a temperature of 65 ± 0.1°C by a temperature control unit. The isocratic mobile phase consisted of pH 2.28 aqueous sulfuric acid at a flow rate of 0.45 mL/min. Injection volumes of both 10 μL (for analysis of organic acids and sugars) and 5 μL (for ethanol and glycerol) were used to avoid overloading the detector. The total run time per sample was 60 minutes.

Citric, tartaric, malic, lactic, and succinic acids were detected at 210 nm by the PDA detector, and glucose, fructose, ethanol, and glycerol were detected at 410 nm by the differential refractometer detector. Analytes in samples were identified and quantified using external calibration curves based on peak area estimation with baseline integration. Results were expressed as milligrams analyte per 100 mL wine for organic acids and residual sugars, grams per liter wine for glycerol, and % v/v (alcohol by volume, ABV) for ethanol. Total residual

sugars were calculated as the sum of glucose and fructose, and total organic acids was calculated as the sum of citric, tartaric, malic, lactic, and succinic acids.

Anthocyanin attributes analysis

The anthocyanin attributes analysis of the wines included total and individual anthocyanins. Analysis was done on each wine sample (Skin Contact and Enzyme treatment) during storage (0, 3, and 6 months at 15°C), and samples were measured in analytical duplicates.

Anthocyanin quantification. The anthocyanin content of wines was analyzed using the HPLC-PDA method of Cho et al. (2004). Samples were passed through a 0.45 μm PTFE syringe filter before injection onto a Waters Alliance HPLC system equipped with a Waters model 996 PDA detector and Millennium version 3.2 software. A 4.6 x 250 mm Symmetry® C_{18} column (Waters Corporation) with a 3.9 mm x 20 mm Symmetry® C_{18} guard column was used to separate analytes. The mobile phase consisted of a binary gradient with 5% (v/v) formic acid in water (solvent A) and methanol (solvent B) at a flow rate of 1.0 mL/min. A gradient was used with 2% to 60% B from 0-60 minutes, 60% to 2% B from 60-65 minutes, then holding at 2% B from 65-80 minutes. A 50 μL injection volume was used, and the total run time per sample was 80 minutes. Anthocyanins were detected at 510 nm.

Anthocyanins were identified using external calibration curves and quantified as the anthocyanidin-3-glucoside of their major aglycone (cyanidin, delphinidin, peonidin, petunidin, or malvidin) using external calibration curves based on peak area estimation with baseline integration. Total anthocyanins were determined by summing the concentrations of individual anthocyanin compounds. Results were expressed as mg/100 mL wine.

Color attributes analysis

The color attributes analysis of the wines included L*, hue angle, chroma, red color, brown color, and color density. Analysis was done on each wine sample (Skin Contact and Enzyme treatment) during storage (0, 3, and 6 months at 15°C), and samples were measured in analytical duplicates.

L*, hue angle, and chroma. Wine color analysis was conducted using a ColorFlex system (HunterLab, Reston, VA, USA). The ColorFlex system uses a ring and disk set (to control liquid levels and light interactions) for measuring translucent liquids in a 63.5-mm glass sample cup with an opaque cover to determine Commission Internationale de l'Eclairage (CIE) Lab transmission values of L*=100, a*=0, and b*=0 (Commission Internationale de l'Eclairage (CIE) 1986). The CIELAB system describes color variations as perceived by the human eye. CIELAB is a uniform three-dimensional space defined by colorimetric coordinates, L*, a*, and b*. The vertical axis L* measures lightness from completely opaque (0) to completely transparent (100), while on the hue-circle, +a* red, -a* green, +b* yellow, and -b* blue are measured. Hue angle, calculated as $\tan^{-1}\frac{b*}{a*}$, described color in angles from 0 to 360°: 0° is red, 90° is yellow, 180° is green, 270° is blue, and 360° is red. For samples with hue angles <90°, a 360° compensation (hue + 360°) was used to account for discrepancies between red samples with hue angles near 0° and those near 360° (McLellan et al. 2007). Chroma, calculated as $\sqrt{a*^2 + b*^2}$, identified color by which a wine appeared to differ from gray of the same lightness and corresponds to saturation (intensity/purity) of the perceived color.

Red color, brown color, and color density. Red color and brown color of wines were measured spectrophotometrically as absorbance at 520 nm and 420 nm, respectively, and color density was measured as red color + brown color (Iland et al. 1993). Absorbance values were measured using

a Hewlett-Packard 8452A Diode Array spectrophotometer equipped with UV-Visible ChemStation software (Agilent Technologies, Inc., Santa Clara, CA). Samples were diluted five times with deionized water prior to analysis and were measured against a blank sample of deionized water. A 1-cm cell was used for all spectrophotometer measurements.

Aroma attributes analysis

The volatile aroma profiles analysis of 2018 Noble muscadine wines was conducted at Graz University of Technology (Graz, Austria) Institute of Analytical Chemistry and Food Chemistry. Wines were packaged in 20-mL clear glass vials, sealed with a polypropylene cap with a polytetrafluoroethylene-line silicon septum, wrapped with Parafilm® flexible film (Bemis Company, Inc., Neenah, WI), and shipped to Graz University of Technology for analysis. Volatile aroma profiles were determined at 3-months storage at 15°C. Analysis was done on each wine sample (Skin Contact and Enzyme treatment), and samples were measured in analytical triplicates.

Determination of volatile aroma profiles. To identify the volatile aroma compounds in wines, volatile compounds were extracted from 1 mL of wine in a 10-mL glass vial using solid-phase microextraction (SPME) with a 2-cm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber for 30 minutes at 40°C. A gas chromatography-mass spectrometry (GC-MS) system equipped with a Shimadzu GC 2010 (Shimadzu Corporation, Kyoto, Japan), Shimadzu QP 2010 MS, and a PAL HTX autosampler (CTC Analytics AG, Zwingen, Switzerland) was used to separate and identify volatile compounds. Samples were extracted/injected in analytical triplicate. Volatiles were separated on a nonpolar Restek Rxi 5MS column (30 m x 0.25 mm x 1 μm; Restek, Bellefonte, PA) 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 were recorded in the scan mode (*m/z* 35-350) with a 9.8 minute solvent cut time and a detector voltage relative to the tuning result.

Data was analyzed using the Shimadzu GCMS Postrun Analysis software. Compounds were identified using comparison of mass spectra with NIST14 (National Institute of Standards and Technology, Gaithersburg, MD), Flavors and Fragrances of Natural and Synthetic Compounds (FFNSC3, John Wiley & Sons, Inc., Hoboken, NJ), and Adam's Essential Oils (Adams 2007) mass spectral libraries and comparison of calculated Kovats retention indices (Kováts 1958) with values reported in the Flavornet (Acree and Arn 2004) and Pherobase (Sayed 2003) databases. A matching library result and a retention index 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.

Sensory attributes analysis

Only the aroma attributes of the wines were evaluated for sensory attributes and included evaluation of aroma intensity and aroma liking by a consumer panel. There were six wines evaluated (3 Skin Contact treatments x 2 Enzyme treatments), and analysis was done on each wine sample at 3-months storage at 15°C. For the sensory evaluations, the replications of each treatment were combined.

Consumer sensory panel. The consumer sensory panel was conducted at the UA System Department of Food Science (Institutional Review Board protocol # 1908209641; Figure 1). Panelists were recruited from the Department of Food Science and from an annual meeting of the Arkansas Association of Grape Growers (AAGG). In total, 68 panelists evaluated the wines for intensity and liking of Noble muscadine wine aroma attributes. Overall, 50% of panelists were female, 50% were male, 3% were 18-21 years of age, 41% were 22-34, 14% were 35-44, 11%

were 45-54, 23% were 55-64, and 8% were 65 or older. Thirty-four percent of panelists indicated that currently worked or had previously worked in the grape/wine industry, while 66% did not. Sixty-five percent of panelists had previously consumed muscadine wine and 35% had not.

Panelists evaluated 30-mL of each wine, and each wine was evaluated one time. The wines were served at the same time at room temperature (25°C) in wine glasses labeled with three-digit codes in a randomized complete block design. Serving order was randomized among panelists to prevent presentation order bias. Each wine glass was covered with a food-grade plastic disc to prevent dissipation of aromas. Panelists were instructed to remove the disc before evaluating each sample, and then replace the disc before evaluating the next sample. The panelists used a 15-cm line scale with anchors (none, moderate, and very strong) to indicate the intensity of wine overall aroma, fruity aroma, green/herbaceous aroma, and floral aroma, and a nine-point hedonic scale (1 = dislike extremely and 9 = like extremely) to indicate their overall liking of the wine aroma. In addition, panelists were instructed to list one-to-five words to describe the aroma of each wine. An example of a ballot presented to consumer panelists is shown in Figure 2.

Design and statistical analysis

After harvest, Noble muscadine grapes were randomized for Skin Contact treatments (0, 3, and 7 days) in duplicate. Each Skin Contact treatment was split after fermentation into two Enzyme treatments (No BG and BG). There were six treatments (3 Skin Contact treatments x 2 Enzyme treatments) with two replications. The wines were bottled (125-mL bottles) and stored at 15°C. The wines were analyzed at 0-, 3-, and 6-months storage at 15°C for composition, anthocyanin, and color attributes, and at 3-months storage at 15°C for aroma and sensory attributes. For composition, anthocyanin, color, and aroma attributes, samples were taken from

one 125-mL bottle, and for sensory evaluations, 375-mL bottles of each replication were combined. Bottles of wine were treated as individual experimental units in a full factorial design. There were 36 samples when wines were analyzed for composition, anthocyanin, and color attributes during storage, 12 samples when wines were analyzed at 3-months storage for aroma attributes, and six samples when wines were analyzed at 3-months storage for sensory attributes. Statistical analyses were conducted using JMP® Pro statistical software (version 15.0.0, SAS Institute, Cary, NC, USA). Additional information on the statistical analyses is provided below.

Composition, anthocyanin, and color attributes. For the Noble muscadine wines at 0-, 3-, and 6-months storage, a split-plot analysis was used with the Skin Contact treatments and replicates as the whole plots and the enzyme treatments as the subplots. A univariate analysis of variance (ANOVA) was conducted with the fixed effects to determine the significance of the main factors (Skin Contact, Enzyme, and Storage) and their interactions. All factors were treated as categorical. Tukey's Honest Significant Difference (HSD) test and student's t-test were used to detect differences among means ($p<0.05$). Figures were created in JMP®, and error bars represented one standard error from the mean.

Aroma attributes. Peak areas (TIC) for each positively-identified compound in Noble muscadine wines at 3-months storage at 15°C were used as a semi-quantitative measure for multivariate statistics. Each compound was assigned a general aroma group based on descriptors reported in the Flavornet (Acree and Arn 2004) and Pherobase (Sayed 2003) databases. The areas of compounds within each group were summed to create general "aroma category" variables. This was done so that the model did not overfit to noise, which occurs when the number of parameters is greater than the number of variables. An initial hierarchical clustering analysis with Ward's minimum variance cluster criterion was conducted to determine groupings of observations based

on aroma categories. A principal components analysis (PCA), based on the aroma categories, was used to explore the relationship between Skin Contact and Enzyme treatments and volatile aroma profiles.

Sensory attributes. For the consumer sensory panel evaluation of Noble muscadine wines at 3-months storage at 15°C, a univariate ANOVA was used to detect the significance of the main effects (Skin Contact and Enzyme) and their interaction for overall aroma, fruity aroma, green/herbaceous aroma, and floral aroma intensity and overall aroma liking. Panelist was included in the model as a random effect to account for between-panelist variation. Tukey's HSD was used to detect significant differences among means ($p<0.05$). For the aroma descriptor terms provided by panelists for each wine, the Text Explorer platform in JMP® was used to determine the most frequently-used descriptors across all wines 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 for each wine were determined. A PCA was conducted, based on the descriptor frequencies, to explore the relationship between Skin Contact and Enzyme treatments and wine aroma. Figures were created in JMP®, and error bars represented one standard error from the mean.

Results and Discussion

The composition of Noble muscadine grape musts was similar among the Skin Contact treatments. Musts had 16.0% SS, 3.33 pH, and 0.65% TA. The SS levels of the musts were adjusted to 21% prior to fermentation. Zhang et al. (2017) found 18.1% SS, 3.82 pH, and 0.30% TA for Noble muscadine grape musts from Mississippi, and Striegler et al. (2005) and Threlfall et al. (2005) reported 16.5-19.7% SS, 3.45 pH, and 0.37% TA for black muscadine grapes grown

in Arkansas. Although the SS levels were less ideal for winemaking in the present study, the acid levels were higher, and thus more ideal for winemaking, than those in the previous studies.

After about eight months of fermentation at 15°C, the wines were bottled in May 2019 and stored at 15°C. The impacts of skin contact time and β-glucosidase enzyme addition on Noble muscadine wine composition, anthocyanin, and color attributes were evaluated during storage (0, 3, and 6 months at 15°C), and the effects of skin contact and enzyme addition were evaluated on wine aroma and sensory attributes at 3-months storage at 15°C.

Analysis of composition, anthocyanin, and color attributes during storage

At bottling (0-months storage at 15°C) in 2018, Noble muscadine wines had acceptable compositions with sugar and organic acid levels within the typical ranges for dry-red table wines. Wines with longer skin contact times had higher TA and residual sugars, lower ethanol content, and higher anthocyanins leading to darker, more red colors. Addition of a β-glycosidic enzyme at bottling lead to wines with higher glucose contents, but did not impact other composition, anthocyanin, or color attributes. While the composition of Noble muscadine wines remained stable during storage, a decrease in anthocyanin content and color quality was observed. However, there was no increase in brown color over 6-months storage.

Composition. Noble muscadine wines were analyzed during storage (0, 3, and 6 months at 15°C) for pH, TA, glycerol, ethanol, glucose, fructose, total residual sugars, tartaric acid, malic acid, citric acid, succinic acid, lactic acid, and total organic acids. At 0-months storage, wines had 3.1 pH, 0.6-0.7% TA, 10.7-12.5 g/L glycerol, 10.5-11.9% ethanol, 0-12 mg/100 mL glucose, 27-97 mg/100 mL fructose, 27-106 mg/100 mL total residual sugars, 260-362 mg/100 mL tartaric acid, 90-137 mg/100 mL malic acid, 54-82 mg/100 mL citric acid, 149-158 mg/100 mL succinic acid, 40-95 mg/100 mL lactic acid, and 663-774 mg/100 mL total organic acids (data not shown). The

composition of Noble muscadine wines in the present study was within the 2.9-3.3 pH and 0.4-0.6% TA ranges reported for Arkansas Noble wines in the literature (Sims and Morris 1984, 1985, 1986). Most of the composition attributes had significant interactions, except for pH, glycerol, fructose, and malic acid (Table 1).

The Storage main effect was significant for pH. The wines at 0-months storage (3.11) had a higher pH than the wines at 6-months storage (2.99). The Skin Contact x Enzyme, Skin Contact x Storage, and Enzyme x Storage interactions were significant for TA. The wine with 3-days skin contact and no BG enzyme had a higher TA (0.78%) than the 7-days no BG (0.75%), 0-days no BG (0.66%), and 0 days BG (0.66%) wines (Figure 3a). There was no difference in TA between Enzyme treatments within any of the Skin Contact treatments. All wines with 3- and 7-days skin contact had higher TAs than the 0-days skin contact wines. This was contradictory to the findings of Arnold and Noble (1979), Ough (1969), and Singleton et al. (1975), who concluded that TA decreases and pH increases with increasing skin contact. However, these studies were conducted on *Vitis vinifera* white grape wines. Unlike wines produced from other *Vitis* species, muscadine wines tend to increase in acidity, and therefore decrease in pH, during fermentation and storage (Lamikanra 1997). This atypical storage-acidity relationship of muscadine wines could have disrupted the correlation between skin contact time and acidity seen with other grape species. The 0-days skin contact wines had a lower TA than wines with 3- and 7-days skin contact within each Storage time (Figure 3b). The 3-days (0.84%) and 7-days (0.83%) skin contact wines at 3-months storage had higher TA values than all other wines, and the 0-days skin contact wine at 0-months storage (0.59%) had the lowest TA of all wines. The no BG (0.80%) and BG (0.80%) wines at 3-months storage had higher TAs than no BG (0.72%) and BG (0.71%) wines at 6-months storage and the no BG (0.67%) and BG (0.69%) wines at 0-

months storage (Figure 3c). There was no difference in TA between Enzyme treatments at any of the storage times. The increase in TA from 0- to 3-months storage is consistent with the increase in acidity during storage seen in muscadine wines (Lamikanra 1997).

The Skin Contact x Enzyme x Storage interaction was significant for glycerol and ethanol. In general, regardless of Enzyme treatment or Storage time, the wines with 3- and 7-days skin contact had higher glycerol contents than the wines with 0-days skin contact (Figure 4a). There did not appear to be any effects of Enzyme or Storage on glycerol. In general, regardless of Enzyme treatment, the wines with 0-days skin contact tended to have higher ethanol levels than wines with 3- or 7-days skin contact (Figure 4b). The 0-days skin contact wine without BG at 6-months storage (11.94% v/v) and the 0-days skin contact wines with BG at 3-months storage (12.08% v/v) had higher ethanol contents than any of the wines with 3-days or 7-days skin contact, regardless of Enzyme treatment or storage time. There did not appear to be any major effects of Storage or Enzyme on ethanol content. Despite some differences among Skin Contact, Enzyme, and/or Storage treatments, the pH, TA, glycerol, and ethanol content of all Noble muscadine wines in the present study were similar to the typical values of pH < 3.6, 0.5-0.8% TA, 7-10 g/L glycerol, and 9-13% ethanol for a dry red table wine (Liu and Davis 1994, Waterhouse et al. 2016).

The concentrations of fructose were approximately 10 times greater than those of glucose in all wines. This was likely because yeast preferentially ferment glucose, thus decreasing concentration throughout fermentation (Waterhouse et al. 2016). The Skin Contact x Enzyme x Storage interaction was significant for glucose. In general, wines with BG enzyme had higher glucose concentrations than wines without BG (Figure 5). This was likely because addition of the glycosidic enzyme cleaved the bond between glucose and aroma compound aglycones,

increasing the free glucose content of the wine (Maicas and Mateo 2005). Wines with 0-days skin contact and BG enzyme at 3-months storage (18.20 mg/100 mL) had a higher glucose concentration than all wines without the BG enzyme. There was no consistent effect of Skin Contact or Storage on glucose levels. Regardless of treatment, the concentrations of glucose in Noble muscadine wine were below the detection threshold of 360-1200 mg/100 mL (Belitz et al. 2009, Hufnagel and Hofmann 2008, Noble and Bursick 1984). Therefore, addition of a β-glycosidic enzyme to Noble muscadine wine likely did not increase the perceived sweetness. The Skin Contact x Enzyme interaction and the Storage main effect were significant for fructose and total residual sugars. The concentrations of fructose and total residual sugars decreased from 0-months (51.44 and 56.16 mg/100 mL, respectively) to 6-months storage (32.65 and 35.40 mg/100 mL, respectively). This decrease in sugars could have been caused by oxidation or reduction of sugars to sugar acids or alcohols, respectively, or the formation of sugar-bisulfite adducts (Waterhouse et al. 2016). Wines with 0-days skin contact and BG enzyme had higher fructose (97.92 mg/100 mL) and total residual sugar (109.48 mg/100 mL) concentrations than all other wines (Figure 6). The only difference between enzyme levels was seen at 0-months storage, where BG wines had higher fructose and total residual sugars than no BG wines (74.87 and 74.87 mg/100 mL, respectively). In general, wines with 0-days skin contact had higher fructose and total residual sugar levels than wines with 3- or 7-days skin contact. This could indicate that the sugars were consumed at a higher rate during fermentation in the wines that were fermented on the skins. However, this was not reflected in the ethanol content. In fact, wines with 0-days skin contact generally had the highest ethanol contents. Therefore, it is possible that the additional phenolic compound aglycons extracted during maceration bound some of the free sugars in the wine (Sims and Morris 1985, Waterhouse et al. 2016).

The Storage main effect was significant for malic acid. Noble muscadine wines at 6-months storage (149.68 mg/100 mL) had the highest malic acid content, followed by those at 0-months storage (108.29 mg/100 mL) and 3-months storage (98.63 mg/100 mL). The concentration of malic acid in all wines was less than the 200-700 mg/100 mL range typically found in non-muscadine red wines (Da Conceicao Neta et al. 2007, Fowles 1992, Sowalsky and Noble 1998). Lamikanra (1997) found that while malic and tartaric acids were the most predominant organic acids in Noble muscadine wines prior to fermentation, their concentrations decreased throughout fermentation.

The Skin Contact x Storage interaction was significant for tartaric acid, citric acid, succinic acid, lactic acid, and total organic acids. At 0-months storage, the wines with 0-days skin contact had a lower tartaric acid content (259.80 mg/100 mL) than wines with 3-days (335.38 mg/100 mL) or 7-days (355.16 mg/100 mL) skin contact (Figure 7). Similarly, at 3-months storage, the 0-days skin contact wines (386.21 mg/100 mL) had lower tartaric acid than the 7-days skin contact wines (404.70 mg/100 mL). This was consistent with the lower TA seen in 0-days skin contact wine at 0- and 3-months storage (Figure 3b). There was no apparent effect of Storage on tartaric acid. The tartaric acid concentrations of all Noble muscadine wines fell within the typical range of 200-600 mg/100 mL for dry-red wines (Da Conceicao Neta et al. 2007, Fowles 1992, Sowalsky and Noble 1998). The 7-days skin contact wines at 6-months storage had higher citric acid (309.83 mg/100 mL) than all other wines. There was no difference between Skin Contact treatments at 0- or 3-months storage. Succinic acid is the predominant organic produced in wines during fermentation, and muscadine wines tend to have higher succinic acid levels than other *Vitis* wines (Lamikanra 1997). At 6-months storage, the 0-days skin contact wines had higher succinic acid levels (195.16 mg/100 mL) than wines with 3-days

(125.33 mg/100 mL) or 7-days (122.94 mg/100 mL) skin contact, but differences among Skin Contact treatments were not seen at 0- or 3-months storage. The succinic acid levels remained fairly steady during storage and were similar to the 180 mg/100 mL succinic acid reported in Noble muscadine wine by Lamikanra (1997). High succinic acid levels can give wine a bitter taste, and levels reported in the present study were higher than the 3.5 mg/100 mL detection threshold (Amerine et al. 1979). There were no differences in lactic acid concentration among Skin Contact treatments at any Storage times. Lactic acid levels in wines remained steady during storage and were within the typical 0-300 mg/100 mL range for dry table wines (Da Conceicao Neta et al. 2007, Fowles 1992, Sowalsky and Noble 1998). The 0-days skin contact wines at 6-months storage (1,235.34 mg/100 mL) had higher total organic acids than 0-days skin contact wines at 0-months (670.05 mg/100 mL) and 3-months (713.04 mg/100 mL) storage. With the exception of the 0-days skin contact wine at 6-months storage, total organic acid levels in the present study were similar to the 750 mg/100 mL total organic acids reported by Lamikanra (1997) in Noble muscadine wines. There was no effect of Enzyme treatment on individual or total organic acid concentrations.

Anthocyanins. Noble muscadine wines were analyzed during storage (0, 3, and 6 months at 15°C) for individual and total anthocyanins. Anthocyanins in wines included delphinidin-3,5-diglucoside, petunidin-3,5-diglucoside, peonidin-3,5-diglucoside, malvidin-3,5-diglucoside, and cyanidin-3,5-diglucoside. The anthocyanin profiles of Noble muscadine wines in the present study were consistent with the non-acylated, 3,5-diglucosides typically found in *Vitis rotundifolia* grapes and wine (Sims and Bates 1994). These diglucoside anthocyanins are more susceptible to color degradation during storage than their monoglucoside counterparts because they are unable to form stabilized polymeric pigment complexes and have lower pK_a values and

will thus lose color more readily due to increases in pH (Robinson et al. 1966, Sims and Morris 1986). Sims and Morris (1985) showed that there was very little, if any, formation of anthocyanin-tannin pigments in Arkansas Noble muscadine wine during storage.

Delphinidin-, petunidin-, and peonidin-3,5-diglucoside comprised approximately 85% of total anthocyanins across Skin Contact and Enzyme treatments at 0-months storage, and thus only these three individual anthocyanins, along with total anthocyanins, were discussed in this study. At 0-months storage, wines had 48-103 mg/100 mL delphinidin-3,5-diglucoside, 32-76 mg/100 mL petunidin-3,5-diglucoside, 29-50 mg/100 mL peonidin-3,5-diglucoside, and 142-290 mg/100 mL total anthocyanins (data not shown). Total anthocyanins in the present study were higher than those reported in the literature for Noble muscadine wine. Zhang et al. (2017), Sims and Bates (1994), and Talcott and Lee (2002) reported 59-92 mg/100 mL total anthocyanins in Noble muscadine wines from the southeastern United States. Delphinidin-3,5-diglucoside was the most predominant individual anthocyanin in the present study, comprising 35% of the total anthocyanin content. This was consistent with the results of Nesbitt et al. (1974), who found that delphinidin-3,5-diglucoside was the most prevalent anthocyanin across different varieties of red muscadine wine.

The Skin Contact x Storage interaction was significant for all anthocyanin attributes (Table 2). In general, the individual and total anthocyanin content of Noble muscadine wines decreased from 0-months to 6-months storage (Figure 8). This was likely due to degradation of anthocyanins from bisulfite bleaching or hydration, as diglucoside anthocyanins are not able to form stable polymeric pigment complexes (Ballinger et al. 1973, Waterhouse et al. 2016). At 0-months storage, wines with 0-days skin contact had lower delphindin-3,5-diglucoside (48.10 mg/100 mL), petunidin-3,5-diglucoside (32.48 mg/100 mL), peonidin-3,5-diglucoside (28.92

mg/100 mL), and total anthocyanins (142.13 mg/100 mL) than wines with 3-days (100.52, 69.91, 48.27, and 278.36 mg/100 mL, respectively) or 7 days (102.65, 75.71, 49.66, and 289.69 mg/100 mL, respectively) skin contact. Similar patterns were seen at 3- and 6-months storage. There was no difference in delphinidin-3,5-diglucoside content between wines with 3- and 7-days skin contact at any Storage times. This was consistent with the results of Sims and Bates (1994), who saw an increase in anthocyanin extraction from Noble muscadine grapes from 0-4 days skin contact, but then saw levels remain steady from 4-6 days. Wines with 7-days skin contact had higher petundinin-3,5-diglucoside at 0-months (75.71 mg/100 mL) and 3-months storage (59.36 mg/100 mL) than wines with 3-days skin contact (69.61 and 55.32 mg/100 mL, respectively). A similar trend was seen with total anthocyanins. This was logical, as anthocyanin content generally increases with increasing skin contact time (Arnold and Noble 1979, Schmidt and Noble 1983). Therefore, it is likely that Noble muscadine wines with higher skin contact times will have more intense red color, and that this red color will degrade during storage.

The Enzyme x Storage interaction was significant for petunidin-3,5-diglucoside and peonidin-3,5-diglucoside. The petunidin- and peonidin-3,5-diglucoside content decreased during storage (Figure 9). There was no difference among enzyme levels for petunidin-3,5-diglucoside at any Storage times. A similar trend was seen with peonidin-3,5-diglucoside. However, at 3-months storage, the wines with BG enzyme (35.52 mg/100 mL) had a higher peonidin-3,5-diglucoside concentration than the wines without BG enzyme (34.75 mg/100 mL).

Color. Noble muscadine wines were analyzed during storage (0, 3, and 6 months at 15°C) for L*, hue angle, chroma, red color, brown color, and color density. At 0-months storage, wines had 4.9-24.0 L*, 360-361° hue angle, 30-64 chroma, 1.5-4.0 red color, 2.6-8.7 brown color, and 4.1-12.7 color density (data not shown).

The Skin Contact x Enzyme x Storage interaction was significant for all color attributes (data not shown). Wines with 0-days skin contact had higher L* values (lighter color) than all wines with 3- or 7-days skin contact (Figure 10a). This demonstrated that the color intensity of Noble muscadine wines increased significantly with increasing skin contact time, due to increases in anthocyanin extraction (Schmidt and Noble 1983). For all Storage and Enzyme treatments, the 0-days skin contact wines had a higher (less red) hue angle than the 3- or 7-days skin contact wines (Figure 10b). Nesbitt et al. (1974) found that red muscadine wines with lower L* (darker color) and redder hue angles were judged as having more desirable color. Therefore, the color of the 3- and 7-days skin contact wines would likely be preferred over that of the 0-days skin contact wines. For the wines without BG enzyme, the 3-days skin contact wine at 6-months storage (360.36°) had a higher (less red) hue angle than the 7-days skin contact wine at 6-months storage (360.30°). For the wines with BG enzyme, the 3-days skin contact wines at 0-months (360.33°) and 6-months storage (360.36°) had higher hue angle than the 7-days skin contact wines at 0-months (360.27°) and 6-months (360.30°) storage. There was no effect of Enzyme treatment on hue angle. For all Storage and Enzyme treatments, the 0-days skin contact wines had a higher chroma (more saturated color) than the 3- or 7-days skin contact wines (Figure 9b). This indicated that color saturation/purity decreased with increasing skin contact time. Muscadine juices and wines with no fermentation on the skins tend to have a bright red, almost pinkish color, whereas fermentation on the skins yields wine with darker red, more complex colors. This explains the decrease in chroma as wines were fermented on the skins. Chroma remained steady during storage, and there was no impact of Enzyme treatment on chroma.

In general, the red color, brown color, and color density of the Noble muscadine wines increased with increasing skin contact time (Figure 11). The 0-days skin contact wine without BG enzyme at 0-months storage had the lowest red color (1.50), and the 7-days skin contact wine with BG enzyme at 3-months storage had the highest (4.11). Red color values in the present study were similar to the 1.8-2.8 red color range reported by Sims and Bates (1994) for Noble muscadine wines over one year of storage. Regardless of Enzyme or Skin Contact treatment, the red color of wines increased slightly from 0- to 3-months storage, but then decreased from 3-months to 6-months storage. This decrease in red color was likely due to degradation of the less-stable diglucoside anthocyanins found in Noble muscadine wine. While there were slight decreases in color density during storage, there was no increase in brown color observed. This was significant, as muscadine wines typically experience significant browning during storage that limits their shelf-life and consumer acceptability (Sims and Morris 1986). There was no impact of Enzyme treatment on red color, brown color, or color density.

Analysis of aroma attributes

Noble muscadine wines were analyzed at 3-months storage at 15°C for volatile aroma compound profiles. There were 45 volatile aroma compounds positively identified in Noble muscadine wines. Initial exploration of volatile aroma chromatograms showed that wines had similar chromatogram peaks regardless of Skin Contact or Enzyme treatment, but peak areas differed (data not shown). Table 3 shows the compounds identified in the wines, their compound class, the aroma category each was grouped into, and more detailed aroma descriptors. Compounds included chemical, floral, green/fat (waxy, rancid), and woody alcohols, floral, green/fat, and roasted/caramelized aldehydes, vegetal alkyl sulfides, unpleasant carboxylic acids, floral, fruity, and herbal/spicy esters, fruity glycols, green/fat ketones, floral and herbal/spicy

terpenes, and herbal/spicy oxabicycloalkanes. The esters were the largest class of compounds in all wines. Esters are characteristic byproducts of alcoholic fermentation and are critical for the aroma of most wines (Waterhouse et al. 2016). Lamikanra (1987) determined that fatty acid ethyl esters and higher alcohols were numerically the largest class of compounds in Noble muscadine wines. Baek and Cadwallader (1999) identified esters as being odor-active in muscadine grape juice but concluded that they would be more associated with muscadine wine aroma, as their concentration would increase during fermentation. Higher alcohols were also prevalent in Noble muscadine wines. With the exception of 2-phenylethanol, the overall contributions of higher alcohols to wine aroma was likely low, as these compounds have high detection thresholds (Baek and Cadwallader 1999). The compound 2-Phenylethanol is known to be influential for muscadine wine aroma, contributing a rose and honey-like aroma (Lamikanra 1987, Lamikanra et al. 1996). This alcohol is also a significant contributor to muscadine grape juice aroma (Baek and Cadwallader 1999). The muscadine grape is the only grape with significant amounts of 2-phenylethanol, as this compound is produced primarily as a secondary aroma compound during fermentation in most wines (Lamikanra et al. 1996).

PCA was used to reduce dimensionality of the data and to elucidate relationships between aroma categories and Skin Contact and Enzyme treatments. The TIC areas were summed for compounds within each aroma category. Examining the PCA results, distinctions could be made among wines with different Skin Contact and Enzyme Treatments (Table 4). Four components explained over 85% of the variation in the dataset. PC1 (42.4%) had positive loadings for herbal/spicy, green/fat, and chemical aroma categories, and wines that loaded positively on PC1 were all wines with 3-days skin contact and wines with BG enzyme and 7-days skin contact. Unpleasant, fruit, and roasted/caramelized aroma categories and all wines with 0-days skin

contact loaded negatively on PC1. This indicated that wines with greater skin contact time could potentially have more herbal, green, and unripe aroma notes, whereas wines with no fermentation on the skins could be perceived as fruitier and roasted/caramelized, although possibly more unpleasant (cheesy and pungent carboxylic acid aromas) as well. This was consistent with the findings of Sims and Bates (1994), who determined that Noble muscadine wines with 0-days skin contact had higher fruity aroma intensities compared to wines with longer skin contact times.

PC2 (22.6%) had positive loadings for floral and chemical aroma categories and 0-days and 3-days wine with BG enzyme. The woody aroma category and the all wines with 7-days skin contact loaded negatively on PC2. This indicated that wines with longer skin contact times had higher woody aromas, whereas wines with shorter skin contact times had floral aromas characteristic of muscadine wines and juices. PC3 (13.6%) had positive loadings for the vegetal aroma category and wines without the BG enzyme and 3- and 7-days skin contact. The wines with BG enzyme and 0- and 3-days skin contact loaded negatively on PC3. Thus, PC3 represented separation between wines with and without the glycosidic enzyme, and wines without the enzyme were more associated with vegetal aromas. Baek and Cadwallader (1999) found that application of β-glucosidase enzyme to muscadine grape juice increased the concentrations of fruity and floral aroma compounds. Therefore, it was possible that application of the BG enzyme in the present study lead to wines with more fruity and floral aroma notes, whereas wines without the enzyme would be perceived as more vegetal/green. Floral and unpleasant aroma categories and all wines with 3-days skin contact loaded positively on PC4 (9.3%), and the 0-days skin contact wine with BG enzyme loaded negatively on PC4. Therefore, it is possible that the wines with 3-days skin contact had more floral and unpleasant aroma notes.

Sensory attributes analysis

The aroma intensity and aroma liking of Noble muscadine wines at 3-months storage at 15°C were evaluated by a consumer panel (n = 68). Consumer panelists evaluated overall aroma intensity, fruity aroma intensity, green aroma intensity, floral aroma intensity, and overall aroma liking. In addition, panelists were asked to list descriptor terms for the aroma of each wine. Wines without the β-glucosidase enzyme were perceived as having a fruitier, more pleasant aroma than wines with the enzyme, and panelists liked the aroma of wines with 3-days skin contact the most. Fruity, floral, earthy, and candy were the most commonly-used descriptors for the aroma of muscadine wines. Wines with higher skin contact times were associated with spicy, dark fruit aromas, whereas wines with no skin contact were perceived as having strawberry, candy, and artificial aroma notes. Addition of the glycosidic enzyme lead to wines with more unpleasant hay/chemical notes, whereas pleasant red fruit notes were more noticeable in wines without enzyme application.

Aroma intensity and liking. Panelists evaluated the overall aroma intensity, fruity aroma intensity, green aroma intensity, floral aroma intensity, and overall aroma liking at 3-months storage at 15°C. There was no effect of Skin Contact or Enzyme treatment on overall aroma intensity, green aroma intensity, or floral aroma intensity (Table 5). The Enzyme main effect was significant for fruity aroma intensity, and wines without BG enzyme were perceived as fruitier than wines with BG enzyme. Although BG enzyme is reported to increase the fruity aroma compounds of muscadine juices, it also increases the concentration of other glycosidically-bound compounds (Baek and Cadwallader 1999). Muscadine wines have a unique, excessively fruity character (Sims and Bates 1994), and application of the glycosidic enzyme could have released some compounds that masked this natural fruitiness. In addition, Baek and Cadwallader (1999)

determined that most esters in muscadine juice were present only in the free form. As esters are the compounds primarily responsible for the fruitiness of muscadine wines, it is possible that they were present mostly in the free (unbound) form in Noble muscadine wines, and therefore application of a glycosidic enzyme would not affect their odor perception.

The Skin Contact and Enzyme main effects were significant for overall aroma liking. Panelists liked the aroma of the 3-days skin contact wine the most, followed by the 7-days skin contact wine, and the 0-days skin contact wine. Sims and Bates (1994) determined that lower skin contact times were more ideal for preserving the typical light, fruity character of muscadine wines. The results of volatile aroma profile analysis in the present study indicated that wines with lower skin contact times were associated with fruity aroma compounds, whereas wines with higher skin contact times were associated with herbal/spicy and green aroma compounds (Table 4). Therefore, panelists could have rated their liking of the 3-days skin contact wine the highest because they preferred balance between the simpler fruity aromas of the 0-days skin contact wine and the more complex notes of the wines with higher skin contact. The overall aroma liking was higher for the wines without BG enzyme. This could be due to the higher perceived fruity aroma of the no BG wines. It is also possible that application of the glycosidic enzyme increased the concentrations of some unpleasant aroma compounds. Baek and Cadwallader (1999) determined that while β-glycosidase application increased the concentrations of some pleasant aroma compounds in muscadine juice, it also increased the concentrations of some unpleasant aroma compounds, such as *p*-vinylguaiacol (curry-like aroma).

Aroma descriptors. There were 37 terms used to describe the aroma of 2018 Noble muscadine wines. Terms used less than five times overall were excluded from analysis. Figure 12 shows a word cloud for the descriptors used for Noble muscadine wine aroma, across all Skin Contact

and Enzyme treatments. The size of each term in the word cloud indicates its frequency of use. The most commonly-used term across all wines was fruity (n = 101), followed by floral (n = 47), earthy (n = 29), candy (n = 28), alcohol (n = 27), concord (n = 24), berry (n = 22), fresh (n = 22), herbal (n = 21), jam (n = 20), and rose (n = 19). These descriptors were in line with the typical red fruit, candy/artificial, floral, and foxy aroma character of red muscadine wines (Gürbüz et al. 2013, Lamikanra et al. 1996, Sims et al. 1995).

The number of times each descriptor was used for each wine was determined and used for PCA to determine the effect of Skin Contact and Enzyme treatments on Noble muscadine wine aroma characteristics. Four components explained over 85% of the variation in the dataset (Table 6). Spice, plum, metallic, bubblegum, blackberry, medicinal, and alcohol descriptors loaded positively on PC1 (26.7%), and strawberry, grass, candy, rubber, and artificial descriptors loaded negatively on PC1. It was determined that PC1 represented high levels of dark fruit and spicy aromas, and low levels of typical muscadine wine/juice aromas (strawberry, candy, artificial). The wines with 3- and 7-days skin contact had positive loadings on PC1, and wines with 0-days skin contact had negative loadings. Therefore, wines with higher skin contact were associated with more complex, dark fruit aromas characteristic of red wines, whereas muscadine wines with no skin contact had typical muscadine juice aromas. PC 2 (22.2%) had positive loadings for cooked fruit, hay, chemical, and perfume aromas, and negative loadings for berry, jam, fruity, and pleasant aromas. Wines with BG enzyme loaded positively on PC2 and wines without BG enzyme loaded negatively. Therefore, wines without BG enzyme were associated with pleasant fruity aromas, whereas wines with BG enzyme were associated with the more unpleasant hay and chemical aromas. This could explain why panelists perceived higher fruity aroma intensity for wines without BG enzyme and liked the overall aroma of these wines more.

PC3 (20.8%) and PC4 (19.8%) were also considered, but there were no obvious patterns concerning aroma descriptors and Skin Contact and Enzyme treatments.

Conclusions

In 2018, Noble muscadine wines had compositions at bottling within typical ranges for dry red table wines, remaining mostly stable during six months of storage at 15°C. The acidity of wines increased during storage, and wines with longer skin contact times had higher TA values and lower residual sugars. Addition of a glycosidic enzyme at bottling led to wines with higher glucose and total residual sugar levels, and the residual sugar levels of all wines decreased during storage.

Only diglucoside anthocyanins were identified in Noble muscadine wines, and delphinidin-3,5-diglucoside was the most predominant anthocyanin. The individual and total anthocyanin content of wines decreased during storage, likely due to degradation of anthocyanins from bisulfite bleaching or hydration. Anthocyanin content of wines increased with skin contact time, with the greatest increase observed from 0- to 3-days skin contact. The color intensity, red hue, red color, brown color, and color density increased with skin contact time. The color density of muscadine wines decreased during storage, but a corresponding increase in brown color density was not observed.

Fruity esters were the largest class of volatile aroma compounds in Noble muscadine wines, followed by higher alcohols, notably 2-phenylethanol (rose-like character). Wines with higher skin contact times were associated with herbal, green, and unripe aroma notes, whereas wines with no skin contact time were associated with fruitier aromas. Enzyme addition lead to wines that could potentially be perceived as less vegetal than those without enzyme addition.

The consumer sensory panel found differences among the wines with different Skin Contact and Enzyme treatments. Wines without enzyme addition had fruitier, more pleasant aromas than those with the enzyme. Panelists liked the aroma of the wines with 3-days skin contact the most. The most-commonly used descriptors for muscadine wine aroma were fruity, floral, earthy, and candy. Wines with higher skin contact times were associated with spicy, dark fruit aroma descriptors, whereas wines with no skin contact were perceived as having strawberry, candy, and artificial aromas characteristic of muscadine grape juice. Addition of glycosidic enzyme led to wines with more unpleasant hay/chemical notes, whereas pleasant red fruit notes were more noticeable in wines without enzyme application. Therefore, variations in skin contact time and addition of a glycosidic enzyme impacted the composition, anthocyanin, color, volatile aroma, and sensory properties of wines produced from Arkansas-grown Noble muscadine grapes.

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Tables

Table 1. Main and interaction effects from ANOVA on composition attributes for Noble muscadine wines with different Skin Contact times (0, 3, or 7 days), β-glucosidase (BG) Enzyme levels (no BG, BG), and Storage times (0, 3, and 6 months at 15°C) (Arkansas, 2018).

Effects	pH	Titratable acidity (%)	Glycerol (g/L)	Ethanol (% v/v)	Glucose (mg/100 mL)	Fructose (mg/100 mL)	Total residual sugars (mg/100 mL)	Tartaric acid (mg/100 mL)	Malic acid (mg/100 mL)	Citric acid (mg/100 mL)	Succinic acid (mg/100 mL)	Lactic acid (mg/100 mL)	Total organic acids (mg/100 mL)
Skin Contact (SC)													
0 days	3.09 a[a]	0.66 b	10.97 b	11.81 a	5.83 a	86.35 a	92.17 a	316.50 a	163.24 a	166.72 a	159.76 a	66.59 a	872.81 a
3 days	3.05 a	0.78 a	12.20 a	11.00 b	1.46 a	19.08 a	20.54 a	352.77 a	99.41 a	94.66 b	133.73 a	79.30 a	759.87 a
7 days	3.08 a	0.76 a	12.12 a	10.93 b	4.97 a	18.65 a	23.61 a	362.54 a	93.95 a	63.20 b	134.39 a	84.85 a	738.94 a
P value	*0.2560*	***0.0019***	*0.0534*	***0.0105***	*0.2312*	***0.0547***	*0.0532*	*0.0924*	*0.3790*	*0.3574*	*0.3341*	*0.7085*	*0.6506*
Enzyme (E)													
No BG	3.08 a	0.73 a	11.79 a	11.29 a	0.13 b	37.10 b	37.23 b	342.06 a	128.84 a	112.25 a	145.66 a	78.04 a	806.85 a
BG	3.07 a	0.73 a	11.73 a	11.21 a	8.04 a	45.61 a	53.66 a	345.81 a	108.90 a	104.14 a	139.59 a	75.79 a	774.23 a
P value	*0.0805*	*0.5505*	*0.2145*	*0.1163*	***<0.0001***	***0.0029***	***<0.0001***	*0.6512*	*0.1160*	*0.6228*	*0.2899*	*0.7234*	*0.4628*
Storage (S)													
0 months	3.11 a	0.68 c	11.72 a	11.14 b	4.72 a	51.44 a	56.16 a	316.78 b	108.29 b	65.10 b	153.27 a	73.80 a	717.24 b
3 months	3.12 a	0.80 a	11.77 a	11.33 a	4.78 a	39.98 b	44.76 a	352.35 a	98.63 b	104.01 ab	126.80 b	69.91 a	751.70 b
6 months	2.99 b	0.72 b	11.80 a	11.27 ab	2.75 a	32.65 b	35.40 c	362.68 a	149.68 a	155.47 a	147.81 a	87.04 a	902.68 a
P value	***<0.0001***	***<0.0001***	*0.3651*	***0.0178***	*0.1562*	***<0.0001***	***<0.0001***	***<0.0001***	***0.0036***	***0.0002***	***0.0010***	*0.0767*	***0.0027***
SC x E (P value)	*0.4866*	***0.0197***	***0.0015***	*0.1162*	***0.0016***	***0.0020***	***0.0006***	*0.6893*	*0.8497*	*0.8725*	*0.5204*	*0.9960*	*0.8949*
SC x S (P value)	*0.0656*	***<0.0001***	*0.1079*	*0.4714*	*0.0618*	*0.3508*	*0.1190*	***<0.0001***	*0.1042*	***<0.0001***	***0.0001***	***<0.0001***	***<0.0001***
E x S (P value)	*0.0599*	***0.0154***	*0.1472*	*0.2276*	*0.2098*	*0.8311*	*0.8956*	*0.9868*	*0.4015*	*0.8438*	*0.5412*	*0.9416*	*0.7504*
SC x E x S (P value	*0.0880*	*0.1324*	***0.0009***	***0.0001***	***0.0428***	*0.2590*	*0.1523*	*0.2305*	*0.3690*	*0.9467*	*0.9763*	*0.9914*	*0.8852*

[a] Means with different letters for each attribute within effects are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

Table 2. Main and interaction effects from ANOVA on anthocyanin attributes for Noble muscadine wines with different Skin Contact times (0, 3, or 7 days), β-glucosidase (BG) Enzyme levels (no BG, BG), and Storage times (0, 3, and 6 months at 15°C) (Arkansas, 2018).

Effects	Delphinidin-3,5-diglucoside (mg/100 mL)	Petunidin-3,5-diglucoside (mg/100 mL)	Peonidin-3,5-diglucoside (mg/100 mL)	Total anthocyanins (mg/100 mL)
Skin Contact (SC)				
0 days	36.89 b[a]	25.83 b	23.34 b	112.33 b
3 days	73.99 a	53.07 a	37.47 a	210.87 a
7 days	74.88 a	56.79 a	37.74 a	216.78 a
P value	***0.0002***	***0.0002***	***<0.0001***	***0.0001***
Enzyme (E)				
No BG	61.91 a	45.27 a	32.82 a	180.01 a
BG	61.92 a	45.19 a	32.89 a	179.98 a
P value	*0.9708*	*0.5100*	*0.4983*	*0.9622*
Storage (S)				
0 months	83.75 a	59.37 a	42.29 a	236.72 a
3 months	65.01 b	47.45 b	35.13 b	189.94 b
6 months	36.99 c	28.87 c	21.14 c	113.32 c
P value	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
SC x E (P value)	*0.5189*	*0.5302*	*0.1228*	*0.6508*
SC x S (P value)	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
E x S (P value)	*0.4013*	***0.0312***	***<0.0001***	*0.2725*
SC x E x S (P value)	*0.3636*	*0.1993*	*0.1422*	*0.4180*

[a] Means with different letters for each attribute within effects are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

Table 3. Volatile aroma compounds identified in Noble muscadine wines at 3-months storage at 15°C with different Skin Contact times (0, 3, or 7 days) and β-glucosidase (BG) Enzyme levels (no BG, BG) (Arkansas, 2018).

Compound[a]	Compound class	Aroma category	Aroma descriptors[b]
Octanol	Alcohol	Chemical	Chemical, metal
2-Ethylhexanol	Alcohol	Floral	Rose, citrus
2-Phenylethanol	Alcohol	Floral	Honey, rose
1-Decanol	Alcohol	Green/fat	Fat
1-Dodecanol	Alcohol	Green/fat	Fat, wax
1-Hexanol	Alcohol	Green/fat	Green, herbal
1-Nonanol	Alcohol	Green/fat	Fat, green
cis-3-Hexen-1-ol	Alcohol	Green/fat	Grass, leaf
Nerolidol	Alcohol	Woody	Wood, flower, wax
Phenylacetaldehyde	Aldehyde	Floral	Floral, honey, rose
Decanal	Aldehyde	Green/fat	Soap, orange peel
Nonanal	Aldehyde	Green/fat	Fat, citrus, green
Furfural	Aldehyde	Roasted/caramelized	Almond, caramel
Methionol	Alkyl sulfide	Vegetal	Cooked potato
2-Methylbutyric acid	Carboxylic acid	Unpleasant	Cheese, sweat
Isovaleric acid	Carboxylic acid	Unpleasant	Sweat, cheese
Octanoic acid	Carboxylic acid	Unpleasant	Sweat, cheese, fat
Neryl formate	Ester	Floral	Rose, floral
2-Methylbutyl acetate	Ester	Fruity	Fermented fruit, banana, rum
Diethyl succinate	Ester	Fruity	Wine, fruit, watermelon
Ethyl 2-furoate	Ester	Fruity	Fruit, floral
Ethyl 2-methylbutyrate	Ester	Fruity	Apple, strawberry
Ethyl butanoate	Ester	Fruity	Apple, strawberry, bubblegum
Ethyl decanoate	Ester	Fruity	Grape
Ethyl dodecanoate	Ester	Fruity	Mango, leaf
Ethyl heptanoate	Ester	Fruity	Fruit
Ethyl hexanoate	Ester	Fruity	Apple peel, strawberry, anise
Ethyl isobutyrate	Ester	Fruity	Strawberry
Ethyl isovalerate	Ester	Fruity	Anise, apple, black currant
Ethyl nonanoate	Ester	Fruity	Tropical fruit, rose
Ethyl octanoate	Ester	Fruity	Fruit, floral
Ethyl *trans*-4-decenoate	Ester	Fruity	Fruit, wax, cognac
Hexyl acetate	Ester	Fruity	Fruit, herb, wine
Isoamyl acetate	Ester	Fruity	Banana, pear
Isobutyl acetate	Ester	Fruity	Apple, banana
Methyl hexanoate	Ester	Fruity	Fruit, fresh, paint thinner
Ethyl cinnamate	Ester	Herbal/Spicy	Cinnamon, honey
2,3-Butanediol	Glycol	Fruity	Fruit, onion

Table 3 (Cont.)

Compound[a]	Compound class	Aroma category	Aroma descriptors[b]
2-Nonanone	Ketone	Green/fat	Hot milk, soap, fat
Citronellol	Terpene	Floral	Rose, citrus, clove
Linalool	Terpene	Floral	Floral, lavender, Earl Grey tea
α-Terpineol	Terpene	Herbal/Spicy	Anise, mint, toothpaste
Eucalyptol	Terpene	Herbal/Spicy	Mint, licorice, pine
p-Cymene	Terpene	Herbal/Spicy	Herbal, spice
1,4-Cineole	Oxabicycloalkane	Herbal/Spicy	Spice

[a] Compounds were identified by comparison of mass spectra with NIST14 (National Institute of Standards and Technology, Gaithersburg, MD, USA), Flavors and Fragrances of Natural and Synthetic Compounds (FFNSC3, John Wiley & Sons, Inc., Hoboken, NJ, USA), and Adam's Essential Oils (Adams 2007) mass spectral libraries and comparison of calculated Kovats retention indices (Kováts 1958) with previously reported values .
[b] Aroma descriptors obtained from the Flavornet (Acree and Arn 2004) and Pherobase (Sayed 2003) databases.

Table 4. Summary of principal components analysis on volatile aroma compound categories in Noble muscadine wines at 3-months storage at 15°C with different Skin Contact times (0, 3, or 7 days) and β-glucosidase (BG) Enzyme levels (no BG, BG) (Arkansas, 2018).

		Component 1 (42.4%)[a]	**Component 2 (22.6%)**	**Component 3 (13.6%)**	**Component 4 (9.3%)**
Positive loadings[b]	Aroma categories[c]	Herbal/spicy Green/fat Chemical	Floral Chemical	Vegetal	Floral Unpleasant
	Key samples	3 days, No BG 3 days, BG 7 days, BG	0 days, BG 3 days, BG	3 days, No BG 7 days, No BG	3 days, No BG 3 days, BG
Negative loadings[d]	Aroma categories	Unpleasant Fruit Roasted/caramelized	Woody	---	---
	Key samples	0 days, No BG 0 days, BG	7 days, No BG 7 days, BG	0 days, BG 3 days, BG	0 days, BG

[a] Percent of variation in data explained by each component.
[b] Loading values >0.5 were considered positive loadings for aroma categories on each component.
[c] Aroma categories represent the sum of the total ion chromatogram (TIC) peak areas of positively identified compounds within each category (Table 5).
[d] Loading values <-0.5 were considered negative loadings for aroma categories on each component.

Table 5. Main and interaction effects from ANOVA on sensory attributes from a consumer sensory panel (68 panelists) for Noble muscadine wines at 3-months storage at 15°C with different Skin Contact times (0, 3, or 7 days) and β-glucosidase (BG) Enzyme levels (no BG, BG) (Arkansas, 2018).

Effects	**Overall aroma intensity[a]**	**Fruity aroma intensity**	**Green aroma intensity**	**Floral aroma intensity**	**Overall aroma liking[b]**
Skin Contact					
0 days	8.2 a[c]	7.2 a	6.1 a	6.5 a	5.2 b
3 days	8.6 a	7.6 a	5.5 a	6.3 a	5.8 a
7 days	8.9 a	7.4 a	6.0 a	6.5 a	5.6 ab
P value	*0.0891*	*0.6433*	*0.2164*	*0.8423*	***0.0215***
Enzyme					
No BG	8.7 a	7.9 a	5.7 a	6.3 a	5.8 a
BG	8.5 a	6.9 b	6.0 a	6.6 a	5.3 b
P value	*0.5559*	***0.0024***	*0.3210*	*0.2947*	***0.0052***
Skin Contact x Enzyme (P value)	*0.9028*	*0.6628*	*0.5048*	*0.7679*	*0.8338*

[a] A 15-cm line scale with anchors (none, moderate, and very strong) was used to evaluate overall, fruity, green, and floral aroma intensity.
[b] A nine-point hedonic scale, converted to a numerical scale (1=dislike extremely and 9=like extremely) was used to evaluate overall aroma liking.
[c] Means with different letters for each attribute within effects are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

Table 6. Summary of principal components analysis on terms used by a consumer sensory panel to describe the aroma of Noble muscadine wines at 3-months storage at 15°C with different Skin Contact times (0, 3, or 7 days) and β-glucosidase (BG) Enzyme levels (no BG, BG) (Arkansas, 2018)

		Component 1 (26.7%)[a]	**Component 2 (22.2%)**	**Component 3 (20.8%)**	**Component 4 (19.8%)**
Positive loadings[b]	Descriptors	Spice	Cooked fruit	Concord grape	Citrus
		Plum	Hay	Fresh	Fermented
		Metallic	Chemical	Red fruit	Pleasant
		Bubblegum	Perfume	Blackberry	Chemical
		Blackberry			Earthy
		Medicinal			
		Alcohol			
	Key samples	3 days, No BG	0 days, BG	3 days, No BG	0 days, BG
		3 days, BG	3 days, BG	7 days, BG	3 days, No BG
		7 days, No BG	7 days, BG		7 days, No BG
		7 days, BG			
Negative loadings[c]	Descriptors	Strawberry	Berry	Herbal	Unpleasant
		Grass	Jam	Green	Vinegar
		Candy	Fruity	Pungent	Rose
		Rubber	Pleasant	Raspberry	
		Artificial			
	Key samples	0 days, No BG	0 days, No BG	0 days, No BG	0 days, No BG
		0 days, BG	3 days, No BG	3 days, BG	3 days, BG
			7 days, No BG	7 days, No BG	7 days, BG

[a] Percent of variation in data explained by each component.
[b] Loading values >0.6 were considered positive loadings for aroma descriptors on each component.
[c] Loading values <-0.6 were considered negative loadings for aroma descriptors on each component.

Figures

To:	Renee Terrell Threlfall FDSC B-3
From:	Douglas James Adams, Chair IRB Committee
Date:	09/11/2019
Action:	**Exemption Granted**
Action Date:	09/11/2019
Protocol #:	1908209641
Study Title:	Impact of production techniques on wines produced from muscadine grapes

The above-referenced protocol has been determined to be exempt.

If you wish to make any modifications in the approved protocol that may affect the level of risk to your participants, you must seek approval prior to implementing those changes. All modifications must provide sufficient detail to assess the impact of the change.

If you have any questions or need any assistance from the IRB, please contact the IRB Coordinator at 109 MLKG Building, 5-2208, or irb@uark.edu.

cc: Sarah Mayfield, Key Personnel

Figure 1. University of Arkansas Institutional Review Board (IRB) protocol approval notice for sensory analysis of Noble muscadine wines at 3-months storage at 15°C with different Skin Contact times (0, 3, or 7 days) and β-glucosidase (BG) Enzyme levels (no BG, BG) (Arkansas, 2018).

PANELIST # _____ SAMPLE CODE _____

MUSCADINE WINE AROMA EVALUATION BALLOT

Swirl the wine in the glass to release the aromatics. Smell the wine.

Rate the intensity of the *OVERALL AROMA* of this wine. Indicate your response by drawing a vertical line on the line scale below.

None Moderate Very strong

Rate the intensity of the *FRUITY AROMA* of this wine.
"Fruity" aroma characteristics in wine are perceived as fresh fruit, cooked fruit, jam, and artificial/candy fruit.

None Moderate Very strong

Rate the intensity of the *GREEN/HERBACEOUS AROMA* of this wine.
"Green/herbaceous" aroma characteristics in wine are perceived as grass, fresh (raw) vegetables, fresh or dried herbs, and fresh-cut hay.

None Moderate Very strong

Rate the intensity of the *FLORAL AROMA* of this wine.
"Floral" aroma characteristics in wine are perceived as fresh or dried flowers, such as roses, violets, or lavendar.

None Moderate Very strong

Which statement best describes your impression of the *OVERALL AROMA* of this wine?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

List **five words** to describe the *AROMA* of this wine.

1. _____
2. _____
3. _____
4. _____
5. _____

Figure 2. Ballot presented to panelists for consumer sensory panel evaluation of Noble muscadine wines at 3-months storage at 15°C with different Skin Contact times (0, 3, or 7 days) and β-glucosidase (BG) Enzyme levels (no BG, BG) (Arkansas, 2018).

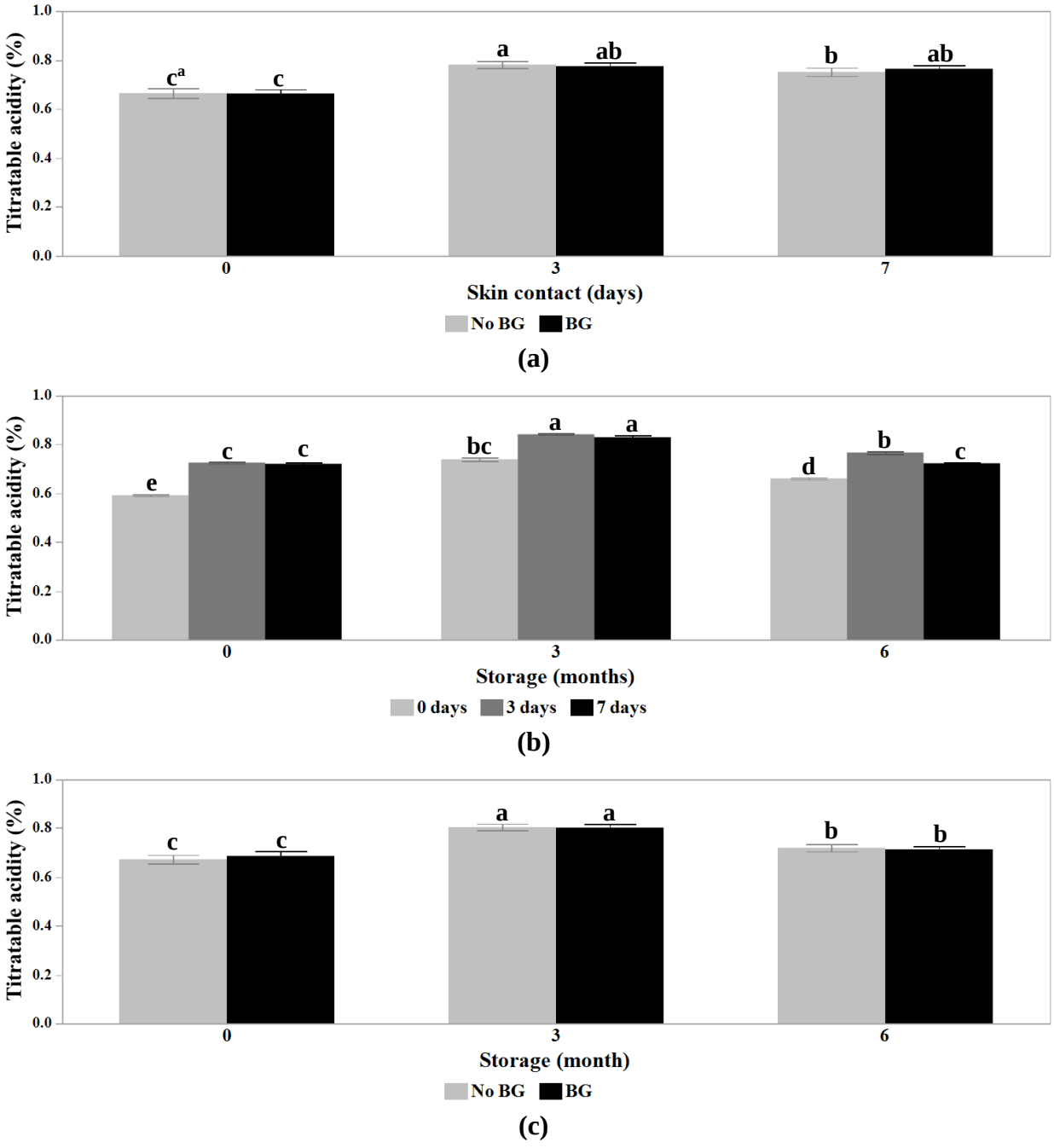

Figure 3. Effect of Skin Contact and Enzyme (a), Skin Contact and Storage (b), and Enzyme and Storage (c) on titratable acidity of Noble muscadine wines with different Skin Contact times (0, 3, or 7 days), β-glucosidase (BG) Enzyme levels (no BG, BG), and Storage times (0, 3, and 6 months at 15°C) (Arkansas, 2018).

[a] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters for each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

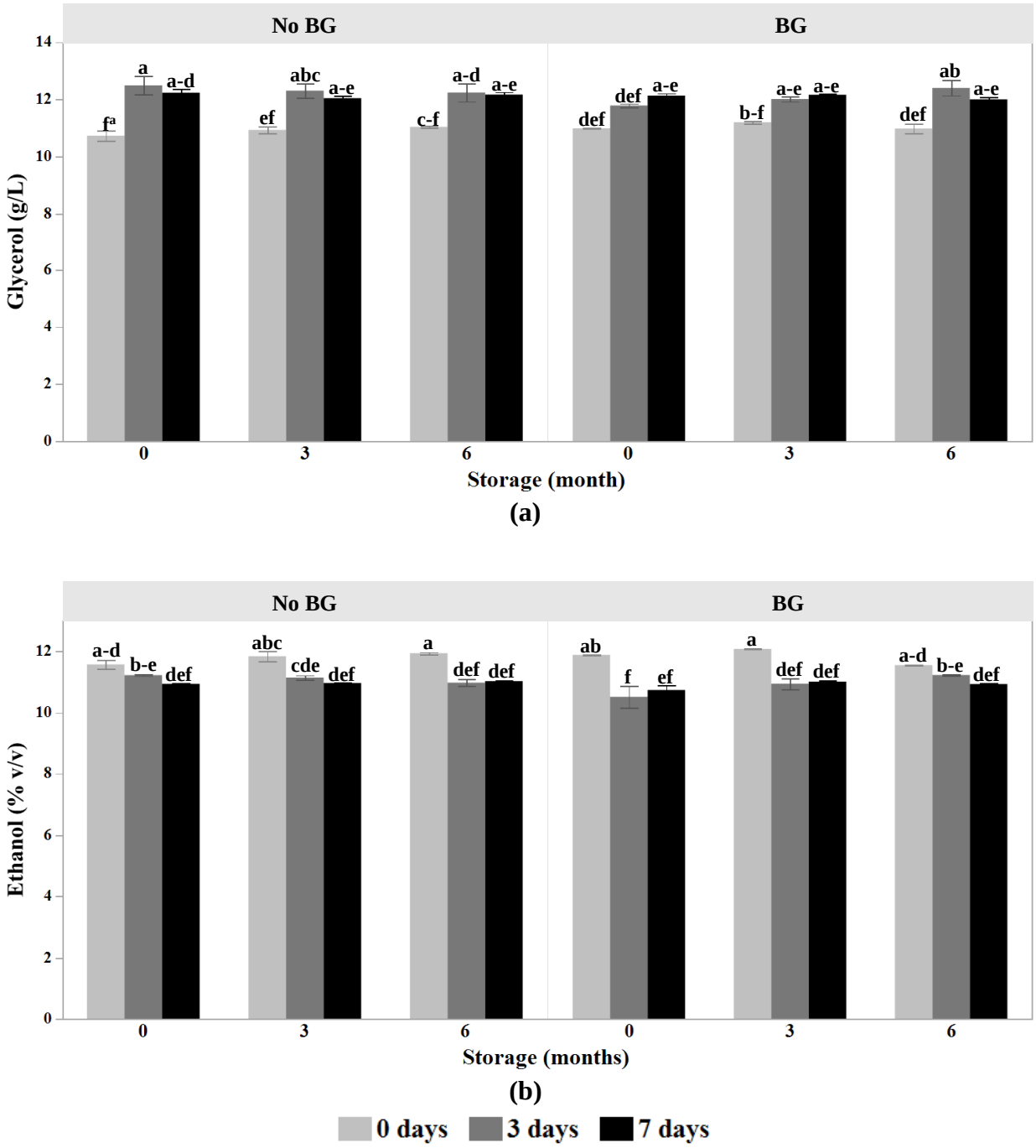

Figure 4. Effect of Skin Contact, Enzyme, and Storage on glycerol content (a) and ethanol content (b) of Noble muscadine wines with different Skin Contact times (0, 3, or 7 days), β-glucosidase (BG) Enzyme levels (no BG, BG), and Storage times (0, 3, and 6 months at 15°C) (Arkansas, 2018).

[a] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters for each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

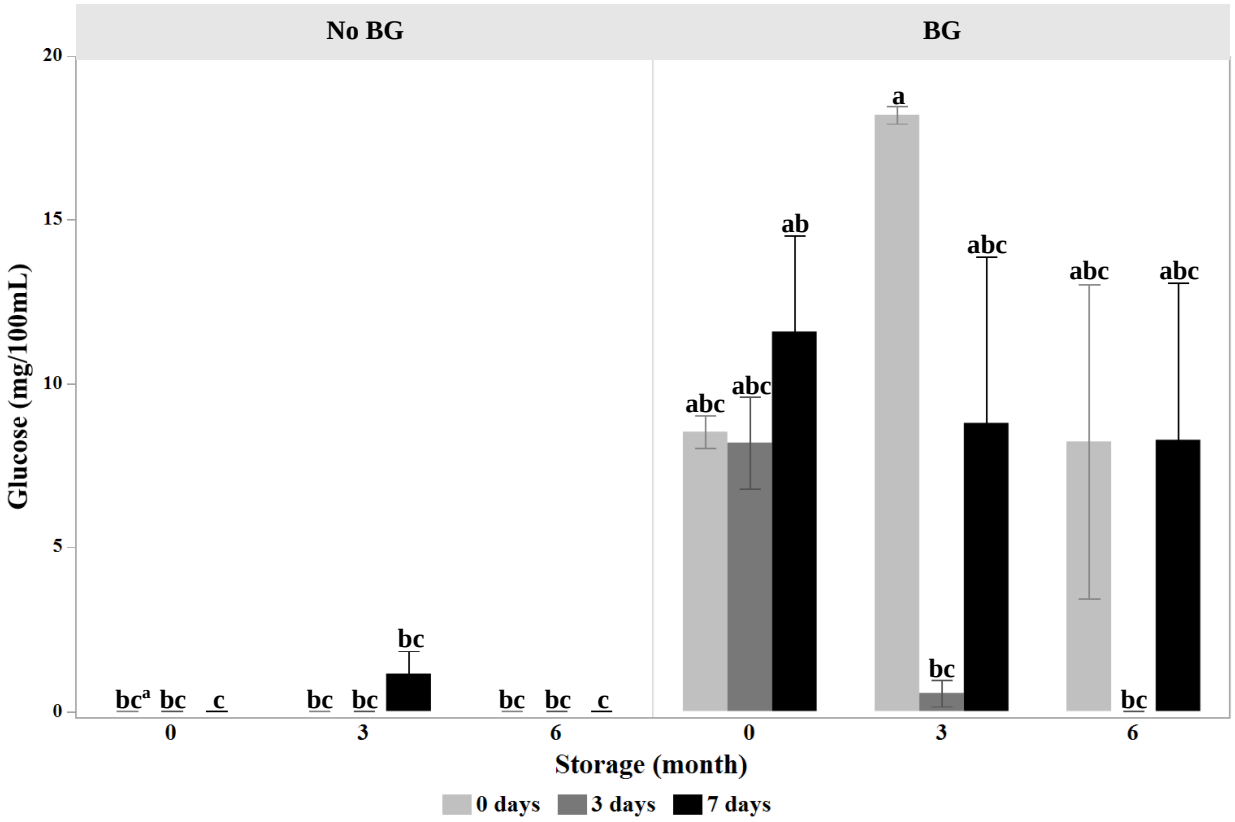

Figure 5. Effect of Skin Contact, Enzyme, and Storage on glucose content of Noble muscadine wines with different Skin Contact times (0, 3, or 7 days), β-glucosidase (BG) Enzyme levels (no BG, BG), and Storage times (0, 3, and 6 months at 15°C) (Arkansas, 2018).
[a] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

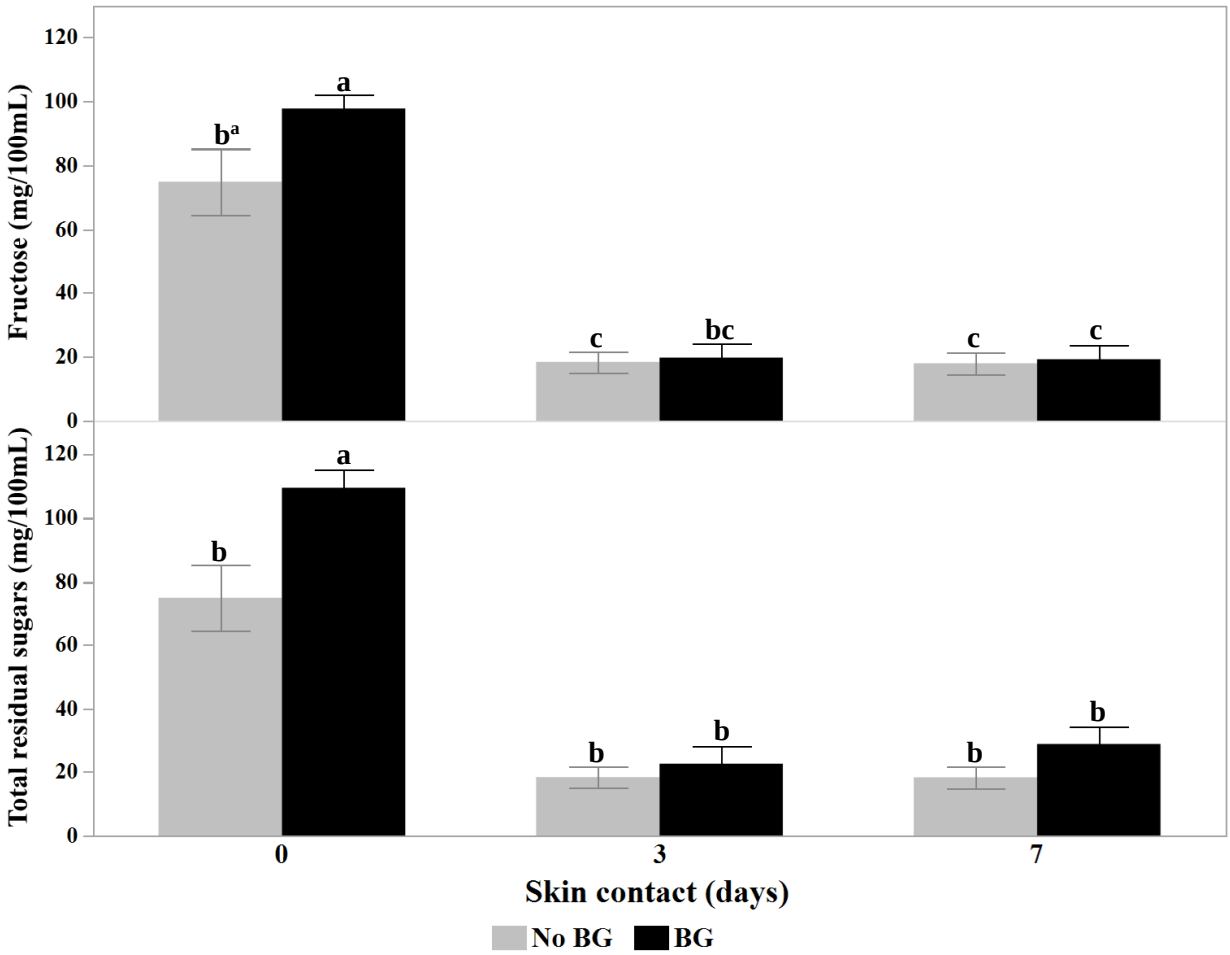

Figure 6. Effect of Skin Contact and Enzyme on fructose and total residual sugars content of Noble muscadine wines with different Skin Contact times (0, 3, or 7 days), β-glucosidase (BG) Enzyme levels (no BG, BG), and Storage times (0, 3, and 6 months at 15°C) (Arkansas, 2018). [a] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters for each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

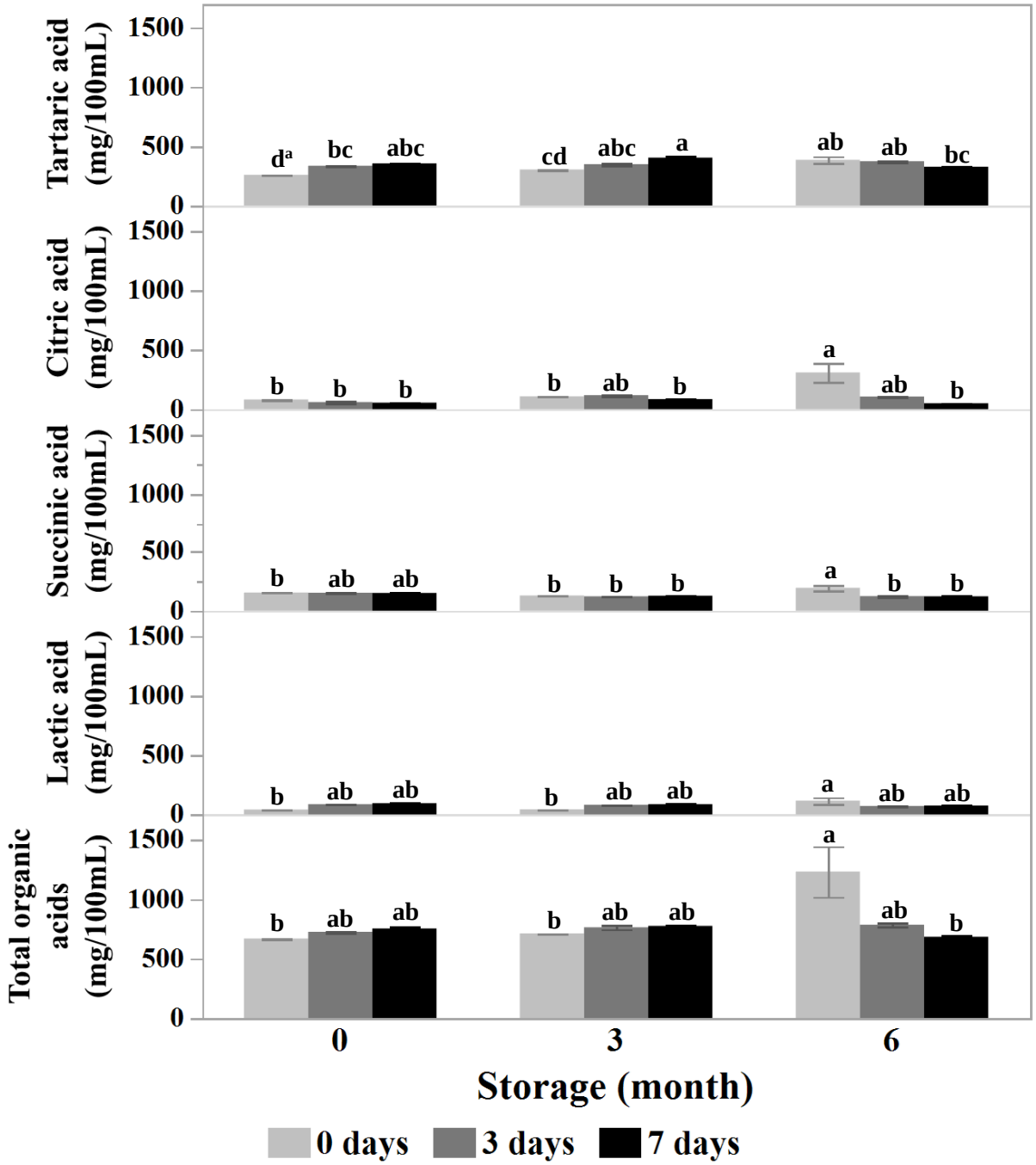

Figure 7. Effect of Skin Contact and Storage on tartaric acid, citric acid, succinic acid, lactic acid, and total organic acid content of Noble muscadine wines with different Skin Contact times (0, 3, or 7 days), β-glucosidase (BG) Enzyme levels (no BG, BG), and Storage times (0, 3, and 6 months at 15°C) (Arkansas, 2018).

[a] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters for each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

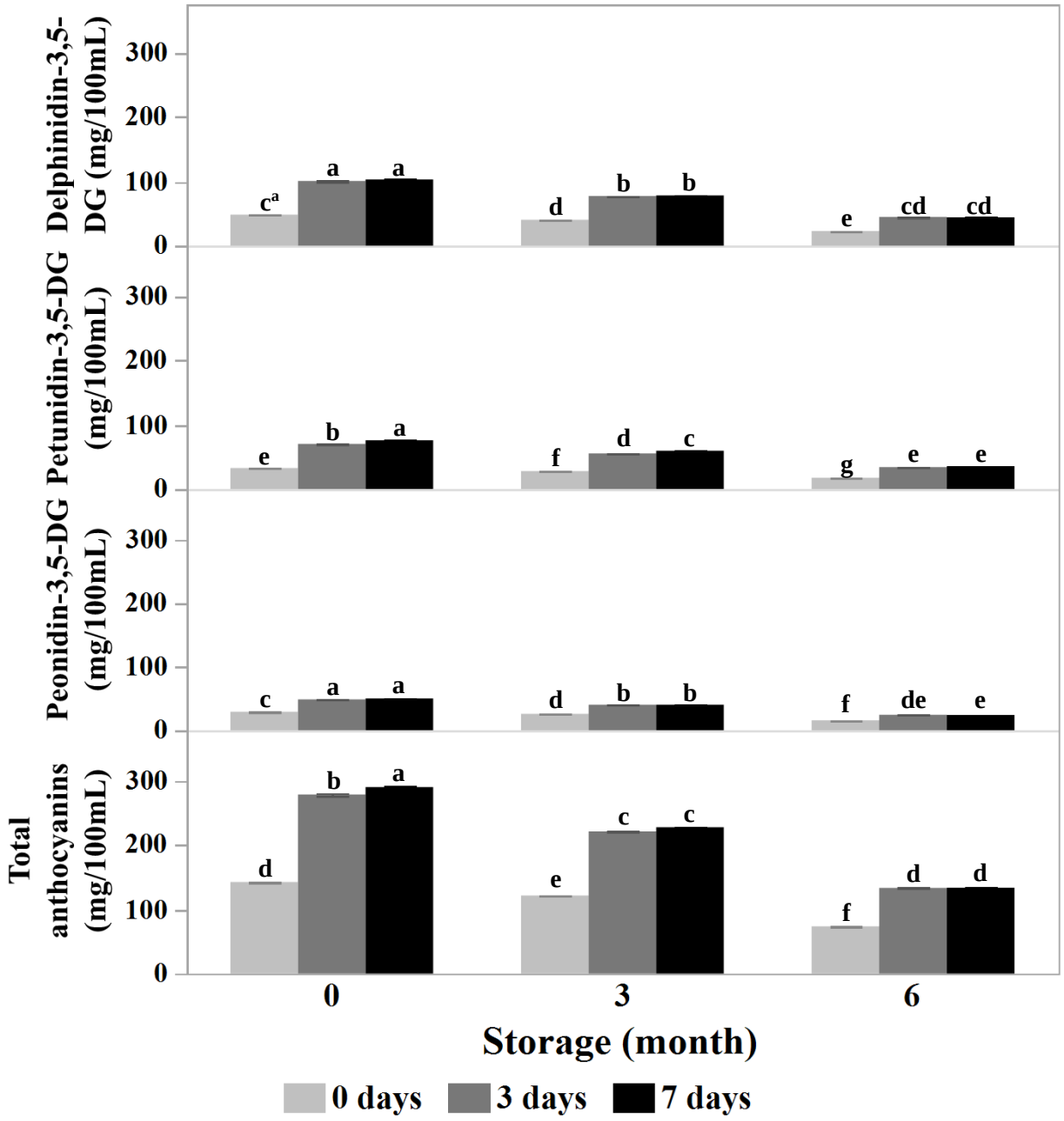

Figure 8. Effect of Skin Contact and Storage on delphinidin-3,5-diglucoside (DG), petunidin-3,5-DG, peonidin-3,5-DG, and total anthocyanin content of Noble muscadine wines with different Skin Contact times (0, 3, or 7 days), β-glucosidase (BG) Enzyme levels (no BG, BG), and Storage times (0, 3, and 6 months at 15°C) (Arkansas, 2018).
[a] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters for each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

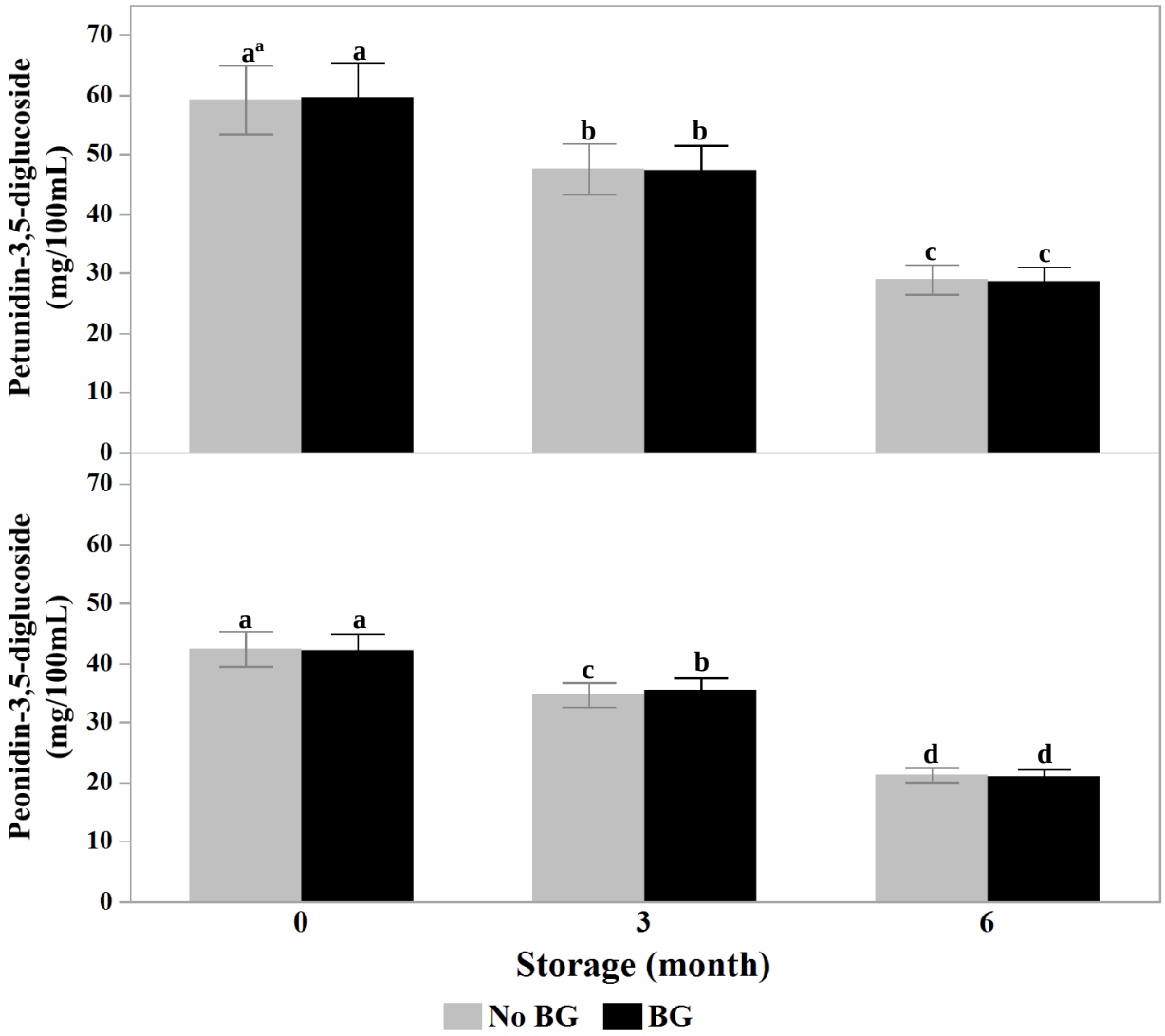

Figure 9. Effect of Enzyme and Storage on petunidin-3,5-diglucosise and peonidin-3,5-diglucoside content of Noble muscadine wines with different Skin Contact times (0, 3, or 7 days), β-glucosidase (BG) Enzyme levels (no BG, BG), and Storage times (0, 3, and 6 months at 15°C) (Arkansas, 2018).

[a] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters for each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

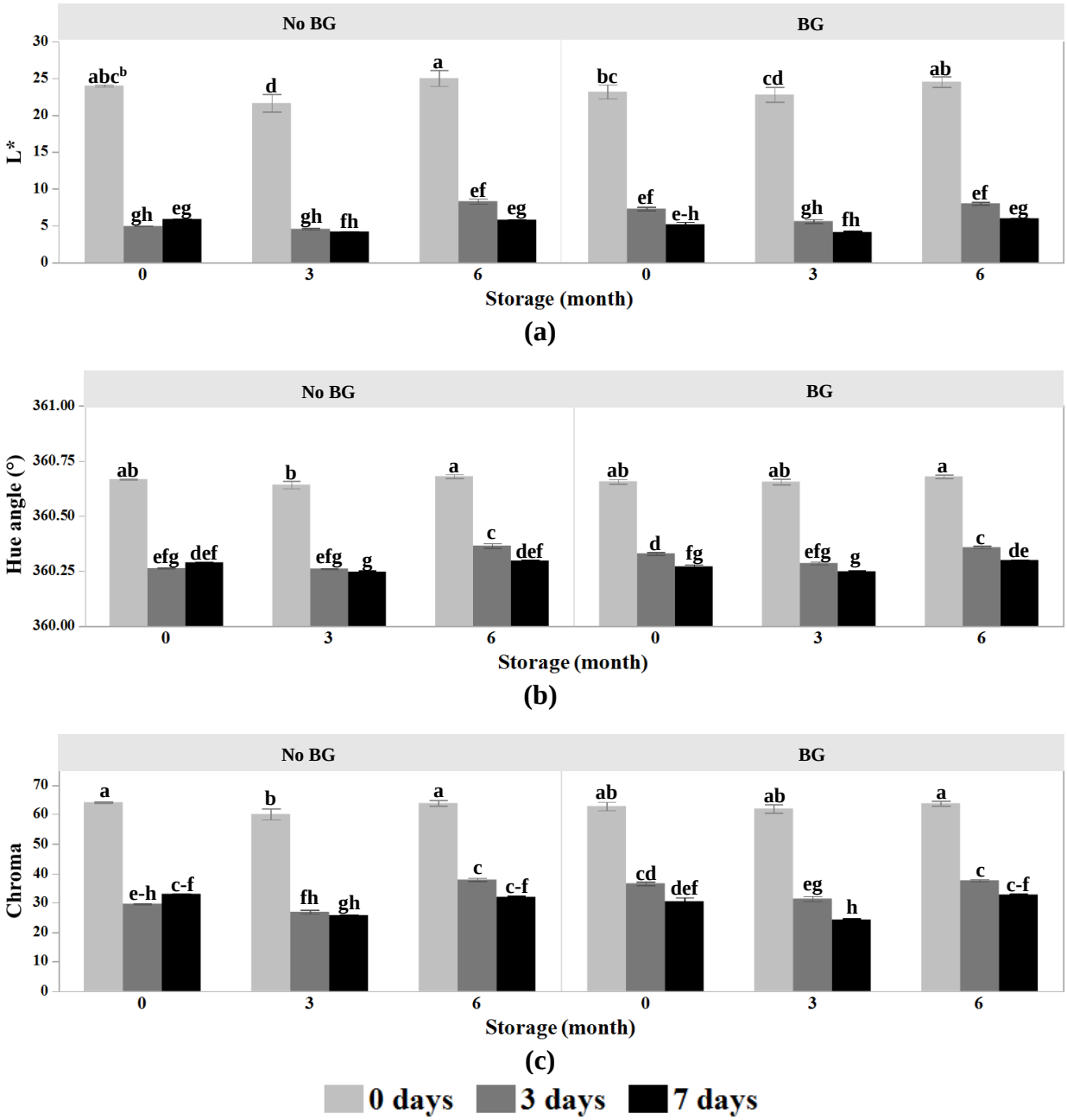

Figure 10. Effect of Skin Contact, Enzyme, and Storage on L* (a), hue angle[a] (b), and chroma (c) of Noble muscadine wines with different Skin Contact times (0, 3, or 7 days), β-glucosidase (BG) Enzyme levels (no BG, BG), and Storage times (0, 3, and 6 months at 15°C) (Arkansas, 2018).

[a] Hue angles <90° were subjected to a 360° compensation to account for discrepancies between red samples near 0° and those near 360°.

[b] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters for each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

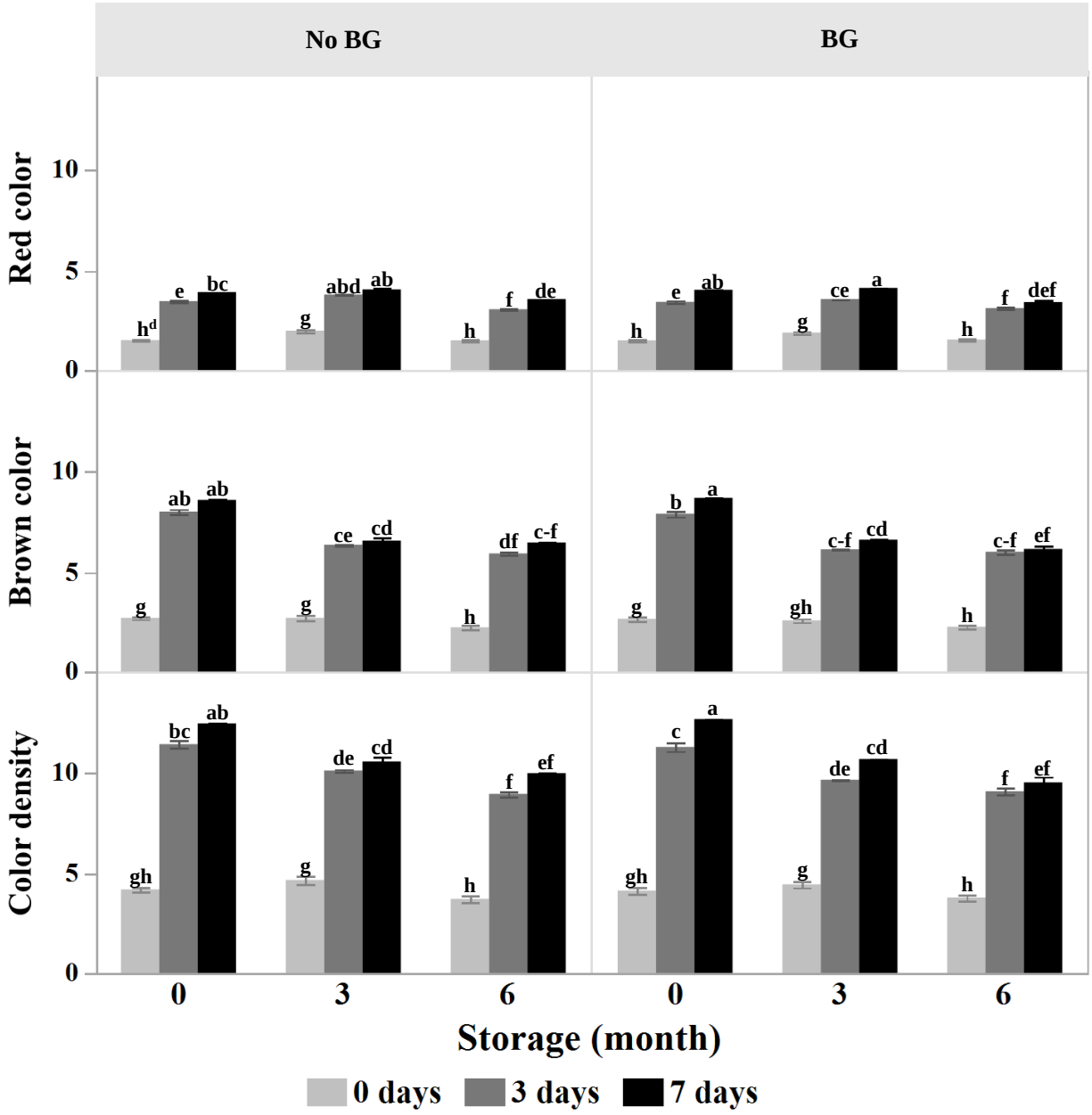

Figure 11. Effect of Skin Contact, Enzyme, and Storage on red color[a], brown color[b], and color density[c] of Noble muscadine wines with different Skin Contact times (0, 3, or 7 days), β-glucosidase (BG) Enzyme levels (no BG, BG), and Storage times (0, 3, and 6 months at 15°C) (Arkansas, 2018).

[a] Red color was calculated as absorbance of wine at 520 nm.

[b] Brown color was calculated as absorbance of wine at 420 nm.

[c] Color density was calculated as absorbance 520 nm + absorbance 420 nm.

[d] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

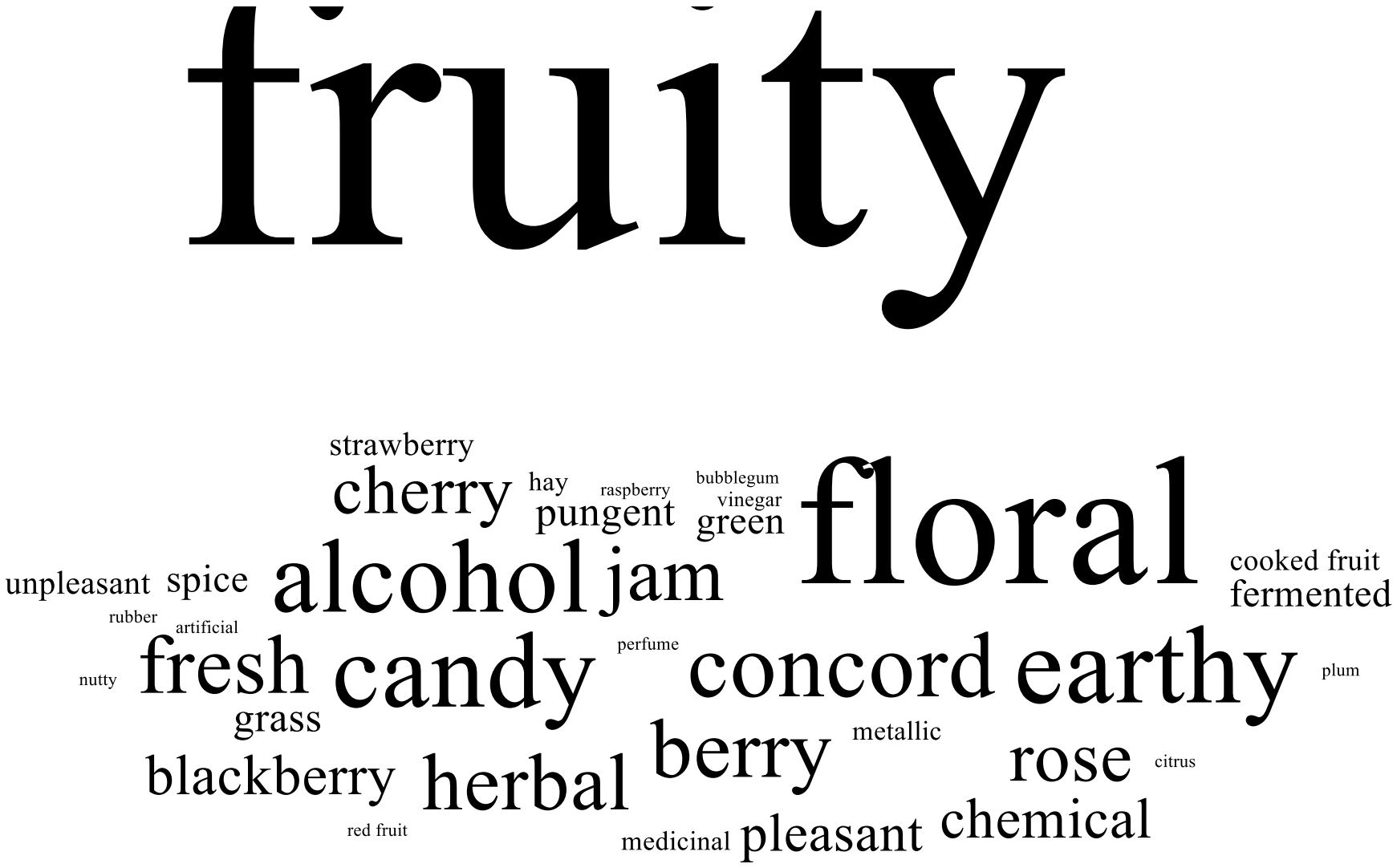

Figure 12. Word cloud for aroma descriptors given by the consumer sensory panelists to describe the aroma of Noble muscadine wines at 3-months storage at 15°C with different Skin Contact times (0, 3, or 7 days) and β-glucosidase (BG) Enzyme levels (no BG, BG) (Arkansas, 2018).

CHAPTER IV

Impact of winemaking methods on composition, anthocyanin, color, and aroma attributes of wine from Enchantment grapes grown in Arkansas

Abstract

Grapevines (*Vitis* spp.) are one of the most widely-planted horticultural crops worldwide, and *V. vinifera* is the most commercially-important species of wine grapes. However, *V. vinifera* grapevines are vulnerable to pests, diseases, and extreme temperatures and are difficult to grow in many regions of the United States, including Arkansas. Enchantment, a *Vitis* hybrid cultivar released from the University of Arkansas System Division of Agriculture (UA System) wine grape breeding program, has *V. vinifera* cultivars in its parentage. This cultivar has teinturier berries with dark purple skins, flesh, and juice, and has shown potential for wine production, yielding wines with *vinifera*-like color/flavor attributes. The objective of this study was to evaluate effects of tannin and oak addition on the composition, anthocyanin, color, and aroma attributes of Enchantment wines during one year of storage. Enchantment grapes were harvested in August 2017 and 2018 from the UA System Fruit Research Station (Clarksville, AR). Wines were produced at the UA System Department of Food Science in 2017 and 2018 with and without the addition of Tannin (no tannin and tannin) and Oak (no oak, American oak, and French oak). The 2017 and 2018 wines were analyzed at 0-months storage for composition, anthocyanin, color, and aroma attributes, and 2017 wines were analyzed during one year of storage (0, 6, and 12 months at 15°C) for composition, anthocyanin, and color attributes. At 0-months storage, both 2017 and 2018 wines had compositions within typical ranges for a dry red wine (3.3-3.4 pH and 0.6-0.7% titratable acidity). Enchantment wines had high levels of

anthocyanins and deep-red color, and only anthocyanin monoglucosides were identified. In 2017 and 2018, malvidin-, petunidin-, and delphinidin-3-glucoside made up a majority of total anthocyanins (70-151 mg/100mL). Tannin and oak addition gave wines higher residual sugar and lower organic acid levels in 2017. The composition of 2017 wines remained mostly stable over time, and all attributes were within commercially acceptable ranges after 12-months storage. Tannin addition lowered pH values of wines over time. Total anthocyanins decreased 65% during storage, regardless of Tannin/Oak treatment, but a corresponding decrease in color quality was not observed. Wine aroma profiles differed among Tannin/Oak treatments both years. Aroma compounds of the wines included green/unripe and floral alcohols, roasted and caramelized aldehydes, unpleasant carboxylic acids, fruity esters, and floral, herbal, and spicy terpenes. The esters were the largest class of compounds in all wines. In 2017, American-oaked wines were associated with traditionally oaky aromas, and in both years, American- and French-oaked wines were associated with roasted and caramelized aromas. In 2018, wines with added tannin were associated with lower amounts of aroma compounds. Overall, these results suggested the potential of Enchantment wine grapes for producing high-quality, deeply red-colored wines with aging potential. Therefore, Enchantment red wine grapes present a unique opportunity for grapes growers and winemakers in Arkansas and the mid-South United States.

Acknowledgements

This research was funded by a Southern Region Small Fruit Consortium (SRSFC) grant (R-16 0403-82467). We would also like to thank the staff at the University of Arkansas System Division of Agriculture Fruit Research Station (Clarksville, AR) for their assistance with this project.

Introduction

Grapevines (*Vitis* spp.) are one of the most widely-planted horticultural crops in the world. In the United States, 95% of grape and wine production occurs in California, Washington, New York, Pennsylvania, and Oregon, but production is focused mostly on *V. vinifera*, which is the most popular species of grapevines (Creasy and Creasy 2009, OIV 2000, TTB 2015, USDA NASS 2019). *V. vinifera* grapevines are highly vulnerable to pests, diseases, and extreme temperatures and are difficult to grow in much of the United States, including Arkansas. The high cost of maintaining *V. vinifera* grapevines in non-ideal climates offsets the profit from producing these wines.

Hybrids (a cross of two or more *Vitis* species) and native species, such as *V. rotundifolia*, are better-adapted to surviving stressors that devastate *V. vinifera* grapes (Reisch et al. 2012). Despite the challenges, grape and wine production contribute significantly to the Arkansas economy. In 2010, the Arkansas grape and wine industry was responsible for 1,700 jobs and over $42 million in wages, and wine-related tourism generated $21 million in revenue (Frank 2010). Grapes grown in Arkansas include mostly native species and hybrids.

The University of Arkansas System Division of Agriculture (UA System) has a Fruit Breeding Program established in 1964 and located at the Fruit Research Station in Clarksville, AR. The program focuses on development of fruit cultivars for Arkansas production of fresh-market fruits and has released about 70 cultivars. This program has released many cultivars of blackberries, peaches and nectarines, table and juice grapes, and blueberries.

In addition, the Fruit Breeding Program began breeding wine grapes over 40 years ago, with a goal to develop new hybrid cultivars that grow well in Arkansas, have desirable flavor attributes, and are suitable for winemaking. The UA System Food Science Department has

worked collaboratively with the Fruit Breeding Program for decades to evaluate 100-150 wine grape genotypes for wine production, with about 20 of these genotypes extensively evaluated. In 2016, the first wine grape cultivars, Opportunity (white-wine cultivar) and Enchantment (red-wine cultivar), were released from the UA System. Enchantment shows potential for regions that have limited productivity of red wine cultivars.

The Enchantment grapevine produces teinturier (red-fleshed) berries with a dark purple color in the flesh and juice of the grape. The female parent of Enchantment, A-1628, resulted from a cross of two *V. vinifera* cultivars, Petit Sirah and Alicante Bouschet, and the male parent, A-1481, was a cross of *V. vinifera*-derived cultivars, Bouschet Petit and Salvador (Clark et al. 2018). In evaluations from 1998-2015, vines had yields of 10.1 kg/vine, cluster weight of 178.3 g, and berry weight of 1.5 g. Vines produced grapes with 18.9% soluble solids, 3.4 pH, and 0.8% titratable acidity (TA) at harvest. Enchantment wine grapes had good composition for wine production, hardiness for growth in the Arkansas climate, and the potential to withstand typical disease pressures of the region (Clark et al. 2018).

Wines were produced from Enchantment at the UA System Department of Food Science from 1998-2015 using small-scale winemaking techniques, and had 11.2% v/v ethanol, 3.4 pH, and 0.9% TA. The primary anthocyanin in Enchantment was identified as the *vinifera*-like malvidin-3-glucoside, which is more stable than the anthocyanin diglucosides typically found in other hybrid wines (Clark et al. 2018). Anthocyanin diglucosides are unable to form stable complexes during aging and are thus more susceptible to color degradation. Monomeric anthocyanins are a major component of young red wines, but disappear during storage as anthocyanins react with tannins, polymers of flavan-3-ol units, to form polymeric pigments (Cheynier et al. 2006, He et al. 2012). Within two years of storage, the majority of red wine color

is derived from such polymeric pigments (de Frietas and Mateus 2010). Tannins can also scavenge and prevent the accumulation of oxidation products and are correlated with the perception of astringency in red wine (Mercurio and Smith 2008, Robichaud and Noble 1990). Wines produced from hybrid grapes typically have lower tannins than those from *V. vinifera* grapes due to lower skin tannins and higher concentrations of tannin-binding proteins (Van Sluyter et al. 2015, Springer and Sacks 2014). Exogenous tannin can be added during wine production to compensate for lower tannin levels in non-*vinifera* wines (Harbertson et al. 2012, Norton et al. 2020).

Another technique to enhance the quality of wines is oak addition. The most notable effect of oak addition is the extraction of smoky, spicy, and vanilla aromas. European/French oak (*Quercus robur*) and American oak (*Q. alba*) are widely used for wine production (Schahinger 2005, Singleton 1995). American oak typically has higher concentrations of oak lactones (coconut, sweet aromas) and possesses more noticeable woody character than French oak (Masson et al. 1995).

Although Enchantment grapes and wine have been preliminarily evaluated over the last 20 years, there have been no published studies on the effects of winemaking techniques on the composition, color stability, and aroma profile of Enchantment wines. Given the potential that Enchantment has shown as a red wine grape for Arkansas and similar regions, the objective of this study was to evaluate the effects of tannin and oak addition on the composition, anthocyanin, color, and aroma attributes of Enchantment wines during one year of storage.

Materials and Methods

Grape harvest

Enchantment grapes were grown at the UA System Fruit Research Station in Clarksville, AR (USDA hardiness zone 7b). The soil type was Linker fine sandy loam (fine-loamy, siliceous, semi active, thermic Typic Hapludult). The grapes were grown on a high-wire bilateral cordon system on own-rooted, variable-age vines. Approximately 100 kg of Enchantment wine grapes were hand harvested in August 2017 and 2018. Harvest date was determined based on ideal composition attributes for Arkansas red wine grapes, as well as past harvest data, weather, and quality of the fruit. Average daily temperature and rainfall for January-August 2017 and 2018 were recorded in Clarksville, AR (Figure 1). The grapes were taken to the UA System Food Science Department in Fayetteville, AR and stored at 4°C overnight for wine production the following day.

Wine production

For wine production, Enchantment grapes were split randomly into two 50-kg batches (no tannin and tannin). Wines were produced according to a traditional red-wine style. Each batch of grapes was passed twice through a crusher/destemmer and 30 mg/L sulfur dioxide (SO_2) as potassium metabisulfite (KBMS) was added at crush. The composition of the must (juice, skins, seeds, and pulp after crushing) was evaluated prior to, during, and at the end of fermentation, and adjustments were made to the must to ensure a complete fermentation. The free SO_2 levels of the wine were evaluated and adjusted as needed. Soluble solids (SS), pH, and titratable acidity (TA) of must were evaluated prior to fermentation. The SS (expressed as %) of juice from the must was determined using a Bausch & Lomb Abbe Mark II refractometer

(Scientific Instruments, Keene, NH). The pH and TA were measured using a Metrohm 862 Compact Titrosampler (Metrohm AG, Herisau, Switzerland) fitted with a pH meter.

The harvest dates of the grapes and initial composition of the musts for 2017 and 2018 wine production are shown in Table 1. The winemaking procedures were similar for both years. Soluble solid levels of the musts were adjusted to 21% using table sugar (sucrose) in both years. In 2018, the TA of the wines was adjusted to 0.9% to reduce the pH of the must <3.6 for fermentation. Scott'Tan™ FT Rouge fermentation tannin (Scott Laboratories, Petaluma, CA) was added at a rate of 500 mg/L estimated juice in the must for the tannin treatment. Musts were inoculated with Lalvin ICV D254® wine yeast (Lallemand, Inc., Montreal, Canada) at a rate of 0.26 g/L estimated juice in the must and fermented on the skins for four days at 15°C. After four days, musts were pressed with a 70-L Enoagricola Rossi Hydropress (Calzolaro, Italy) using three 10-minute press cycles and a pressure of 207 kPa. The wine was collected in a 22.7 L glass carboy fitted with a fermentation lock filled with SO_2 solution to allow release of carbon dioxide and limit oxygen exposure. Fermentation continued at 15°C for approximately 6 months. After fermentation completion, the free SO_2 content of wines was determined using the aeration-oxidation method (Iland et al. 1993) and adjusted to 60 mg/L.

No tannin and tannin wines were each split into six 3.8 L glass jars. Of the six jars per Tannin treatment, there was a control (no oak), French oak, and American oak treatment with duplicates of each. Innerstave French oak and American oak staves (38.3 x 1.5 x 1.5 cm; Innerstave, LLC, Sonoma, CA) were placed in the wines for the oak treatment. Wines were aged on oak for two months at 15°C, then free SO_2 levels were again adjusted to 60 mg/L. The ethanol content of all wines was 11.0-11.4 % v/v at bottling, measured by high performance liquid chromatography (HPLC) (Walker et al. 2003). Wines were bottled into 125-mL glass bottles,

sealed with plastisol-lined lug caps, and stored at 15°C until analysis (0, 6, and 12 months storage). Wines were stored at 15°C for one week prior to month-0 analysis. The 2017 and 2018 Enchantment wines were analyzed at 0-months storage at 15°C for composition, anthocyanin, color, and aroma attributes. The 2017 Enchantment wines were analyzed during storage (0, 6, and 12 months at 15°C) for composition, anthocyanin, and color attributes.

Composition attributes analysis

The composition attributes analysis of the wines included pH, TA, glycerol, ethanol, residual sugars, and organic acids. Analysis was done on each wine sample (Tannin and Oak treatment and replicate) in both years, and samples were measured in analytical duplicates. The 2017 and 2018 wines were analyzed for composition attributes at 0-months storage at 15°C, and the 2017 wines were analyzed during storage (0, 6, and 12 months at 15°C).

pH. The pH of wines was measured using a Metrohm 862 Compact Titrosampler (Metrohm AG, Herisau, Switzerland) fitted with a pH meter. The probe was left in the samples for two minutes to equilibrate before recording the pH value. Wine was degassed prior to analysis.

Titratable acidity (TA). The TA of wines were expressed as % w/v (g/100 mL) tartaric acid and measured using a Metrohm 862 Compact Titrosampler. Six grams of sample was added to 50 mL degassed, deionized water and titrated with 0.1 N sodium hydroxide to an endpoint of pH 8.2. Wine was degassed prior to analysis.

Glycerol, ethanol, residual sugars, and organic acids. The glycerol, ethanol, residual sugars, and organic acids in wines were identified and quantified according to the HPLC procedure of Walker et al. (2003). Samples were passed through a 0.45 μm polytetrafluoroethylene (PTFE) syringe filter (Varian, Inc., Palo Alto, CA, USA) before injection onto an HPLC system consisting of a Waters 515 HPLC pump, a Waters 717 plus autosampler, and a Waters 410

differential refractometer detector connected in series with a Waters 996 photodiode array (PDA) detector (Water Corporation, Milford, MA, USA). Analytes were separated with a Bio-Rad HPLC Organic Acids Analysis Aminex HPX-87H ion exclusion column (300 x 7.8 mm) connected in series with a Bio-Rad HPLC column for fermentation monitoring (150 x 7.8 mm; Bio-Rad Laboratories, Hercules, CA). A Bio-Rad Micro-Guard Cation-H refill cartridge (30 x 4.5 mm) was used as a guard column. Columns were maintained at a temperature of 65 ± 0.1°C by a temperature control unit. The isocratic mobile phase consisted of pH 2.28 aqueous sulfuric acid at a flow rate of 0.45 mL/min. Injection volumes of both 10 µL (for analysis of organic acids and sugars) and 5 µL (for ethanol and glycerol) were used to avoid overloading the detector. The total run time per sample was 60 minutes.

Citric, tartaric, malic, lactic, and succinic acids were detected at 210 nm by the PDA detector, and glucose, fructose, ethanol, and glycerol were detected at 410 nm by the differential refractometer detector. Analytes in samples were identified and quantified using external calibration curves based on peak area estimation with baseline integration. Results were expressed as milligrams analyte per 100 mL wine for organic acids and sugars, grams per liter wine for glycerol, and % v/v (alcohol by volume, ABV) for ethanol. Total residual sugars was calculated as the sum of glucose and fructose, and total organic acids was calculated as the sum of citric, tartaric, malic, lactic, and succinic acids.

Anthocyanin attributes analysis

The anthocyanin attributes analysis of the wines included individual and total anthocyanins. Analysis was done on each wine sample (Tannin and Oak treatment and replicate) in both years, and samples were measured in analytical duplicates. The 2017 and 2018 wines

were analyzed for anthocyanin attributes at 0-months storage at 15°C, and the 2017 wines were analyzed during storage (0, 6, and 12 months at 15°C).

Anthocyanin quantification. The anthocyanin content of wines was analyzed using the HPLC-PDA method of Cho et al. (2004). Samples were passed through a 0.45 μm PTFE syringe filter before injection onto a Waters Alliance HPLC system equipped with a Waters model 996 PDA detector and Millennium version 3.2 software. A 4.6 x 250 mm Symmetry® C_{18} column (Waters Corporation) with a 3.9 mm x 20 mm Symmetry® C_{18} guard column was used to separate analytes. The mobile phase consisted of a binary gradient with 5% (v/v) formic acid in water (solvent A) and methanol (solvent B) at a flow rate of 1.0 mL/min. A gradient was used with 2% to 60% B from 0-60 minutes, 60% to 2% B from 60-65 minutes, then holding at 2% B from 65-80 minutes. A 50 μL injection volume was used, and the total run time per sample was 80 minutes. Anthocyanins were detected at 510 nm.

Anthocyanins were quantified as the anthocyanidin-3-glucoside of their major aglycone (cyanidin, delphinidin, peonidin, petunidin, or malvidin) using external calibration curves based on peak area estimation with baseline integration. Total anthocyanins were determined by summing the concentrations of individual anthocyanin compounds. Results were expressed as mg/100 mL wine.

Anthocyanin identification. An HPLC-electrospray ionization (ESI)-mass spectrometry (MS) system equipped with an analytical Hewlett Packard 1100 series HPLC instrument (Hewlett-Packard Enterprise Company, Palo Alto, CA), an autosampler, a binary HPLC pump, and a UV/VIS detector interfaced to a Bruker Esquire LC/MS ion trap mass spectrometer (Bruker Corporation, Billerica, MA) was used to identify anthocyanin compounds according to the method of Cho et al. (2004). Reverse-phase separation of anthocyanins was conducted using the

same HPLC conditions previously described, and absorption was recorded at 510 nm. Mass spectral analysis was operated in positive ion electrospray mode with a capillary voltage of 4000 V, a nebulizing pressure of 30.0 psi, a drying gas flow of 9.0 mL/min, and a temperature of 300°C. Data was collected with the Bruker software in full scan mode over a range of *m/z* 50-1000 at 1.0 seconds per cycle. Characteristic ions were used for peak assignment.

Color attributes analysis

The color attributes analysis of the wines included L*, hue angle, chroma, red color, and color density. Analysis was done on each wine sample (Tannin and Oak treatment and replicate) in both years, and samples were measured in analytical duplicates. The 2017 and 2018 wines were analyzed for color attributes at 0-months storage at 15°C, and the 2017 wines were analyzed during storage (0, 6, and 12 months at 15°C).

L*, hue angle, and chroma. Wine color analysis was conducted using a ColorFlex system (HunterLab, Reston, VA). The ColorFlex system uses a ring and disk set (to control liquid levels and light interactions) for measuring translucent liquids in a 63.5-mm glass sample cup with an opaque cover to determine Commission Internationale de l'Eclairage (CIE) Lab transmission values of L*=100, a*=0, and b*=0 (Commission Internationale de l'Eclairage (CIE) 1986). The CIELAB system describes color variations as perceived by the human eye. CIELAB is a uniform three-dimensional space defined by colorimetric coordinates, L*, a*, and b*. The vertical axis L* measures lightness from completely opaque (0) to completely transparent (100), while on the hue-circle, +a* red, -a* green, +b* yellow, and -b* blue are measured. Hue angle, calculated as $\tan^{-1}\frac{b^*}{a^*}$, described color in angles from 0 to 360°: 0° is red, 90° is yellow, 180° is green, 270° is blue, and 360° is red. For samples with hue angles <90°, a 360° compensation (hue + 360°) was used to account for discrepancies between red samples with hue angles near 0° and those near

360° (McLellan et al. 2007). Chroma, calculated as $\sqrt{a*^2 + b*^2}$, identified color by which a wine appeared to differ from gray of the same lightness and corresponded to saturation (intensity/purity) of the perceived color.

Red color and color density. Red color of wines was measured spectrophotometrically as absorbance at 520 nm, and color density was measured as red color + yellow/brown color (420 nm) (Iland et al. 1993). Absorbance values were measured using a Hewlett-Packard 8452A Diode Array spectrophotometer equipped with UV-Visible ChemStation software (Agilent Technologies, Inc., Santa Clara, CA). Samples were diluted 10 times with deionized water prior to analysis and were measured against a blank sample of deionized water. A 1-cm cell was used for all spectrophotometer measurements.

Aroma attributes analysis

The volatile aroma profiles analysis of 2017 and 2018 Enchantment wines was conducted at Graz University of Technology (Graz, Austria) Institute of Analytical Chemistry and Food Chemistry. Wines were packaged in 20-mL clear glass vials, sealed with a polypropylene cap with a polytetrafluoroethylene-line silicon septum, wrapped with Parafilm® flexible film (Bemis Company, Inc., Neenah, WI), and shipped to Graz University of Technology for analysis. Volatile aroma profiles were determined in 2017 and 2018 wines at 0-months storage at 15°C. Analysis was done on each wine sample (Tannin and Oak treatment and replicate) in both years, and samples were measured in analytical triplicates.

Determination of volatile aroma profiles. To identify the volatile aroma compounds in wines, volatile compounds were extracted from 1 mL of wine in a 10-mL glass vial using solid-phase microextraction (SPME) with a 2-cm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber for 30 minutes at 40°C. A gas chromatography-mass spectrometry

(GC-MS) system equipped with a Shimadzu GC 2010 (Shimadzu Corporation, Kyoto, Japan), Shimadzu QP 2010 MS, and a PAL HTX autosampler (CTC Analytics AG, Zwingen, Switzerland) was used to separate and identify volatile compounds. Samples were extracted/injected in analytical triplicate. Volatiles were separated on a nonpolar Restek Rxi 5MS column (30 m x 0.25 mm x 1 μm; Restek, Bellefonte, PA) 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 were recorded in the scan mode (*m/z* 35-350) with a 9.8 minute solvent cut time and a detector voltage relative to the tuning result.

Data was analyzed using the Shimadzu GCMS Postrun Analysis software. Compounds were identified using comparison of mass spectra with NIST14 (National Institute of Standards and Technology, Gaithersburg, MD), Flavors and Fragrances of Natural and Synthetic Compounds (FFNSC3, John Wiley & Sons, Inc., Hoboken, NJ), and Adam's Essential Oils (Adams 2007) mass spectral libraries and comparison of calculated Kovats retention indices (Kováts 1958) with values reported in the Flavornet (Acree and Arn 2004) and Pherobase (Sayed 2003) databases. A matching library result and a retention index 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.

Design and statistical analysis

After harvest, Enchantment grape clusters were randomized for Tannin treatments (no tannin and tannin). Each Tannin treatment was then split after fermentation into three Oak treatments (no oak, American oak, and French oak) in duplicate. There were 6 treatments (2 Tannin treatments x 3 Oak treatments) with two replications. The wines were bottled into 125-mL bottles and stored at 15°C. The 2017 and 2018 wines were analyzed at 0-months storage at

15°C for composition, anthocyanin, color, and aroma attributes, and the 2017 wines were analyzed at 0-, 6-, and 12-months storage at 15°C for composition, anthocyanin, and color attributes. There were 12 samples each year when the wines were analyzed at 0-months storage, and there were 36 samples when the wines were analyzed during storage. At each storage time for composition, anthocyanin, color, and aroma attributes, samples were taken from one 125-mL bottle, which was treated as an individual experimental unit in a full factorial design. Statistical analyses were conducted using JMP® Pro statistical software (version 15.0.0, SAS Institute, Cary, NC). Additional information on the statistical analyses is provided below.

Composition, anthocyanin, and color attributes. For the 2017 and 2018 wines at 0-months storage, a univariate analysis of variance (ANOVA) was used to determine the significance of the main factors (Tannin and Oak) and their interaction. For the 2017 wines at 0-, 6-, and 12-months storage, a univariate ANOVA was used to determine the significance of main factors (Tannin, Oak, and Storage) and their interactions. All factors were treated as categorical. Tukey's Honest Significant Difference (HSD) test and student's t-test were used to detect differences among means ($p<0.05$). Figures were created in JMP®, and error bars represented one standard error from the mean.

Aroma attributes. Peak areas (TIC) for each positively identified compound in 2017 and 2018 Enchantment wines at 0-months storage were used as semi-quantitative measures for principal components analysis (PCA). Each compound was assigned a general aroma category based on aroma descriptors reported in the Flavornet (Acree and Arn 2004) and Pherobase (Sayed 2003) databases. The areas of compounds within each category were summed to create general "aroma category" variables. This was done so that the model did not overfit to noise, which occurs when the number of parameters is greater than the number of variables. A PCA, based on the aroma

categories, was used to explore the relationship between Tannin and Oak treatments and volatile aroma profiles.

Results and Discussions

The 2017 and 2018 wine grape production seasons at the Fruit Research Station were relatively mild in terms of temperature and rain (Figure 1). The high and low temperatures were similar in both years from January to August. There was higher rainfall in 2017 than 2018 from April (bud emergence on grapevines) to July prior to harvest. In August of 2017 and 2018, the average daily high temperature was 28.6°C and 30.0°C, respectively. In August, there was less cumulative monthly rainfall in 2017 than 2018 (198.5 mm and 281.7 mm, respectively).

The composition of the Enchantment grapes at harvest varied in both years (Table 1). In 2017, the must had acceptable composition for wine production with a pH of 3.1-3.2 and TA of 0.8% at crush. Acidity was lower in 2018 must with a pH of 3.7-3.8 and TA of 0.7%, so tartaric acid was added to increase the TA by 0.2% for wine production. The SS levels (14.6-14.8% in 2017 and 17.3-17.8% in 2018) of the must in both years were adjusted to 21% prior to fermentation.

After about eight months of fermentation at 15°C in 2017 and 2018, the wines were bottled in May 2018 and 2019 and stored at 15°C. In 2017 and 2018 Enchantment wines, the impacts of tannin and oak additions on composition, anthocyanin, color, and aroma attributes were evaluated at 0-months storage at 15°C. In 2017 Enchantment wines, the composition, anthocyanin, and color attributes were evaluated during storage (0, 6, and 12 months at 15°C).

Analysis of composition, anthocyanin, and color attributes at 0-months storage (2017 and 2018)

At bottling (0-months storage at 15°C) in both 2017 and 2018, Enchantment wines had acceptable compositions with residual sugar and organic acid levels well within the typical ranges for a dry red table wine. Wines had high levels of anthocyanins and a deep red color, and malvidin-3-glucoside was the most prevalent individual anthocyanin. Tannin and Oak addition lead to wines with higher residual sugar levels in 2017, and wines without added tannin had higher amounts of organic acids, in particular the fermentation-evolved malic, succinic, and lactic acids. These trends were not seen in 2018. In 2018 Enchantment wines, tannin addition lead to higher anthocyanins in some wines. In 2017, wines with added tannin had higher red color and color density.

Composition. Enchantment wines from 2017 and 2018 were analyzed at 0-months storage at 15°C for pH, TA, glycerol, ethanol, glucose, fructose, total residual sugars, tartaric acid, malic acid, citric acid, succinic acid, lactic acid, and total organic acids. Regardless of Tannin and Oak treatments, the wines had acceptable minimum and maximum composition values in both years (data not shown). The 2017 wines had 3.4 pH, 0.6% TA, 8 g/L glycerol, 11% (v/v) ethanol, 46-67 mg/100 mL glucose, 150-294 mg/100 mL fructose, 197-362 mg/100 mL total residual sugars, 457-624 mg/100 mL tartaric acid, 320-504 mg/100 mL malic acid, 214-283 mg/100 mL citric acid, 377-843 mg/100 mL succinic acid, 87-391 mg/100 mL lactic acid, and 1505-2557 mg/100 mL total organic acids. The 2018 wines had 3.2-3.3 pH, 0.7% TA, 8 g/L glycerol, 11% (v/v) ethanol, 37-43 mg/100 mL glucose, 90-107 mg/100 mL fructose, 128-151 mg/100 mL total residual sugars, 374-422 mg/100 mL tartaric acid, 211-255 mg/100 mL malic acid, 125-174 mg/100 mL citric acid, 351-370 mg/100 mL succinic acid, 88-105 mg/100 mL lactic acid, and 1,189-1,308 mg/100 mL total organic acids.

In a general comparison of the values from 2017 and 2018, the 2018 wines were slightly more acidic than 2017 wines. In addition, the 2018 wines had lower concentrations of both glucose and fructose than 2017 wines. Total residual sugars in 2018 were approximately half of the total residual sugars measured in 2017. Concentrations of tartaric, malic, citric, succinic, and lactic acids and total organic acids were lower in 2018. In both years, there were no significant Tannin x Oak interactions for any of the attributes, except the pH and TA of the 2018 wine (Table 2). In both years, there were not significant Tannin x Oak interactions or main effects for glucose.

2017 Wines. Tannin and Oak did not impact TA, glycerol, ethanol, or glucose. The Tannin and Oak main effects were significant for pH. Wines with no added tannin (pH 3.44) had a higher pH than wines with added tannin (pH 3.39). The French-oaked wines had a lower pH (3.41) than the unoaked or American-oaked wines (both pH 3.42). TA (0.62%), glycerol (7.78 g/L) and ethanol (11.06% v/v) content of wines were within the typical ranges of 0.5-0.8% TA, 7-10 g/L glycerol, and 9-13% ethanol for a dry table wine (Liu and Davis 1994, Waterhouse et al. 2016).

The concentrations of fructose were 3.5-4.5 times greater than those of glucose in all wines. This was likely because yeast preferentially ferment glucose, decreasing concentration throughout fermentation (Waterhouse et al. 2016). While neither main effect was significant for glucose, both Tannin and Oak affected fructose and total residual sugar concentrations. Wines with added tannin had a greater fructose concentration (254.07 mg/100 mL) than those without additional tannin (184.93 mg/100 mL). French oak-aged wines had the greatest fructose level (262.48 mg/100 mL), followed by American oak-aged (211.17 mg/100 mL), and those without oak addition (184.84 mg/100 mL). Wines with added tannin had greater total residual sugars (312.03 mg/100 mL) than those without additional tannin (238.39 mg/100 mL). French-oaked

wines had the greatest total residual sugar levels (326.02 mg/100 mL), followed by American-oaked (263.92 mg/100 mL), and unoaked (235.68 mg/100 mL). Thus, it is possible that small amounts of sugars were extracted from the oak during aging (del Alamo et al. 2000). Total residual sugar levels for all wines were within the typical range of 70-500 mg/100mL for dry table wines (Liu and Davis 1994).

The Tannin main effect was significant for malic, succinic, and lactic acids. Wines without added tannin had greater amounts of all three acids (458.96, 715.46, and 303.41 mg/100 mL, respectively). The Oak main effect was significant for tartaric acid and citric acid, and the unoaked wines had the lowest levels of these acids (502.77 and 214.66 mg/100 mL, respectively). Tartaric, malic, and citric acids are found in grapes, and succinic and citric acids are formed as by-products of alcoholic fermentation. Lactic acid is formed by lactic acid bacteria during malolactic fermentation (MLF), which also decreases the level of malic acid (Waterhouse et al. 2016). Although Enchantment wines in this study were not intentionally inoculated with lactic acid bacteria, MLF can occur spontaneously in red wines. Thus, the evolution of organic acids in wine is a dynamic process and is affected by factors such as grape composition, fermentation parameters, bacterial activities, and acid additions by the winemaker. For total organic acids, only the Tannin main effect was significant. Wines without added tannin had almost 50% more total acids than the tannin wines (2291.71 and 1637.95 mg/100 mL, respectively). Because all wines came from the same grapes and TA was adjusted multiple times during fermentation (through tartaric acid additions), including at bottling, this difference in total acids was due to the fermentation-evolved malic, succinic, and lactic acids.

2018 Wines. In 2018, the Tannin x Oak interaction was significant for both pH and TA but not for other attributes. Wines had pHs ranging from 3.24-3.25 and TA values of 0.70%. Wines with

added tannin had higher pH values than those with no tannin (Figure 2). There was no obvious relationship between Tannin/Oak levels for TA. Tannin and Oak did not impact glucose, fructose, total residual sugars, tartaric acid, succinic acid, lactic acid, or total organic acids. The Tannin main effect was significant for glycerol, ethanol, malic acid, and citric acid, but oak additions did not impact these attributes. Wines with added tannin had greater glycerol (8.13 g/L) and ethanol (11.42% v/v) content than those with no added tannin (7.82 g/L and 11.17%, respectively), but all wines were within commercially acceptable ranges. Fructose levels were approximately 2.5 times higher than glucose levels.

The Tannin main effect was significant for malic and citric acid. The wines with added tannin had a higher malic acid concentration (251.59 mg/100 mL) and a lower citric acid concentration (139.08 mg/100 mL) than those without added tannin (218.39 and 172.37 mg/100 mL, respectively).

Anthocyanins. Individual and total anthocyanin compounds were identified and quantified in Enchantment wines at 0-months storage at 15°C. Anthocyanins identified in wines included delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, delphinidin-3-(6-O-acetyl)-glucoside, cyanidin-3-(6-O-acetyl)-glucoside, petunidin-3-(6-O-acetyl)-glucoside, peonidin-3-(6-O-acetyl)-glucoside, malvidin-3-(6-O-acetyl)-glucoside, delphinidin-3-(6-*p*-coumaroyl)-glucoside, cyanidin-3-(6-*p*-coumaroyl)-glucoside, petunidin-3-(6-*p*-coumaroyl)-glucoside, and malvidin-3-(6-*p*-coumaroyl)-glucoside (Figure 3). It was of note that only anthocyanin monoglucosides, and not their diglucoside counterparts, were detected in the Enchantment wines. The native and hybrid red wine grapes that typically grow well in Arkansas contain significant amounts of anthocyanin-3,5-diglucosides. For example, Muscadine grapes (*V. rotundifolia*) contain only diglucoside anthocyanins (Sims and Morris

1985), and Chambourcin hybrid grapes contain both diglucoside and monoglucosides anthocyanins (Mayfield and Threlfall 2020, Zhu et al. 2012). Unlike monoglucosides, diglucosides are not able to form copigment and acylated complexes and are thus more susceptible to bisulfite or hydration degradation reactions (Ballinger et al. 1973, Waterhouse et al. 2016). In 2017 and 2018, malvidin-3-glucoside, petunidin-3-glucoside, and delphinidin-3-glucoside made up a majority of the total Enchantment wine anthocyanin content, and thus only these three individual compounds, along with total anthocyanins, were discussed in this study. Enchantment wines in 2017 had 30-39 mg/100 mL malvidin-3-glucoside, 14-18 mg/100 mL petunidin-3-glucoside, 11-14 mg/100 mL delphinidin-3-glucoside, and 70-89 mg/100 mL total anthocyanins (data not shown). In 2018, wines had 51-54 mg/100 mL malvidin-3-glucoside, 20-22 mg/100 mL petunidin-3-glucoside, 16-18 mg/100 mL delphinidin-3-glucoside, and 137-151 mg/100 mL total anthocyanins (data not shown).

In a general comparison of the anthocyanins from 2017 and 2018 wines, the 2018 wines had greater concentrations of malvidin-3-glucoside, petunidin-3-glucoside, delphinidin-3-glucoside, and total anthocyanins than 2017 wines. In 2018, Enchantment grapes had higher SS and lower acid levels at harvest (Table 1), indicating that grapes were riper. This could explain the 80% increase in total anthocyanins from 2017 to 2018, because anthocyanins increase as berries ripen. In addition, environmental factors such as temperature, pests, or rain could have caused the difference in anthocyanin levels between the two years (Kliewer 1977, Spayd et al. 2002). Total anthocyanin concentrations for both years were similar to the levels of 44-164 mg/100 mL found by Revilla et al. (2016) in young red wines from the teinturier grape (and parent of Enchantment) Alicante Bouschet.

The Tannin x Oak interaction was significant for malvidin-3-glucoside, petunidin-3-glucoside, delphinidin-3-glucoside, and total anthocyanins in both years (Figure 4).

2017 Wines. On average, malvidin made up 45% of the total anthocyanins, petunidin made up 20%, and delphinidin made up 15%. Wines had an average total anthocyanin content of 80.08 mg/100 mL. Within each Oak treatment, there was not a difference in any anthocyanin attributes in terms of Tannin treatment, except that the French-oaked wine without tannin had higher malvidin than the wine with tannin. The unoaked wine with added tannin had higher anthocyanin attributes than the French-oaked wine with added tannin, which had the lowest levels (Figure 4a). Higher tannin levels are expected to decrease monomeric anthocyanins, as tannins and anthocyanins combine to form stable polymeric pigments (Cheynier et al. 2006, He et al. 2012). This reaction occurs over time, however, so the effects of additional tannin would likely not be seen in a young red wine at bottling. In fact, after approximately two years of storage, the majority of color in red wine comes from polymeric pigments, rather than monomeric anthocyanins (de Frietas and Mateus 2010)

2018 Wines. Malvidin, petunidin, and delphinidin made up 36%, 15%, and 12%, respectively, of total anthocyanins in 2018 Enchantment wine. The average total anthocyanin content was 144.09 mg/100 mL. Wines with added tannin had higher petunidin- and delphinidin-3-glucoside concentrations relative to wines with no additional tannin across all oak levels (Figure 4b). The tannin wines had greater total anthocyanins for the unoaked and American-oaked wines, and greater malvidin-3-glucoside concentrations for the American-oaked wines. There was no apparent effect of Oak treatment on anthocyanin content.

Color. Enchantment wines were analyzed at 0-months storage at 15°C for L*, hue angle, chroma, red color, and color density in 2017 and 2018. Enchantment wines in 2017 had a 0.6-1.6 L*, 360°

hue angle, 1.1-1.9 chroma, 15-22 red color, and 25-40 color density (data not shown). In 2018, wines had 0.4-0.7 L*, 360° hue angle, 1.9-3.1 chroma, 19-21 red color, and 27-41 color density (data not shown).

In 2018, L* values were lower and chroma values were higher than those in 2017. This indicated that 2018 wines had a darker, more saturated color than the 2017 vintage. In both years, the Tannin x Oak interaction was significant for L*, red color, and color density (Table 3).

2017 Wines. For the unoaked wines, wines with added tannin had a darker color (lower L*) than those without added tannin (Figure 5a). However, for the French-oaked wines, wines with added tannin had a lighter color than those without added tannin. The American-oaked wines were not impacted by Tannin treatment. Neither Tannin or Oak affected hue angle, and the hue of all wines corresponded with that of pure red (360°). Oak affected the chroma of wines, and French-oaked wines had the greatest color saturation (1.73), followed by unoaked (1.61) and American-oaked (1.08) wines. For American and French oak treatments, the wines with additional tannin had higher red color and color density than wines without added tannin (Figure 6a). This could indicate that the tannin added to the wines increased the red and overall color intensity. There was no apparent effect of Oak treatment on red color or color density.

2018 Wines. For the French-oaked treatment, wines with added tannin had a darker color (lower L*) than wines without added tannin (Figure 5b). The French-oaked wine without tannin was lighter in color than the unoaked wine. Oak slightly affected the hue angle of wines in 2018, and American-oaked wines had a lower hue angle (360.16) than French-oaked or unoaked wines (360.20). The Tannin x Oak interaction was significant for chroma. French-oaked wines without added tannin had a higher chroma value (more saturated color) than the no tannin French-oaked wines (Figure 7). For French-oaked wines, the wine with added tannin had greater red color and

overall color density than the no tannin wine (Figure 6b). The French-oaked wine with tannin had a greater red color and color density than the unoaked wines and the American-oaked wine with tannin.

Analysis of composition, anthocyanin, and color attributes during storage (2017)

The composition of Enchantment wines remained mostly stable over time, with the exceptions of a slight increase in pH and decrease in residual sugars. However, all composition attributes remained within commercially acceptable ranges. Wines without added tannin had higher pH values than tannin wines, indicating that tannin addition could help keep pH lower and potentially prevent degradation of color compounds and microbial spoilage. Tannin addition also lead to wines with higher residual sugar concentrations. Monomeric anthocyanin levels decreased over time, but a corresponding decrease in color quality was not observed. This supported the conclusion that anthocyanins formed stable co-pigmentation and polymeric pigment complexes with tannins, flavonols, and other phenolics during aging, suggesting the potential of Enchantment wine grapes for producing aged red wines.

Composition. Enchantment wines were analyzed during storage (0, 6, and 12 months at 15°C) for pH, TA, glycerol, ethanol, total residual sugars, and total organic acids. Individual sugars and acids were considered, but there were no obvious effects of Tannin, Oak, or Storage on these attributes. The Tannin x Storage interaction was significant for pH, TA, and total organic acids (Table 4). Only the Storage main effect was significant for glycerol and ethanol. Both the Tannin and Storage main effects were significant for total residual sugars. The three-way Tannin x Oak x Storage interaction was not significant for any composition attributes, and Oak treatments did not impact composition.

American- and French-oaked wines without tannin at 12-months storage had the highest pH (3.54), and the wines with tannin at 0-months storage had the lowest pH (3.39) (data not shown). The French-oaked wine without tannin at 12-months storage had the lowest TA (0.58%), and the unoaked wine with no tannin at 6-months storage had the highest TA (0.64%) (data not shown). At each storage time, the wines with no added tannin had a higher pH than those with tannin (Figure 8). For example, the wine without added tannin had a pH of 3.49 at 6-months storage, whereas the wine with added tannin had a pH of 3.43 at 6-months storage. In addition, the pH of wines increased slightly with time, but all pH values fell within a commercially acceptable range. The wines with added tannin (0.61%) had a higher TA than those without tannin (0.59%) at 12 months, but not at 0 or 6 months. In wines without added tannin, the TA was lower at 12-months storage (0.59%) than at 0-months (0.62%) or 6-months (0.62%) storage. This was logical, as pH increased and pH and TA are somewhat inversely correlated.

Wines had higher concentrations of both glycerol and ethanol after 12 months. From months 6 to 12, glycerol increased from 7.81 g/L to 8.03 g/L, and ethanol increased from 10.9% to 11.4%. This could indicate that a slight secondary fermentation occurred in the bottle, as both glycerol and ethanol are products of alcoholic fermentation. Wines with added tannin (278.27 mg/100 mL) had higher total residual sugar levels across all time points and Oak treatments relative to wines without tannin (213.70 mg/100 mL). Total residual sugars decreased from 275.21 to 217.48 mg/100 mL from month 0 to 12. This decrease in residual sugars could have been caused by oxidation or reduction of sugars to sugar acids or alcohols, respectively, or the formation of sugar-bisulfite adducts (Waterhouse et al. 2016). The wine without added tannin (2,291.71 mg/100 mL) had higher total organic acids at 0-months storage than the wine with

added tannin (1,637.95 mg/100 mL) (Figure 9). Total organic acid levels remained fairly steady during storage of the wine.

Anthocyanins. Enchantment wines were analyzed during storage (0, 6, and 12 months at 15°C) for malvidin-3-glucoside, petunidin-3-glucoside, delphinidin-3-glucoside, and total anthocyanins. The Storage main effect and Tannin x Oak interaction were significant for all anthocyanin attributes (Table 5).

All anthocyanin attributes decreased from 0- to 12-months storage at 15°C. For example, the malvidin concentration decreased 65%, 35.68 to 12.65 mg/100 mL from 0 to 12 months, and total anthocyanins decreased 66%, 75.28 to 25.81 mg/100 mL. For American- and French-oaked wines, the no tannin wines had higher concentrations of malvidin-3-glucoside (23.97 and 24.71 mg/100 mL, respectively) than the wines with added tannin (21.51 and 19.93 mg/100 mL, respectively) (Figure 10). This was likely because anthocyanins can form complexes with tannins that stabilize color but decrease measurable levels of monomeric anthocyanins. These "polymeric pigments" are more stable during storage, as they are less susceptible to degradation than monomeric anthocyanins (Hayasaka and Kennedy 2003, Waterhouse et al. 2016). The no tannin French-oaked wines had greater petunidin (10.92 mg/100 mL), delphinidin (7.82 mg/100 mL), and total anthocyanin (56.40 mg/100 mL) levels than the tannin French-oaked wines (9.22, 7.01, and 48.88 mg/100 mL, respectively). However, the tannin unoaked wine had higher total anthocyanins (61.29 mg/100 mL) than the no tannin unoaked wine (55.64 mg/100 mL).

Color. Enchantment wines were analyzed during storage (0, 6, and 12 months at 15°C) for color attributes. The Tannin x Oak x Storage interaction was significant for L*, red color, and color density. The Tannin and Storage main effects were significant for hue angle, and the Tannin main effect and Oak x Storage interaction were significant for chroma (Table 6).

For French-oaked wines, no tannin wine had a lower L* (darker color) at month 0 (0.95) than tannin wine (1.6) (Figure 11). The opposite was seen for unoaked wine at month 0: tannin wine had a darker color (L* 0.57) than no tannin wine (L* 0.95). Though not significant, the wines without tannin got darker during storage, regardless of Oak treatment, and the wines with tannin and oak got darker during storage. The wines had a hue angle of pure red (360°), with a slight decrease in hue from 360.30° to 360.16° from months 0 to 12. The wines with added tannin (360.24°) had a higher hue angle than those without tannin (360.21°). The chroma (color saturation) increased slightly from 0 to 12 months, although this increase was mostly insignificant (Figure 12). There was no difference among Oak treatments at 0- or 6-months storage. At 12 months, the unoaked wines had greater color saturation (chroma 3.54) than the French-oaked wines (chroma 1.80).

For American and French oak treatments at 0-months storage, the wines with added tannin (22.38 and 22.22, respectively) had greater red color than the corresponding no tannin wines (15.3 and 15.69, respectively) (Figure 13). This difference between Tannin treatments was not seen at other Storage times or Oak treatments. All wines with added tannin at 0-months storage had higher red color than wines at 6- and 12-months storage, across Tannin and Oak treatments. There was no apparent effect of Oak on red color. There were similar trends for color density. The American- and French-oaked wines with added tannin at 0-months storage had higher color density (39.76 and 39.54, respectively) than the respective no tannin wines (25.34 and 25.93, respectively). The unoaked, American-oaked, and French-oaked wines with added tannin at 0-months storage had higher color density than wines at 6- and 12-months storage, across Tannin and Oak treatments.

Analysis of aroma attributes at 0-months storage (2017 and 2018)

There were 56 volatile aroma compounds positively identified in 2017 Enchantment wines and 54 compounds identified in 2018 wines. Initial exploration of volatile aroma chromatograms of Enchantment wines showed that Oak treatments mainly impacted the presence of volatile aromas. Within each Oak treatment, the Tannin treatments had similar chromatogram peaks, but the peak areas differed (data not shown). Therefore, peaks were identified within each Oak treatment, regardless of Tannin treatment. Table 7 shows the compounds identified in 2017 and 2018 wines, their compound class, the aroma category each was grouped into, more detailed aroma descriptors, and whether or not the compound was identified in wines within each Oak treatment. Compounds included chemical, floral, fruity, green/fat (waxy, rancid), roasted/caramelized, and vegetal alcohols, floral, green/fat, and roasted/caramelized aldehydes, vegetal alkyl sulfides, chemical benzothiazoles, fruity, green/fat, and unpleasant carboxylic acids, floral and fruity esters, chemical ethers, roasted/caramelized furans, fruity glycols, green/fat and vegetal ketones, oaked lactones, and floral and herbal/spicy terpenes. The esters were the largest class of compounds in all wines. Esters are characteristic byproducts of alcoholic fermentation and are critical for the aroma of most wines (Waterhouse et al. 2016). Oak lactone, an aliphatic γ-lactone extracted into wine during contact with oak, was only identified in the 2017 American-oaked wines, and not in the 2017 French-oaked wines or 2018 wines. PCA was used to reduce dimensionality of the data and to elucidate relationships between aroma categories and Tannin/Oak treatments. The TIC areas were summed for compounds within each aroma category.

2017 Wines. Examining the PCA results, distinctions could be made among Tannin and Oak treatments in 2017 wines for aroma categories. Four components explained over 80% of the

variation in the dataset (Table 8). PC1 (37.7%) had positive loadings for green/fat, unpleasant, vegetal, floral, and fruity aroma categories, and it was determined that PC1 represented high levels of aroma compounds in general. The unoaked wine with tannin loaded positively on PC1, and American oaked wines with and without tannin loaded negatively on PC1. This indicated that unoaked wine with tannin could have a higher overall aroma impact than the American oaked wines. PC2 (18.5%) had positive loadings for the chemical aroma category and the unoaked and American-oaked wines without tannin. The unoaked and French-oaked wines with tannin loaded negatively on PC2. Therefore, PC2 represented distinction among wines with and without tannin, and the wines with added tannin could potentially be associated with less chemical-smelling aromas.

PC3 (17.2%) had positive loadings for the roasted/caramelized aroma category and all American-oaked and French-oaked wines. The herbal/spicy aroma category and unoaked wines loaded negatively on PC3. This association of oaked wines with roasted/caramelized aromas could mean that oak addition gave Enchantment wines more roasty, complex aromas, whereas unoaked Enchantment wines had more raw, herbal aromas. PC4 (10.3%) had positive loadings for the oaked aroma category and American-oaked wines. The unoaked wine without tannin and the French-oaked wines loaded negatively on PC4. The correlation of American-oaked wines with oaky aromas was notable, as American oak (*Quercus alba*) has a reputation for producing more intense coconut/oaky aromas in wine than French oak (*Q. robur* and *Q. petraea*) (Masson et al. 1995).

2018 Wines. Distinctions could be made among Tannin/Oak treatments for aroma categories in 2018 wines. Four components explained over 80% of the variation in the dataset. PC1 (45.5%) had positive loadings for green/fat, fruity, unpleasant, chemical, floral, and vegetal aroma

categories. Similar to 2017, PC1 likely represented high amounts of aroma compounds in general. All wines without added tannin had positive loadings on PC1, whereas all wines with tannin had negative loadings. Therefore, tannin addition could have led to wines with lower overall aroma impacts. PC2 (16.4%) had positive loadings for herbal/spicy aroma categories and unoaked wines with and without tannin and American-oaked wine without tannin. French-oaked wines loaded negatively on PC2. Thus, similar to 2017, French-oaked wines were associated with lower amounts of herbal/spicy aromas.

Roasted/caramelized aromas and American-oaked wines loaded positively on PC3 (14.7%), and unoaked wines loaded negatively on PC3. This indicated that American-oaked wines were associated with higher amounts of complex roasty and caramelized aromas and agreed with the 2017 finding that American-oaked wines had more roasted/caramelized and oaked aromas. American-oaked wines loaded positively on PC4 (8.7%) and unoaked wine without tannin and French-oaked wine with tannin loaded negatively on PC4. However, no aroma categories loaded positively or negatively on PC4.

Conclusions

In both 2017 and 2018, Enchantment wines had compositions at bottling within typical ranges for a dry red table wine, remaining mostly stable during one year of storage at 15°C. Wines from 2018 were more acidic and had less residual sugar than 2017 wines. There were no consistent trends between 2017 and 2018 for the effects of tannin and oak addition on the composition of Enchantment wines at 0-months storage. The addition of tannin lead to wines with lower pH values and higher sugar levels after 12-months storage.

Only anthocyanin-3-glucosides, and not their diglucoside counterparts, were identified in Enchantment wine. Malvidin-3-glucoside was the predominant anthocyanin. Wines from 2018 had greater amounts of anthocyanin compounds and a darker, more saturated color than 2017 wines. There was no decrease in color quality observed over 12-months storage, supporting the conclusion that Enchantment anthocyanins formed stable pigment complexes with other phenolic compounds during aging.

Fruity esters were the largest class of volatile aroma compounds in Enchantment wine. Wine treatments could be distinguished based on their aroma profiles, and American- and French-oaked wines were associated with higher amounts of roasted and caramelized aromas and lower amounts of raw, herbal aromas. In 2017, American-oaked wines were associated with oaky aromas. Tannin addition led to wines associated with lower overall aroma impacts in 2018.

Overall, these results suggested the potential of Enchantment wine grapes for producing high-quality, deeply red-colored wines with aging potential. Therefore, Enchantment red wine grapes present a unique opportunity for grape growers and wine makers in Arkansas and the mid-South United States as an alternative to the native and hybrid species with less stable color and non-traditional aromas that are typically grown in the area.

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Tables

Table 1. Initial composition of Enchantment grape must Tannin treatments after crushing in 2017 and 2018 (University of Arkansas System Division of Agriculture Fruit Research Station, Clarksville, AR).

Harvest date	Treatment	Soluble solids (%)	pH	Titratable acidity (%)
17 August 2017	No Tannin	14.6	3.14	0.84
	Tannin	14.8	3.17	0.82
8 August 2018	No Tannin	17.3	3.81	0.70
	Tannin	17.8	3.71	0.71

Table 2. Main and interaction effects from ANOVA for Tannin and Oak on wine composition attributes at 0-months storage at 15°C for wines produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (2017 and 2018).

Effects	pH	Titratable acidity (%)	Glycerol (g/L)	Ethanol (% v/v)	Glucose (mg/100 mL)	Fructose (mg/100 mL)	Total residual sugars (mg/100 mL)	Tartaric acid (mg/100 mL)	Malic acid (mg/100 mL)	Citric acid (mg/100 mL)	Succinic acid (mg/100 mL)	Lactic acid (mg/100 mL)	Total organic acids (mg/100 mL)
2017													
Tannin													
No tannin	3.44 a[a]	0.62 a	7.85 a	11.15 a	53.46 a	184.93 b	238.39 b	580.50 a	458.96 a	233.37 a	715.46 a	303.41 a	2291.71 a
Tannin	3.39 b	0.62 a	7.71 a	10.97 a	57.97 a	254.07 a	312.03 a	550.92 a	325.77 b	256.61 a	393.11 b	111.54 b	1637.95 b
P value	***<0.0001***	*0.3728*	*0.4493*	*0.4335*	*0.3119*	***0.0018***	***0.0047***	*0.3826*	***<0.0001***	*0.0599*	***0.0005***	***0.0202***	***0.0026***
Oak													
No oak	3.42 a	0.62 a	7.73 a	10.98 a	50.84 a	184.84 b	235.68 b	502.77 b	405.90 a	214.66 b	593.29 a	251.30 a	1967.92 a
American	3.42 a	0.62 a	7.76 a	11.01 a	52.75 a	211.17 ab	263.92 ab	623.50 a	419.41 a	251.14 a	620.45 a	226.69 a	2141.19 a
French	3.41 b	0.62 a	7.85 a	11.20 a	63.54 a	262.48 a	326.02 a	570.87 ab	351.78 a	269.18 a	449.11 a	144.43 a	1785.38 a
P value	***0.0004***	*0.5525*	*0.8294*	*0.6841*	*0.0587*	***0.0115***	***0.0140***	***0.0265***	*0.0552*	***0.0039***	*0.1647*	*0.4929*	*0.3223*
Tannin x Oak (P value)	*0.1704*	*0.9078*	*0.9799*	*0.9452*	*0.2875*	*0.9596*	*0.9891*	*0.4237*	*0.0790*	*0.0719*	*0.2572*	*0.2796*	*0.2565*
2018													
Tannin													
No tannin	3.24 b	0.70 b	7.82 b	11.17 b	39.03 a	100.67 a	139.69 a	412.92 a	218.39 b	172.37 a	361.74 a	95.50 a	1260.92 a
Tannin	3.26 a	0.70 a	8.13 a	11.42 a	39.00 a	93.87 a	132.86 a	398.37 a	251.59 a	139.08 b	361.10 a	101.66 a	1251.79 a
P value	***<0.0001***	***0.0003***	***<0.0001***	***0.0023***	*0.9920*	*0.2720*	*0.4396*	*0.3262*	***0.0225***	***0.0008***	*0.9590*	*0.5751*	*0.8570*
Oak													
No oak	3.25 b	0.70 b	7.92 a	11.20 a	39.39 a	99.27 a	138.65 a	415.21 a	233.05 a	166.84 a	363.28 a	102.66 a	1281.04 a
American	3.25 a	0.70 a	7.95 a	11.28 a	40.47 a	99.62 a	140.09 a	407.10 a	238.70 a	151.24 a	366.73 a	101.13 a	1264.90 a
French	3.25 a	0.70 a	8.06 a	11.40 a	37.18 a	92.92 a	130.10 a	394.62 a	233.22 a	149.10 a	354.25 a	91.94 a	1223.13 a
P value	***0.0013***	***0.0002***	*0.1358*	*0.0840*	*0.6078*	*0.5998*	*0.6027*	*0.5144*	*0.9253*	*0.1878*	*0.6994*	*0.6866*	*0.6281*
Tannin x Oak (P value)	***0.0395***	***<0.0001***	*0.8449*	*0.5704*	*0.2613*	*0.5772*	*0.4614*	*0.3119*	*0.8024*	*0.1050*	*0.8992*	*0.9975*	*0.6153*

[a] Means with different letters for each attribute within effects and years are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

Table 3. Main and interaction effects from ANOVA for Tannin and Oak on wine color attributes at 0-months storage at 15°C for wines produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (2017 and 2018).

Effects	L*	Hue angle (°)[a]	Chroma	Red color[b]	Color density[c]
2017					
Tannin					
No tannin	0.98 a[d]	360.37 a	1.40 a	15.71 b	26.04 b
Tannin	1.01 a	360.33 a	1.54 a	20.85 a	36.55 a
P value	*0.5531*	*0.1331*	*0.4148*	***<0.0001***	***<0.0001***
Oak					
No oak	0.76 c	360.28 a	1.61 ab	17.04 a	28.59 a
American	0.95 b	360.34 a	1.08 b	18.84 a	32.55 a
French	1.27 a	360.28 a	1.73 a	18.95 a	32.74 a
P value	***<0.0001***	*0.4654*	***0.0135***	*0.0634*	*0.0571*
Tannin x Oak (P value)	***<0.0001***	*0.1597*	*0.3598*	***0.0106***	***0.0122***
2018					
Tannin					
No tannin	0.54 a	360.19 a	2.45 a	19.01 b	27.99 b
Tannin	0.50 a	360.19 a	2.15 b	19.74 a	29.03 a
P value	*0.1200*	*0.8687*	***0.0153***	***0.0225***	***0.0211***
Oak					
No oak	0.51 ab	360.20 a	2.32 a	19.05 a	28.02 a
American	0.48 b	360.16 b	2.12 a	19.28 a	28.36 a
French	0.58 a	360.20 a	2.47 a	19.79 a	29.16 a
P value	***0.0105***	***0.0222***	*0.0712*	*0.1394*	*0.0949*
Tannin x Oak (P value)	***0.0009***	*0.1363*	***<0.0001***	***0.0004***	***0.0004***

[a] Hue angles <90° were subjected to a 360° compensation to account for discrepancies between red samples near 0° and those near 360°.

[b] Red color was calculated as absorbance of wine at 520 nm.

[c] Color density was calculated as absorbance 520 nm + absorbance 420 nm.

[d] Means with different letters for each attribute within effects and years are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

Table 4. Main and interaction effects from ANOVA for Tannin, Oak, and Storage (0, 6, and 12 months at 15°C) on wine composition attributes for wines produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (2017).

Effects	pH	Titratable acidity (%)	Glycerol (g/L)	Ethanol (% v/v)	Total residual sugars (mg/100 mL)	Total organic acids (mg/100 mL)
Tannin (T)						
No tannin	3.49 a[a]	0.61 a	7.91 a	11.19 a	213.70 b	2050.47 a
Tannin	3.44 b	0.61 a	7.84 a	11.02 a	278.27 a	1879.71 b
P value	***<0.0001***	*0.1771*	*0.2873*	*0.0533*	***0.0002***	***0.0396***
Oak (O)						
No oak	3.46 a	0.62 a	7.89 a	11.11 a	236.70 a	1898.22 a
American	3.46 a	0.61 a	7.86 a	11.05 a	237.68 a	2070.61 a
French	3.46 a	0.61 a	7.87 a	11.16 a	263.57 a	1926.44 a
P value	*0.3597*	*0.1881*	*0.9311*	*0.5963*	*0.3141*	*0.1853*
Storage (S)						
Month 0	3.42 c	0.62 a	7.78 b	11.06 b	275.21 a	1964.83 a
Month 6	3.46 b	0.62 a	7.81 b	10.90 b	245.26 ab	1899.47 a
Month 12	3.51 a	0.60 b	8.03 a	11.35 a	217.48 b	2030.98 a
P value	***<0.0001***	***<0.0001***	***0.0095***	***0.0003***	***0.0194***	*0.4207*
T x O (P value)	*0.4041*	*0.0965*	*0.4134*	*0.5322*	*0.6509*	*0.4754*
T x S (P value)	***0.0004***	***0.0095***	*0.5289*	*0.3244*	*0.6989*	***0.0004***
O x S (P value)	*0.1428*	*0.4555*	*0.6106*	*0.3860*	*0.1880*	*0.3689*
T x O x S (P value)	*0.0834*	*0.5215*	*0.6197*	*0.8740*	*0.6910*	*0.1740*

[a] Means with different letters for each attribute within effects are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

Table 5. Main and interaction effects from ANOVA for Tannin, Oak, and Storage (0, 6, and 12 months at 15°C) on wine anthocyanin attributes for wines produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (2017).

Effects	Malvidin-3-glucoside (mg/100 mL)	Petunidin-3-glucoside (mg/100 mL)	Delphinidin-3-glucoside (mg/100 mL)	Total anthocyanins (mg/100 mL)
Tannin (T)				
No tannin	24.37 a[a]	10.70 a	7.64 a	48.40 a
Tannin	22.25 b	10.19 b	7.86 a	46.06 b
P value	***<0.0001***	***0.0280***	*0.2652*	***0.0235***
Oak (O)				
No oak	24.87 a	11.14 a	8.38 a	50.65 a
American	22.74 b	10.12 b	7.46 b	45.97 b
French	22.32 b	10.07 b	7.41 b	45.08 b
P value	***<0.0001***	***0.0004***	*0.0002*	***<0.0001***
Storage (S)				
Month 0	35.68 a	16.20 a	12.10 a	75.28 a
Month 6	21.60 b	9.57 b	6.96 b	40.61 b
Month 12	12.65 c	5.57 c	4.20 c	25.81 c
P value	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
T x O (P value)	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
T x S (P value)	*0.0638*	*0.1640*	*0.3612*	*0.1975*
O x S (P value)	*0.4482*	*0.6463*	*0.5754*	*0.4370*
T x O x S (P value)	*0.0797*	*0.0665*	*0.0724*	*0.0580*

[a] Means with different letters for each attribute within effects are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

Table 6. Main and interaction effects from ANOVA for Tannin, Oak, and Storage (0, 6, and 12 months at 15°C) on wine color attributes for wines produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (2017).

Effects	L*	Hue angle (°)[a]	Chroma	Red color[b]	Color density[c]
2017					
Tannin (T)					
No tannin	0.80 a[d]	360.21 b	1.87 b	14.16 b	23.78 b
Tannin	0.86 a	360.24 a	2.38 a	15.50 a	26.40 a
P value	*0.0566*	***0.0427***	***0.0029***	***0.0004***	***<0.0001***
Oak (O)					
No oak	0.82 a	360.22 a	2.60 a	14.37 a	23.97 a
American	0.81 a	360.24 a	1.88 b	15.06 a	25.66 a
French	0.86 a	360.20 a	1.91 b	15.06 a	25.64 a
P value	*0.3278*	*0.3333*	***0.0008***	*0.1983*	***0.0322***
Storage (S)					
Month 0	0.99 a	360.30 a	1.47 b	18.28 a	31.29 a
Month 6	0.85 b	360.20 b	2.26 a	12.49 c	21.35 b
Month 12	0.64 c	360.16 b	2.66 a	13.72 b	22.63 b
P value	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
T x O (P value)	***0.0002***	*0.9100*	*0.1811*	***0.0178***	***0.0072***
T x S (P value)	*0.8367*	*0.5967*	*0.2880*	***<0.0001***	***<0.0001***
O x S (P value)	***<0.0001***	*0.4252*	***0.0047***	*0.2736*	*0.1177*
T x O x S (P value)	***<0.0001***	*0.0779*	*0.8090*	***0.0301***	***0.0089***

[a] Hue angles <90° were subjected to a 360° compensation to account for discrepancies between red samples near 0° and those near 360°.

[b] Red color was calculated as absorbance of wine at 520 nm.

[c] Color density was calculated as absorbance 520 nm + absorbance 420 nm.

[d] Means with different letters for each attribute within effects are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

Table 7. Volatile aroma compounds identified in unoaked, American-, and French-oaked wines at 0-months storage at 15°C produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (2017 and 2018).

Compound[a]	Compound class	Aroma category	Aroma descriptors[b]	2017			2018		
				No oak	American oak	French oak	No oak	American oak	French oak
Octanol	Alcohol	Chemical	Chemical, metal	✓[c]	✓	✓	✓	✓	✓
2-Ethylhexanol	Alcohol	Floral	Rose, citrus	✓	✓	✓	✓	✓	✓
2-Phenylethanol	Alcohol	Floral	Honey, rose	✓	✓	✓	✓	✓	✓
Benzyl alcohol	Alcohol	Floral	Floral, fruit	✓	✓	✓	✓	✓	✓
1-Pentanol	Alcohol	Fruity	Balsamic, fruit	✓	✓		✓	✓	✓
1-Undecanol	Alcohol	Fruity	Mandarin	✓	✓	✓			
3-Methyl-1-pentanol	Alcohol	Fruity	Wine, cognac	✓	✓	✓	✓	✓	✓
1-Decanol	Alcohol	Green/fat	Fat	✓	✓	✓			
1-Dodecanol	Alcohol	Green/fat	Fat, wax	✓	✓	✓	✓	✓	✓
1-Heptanol	Alcohol	Green/fat	Chemical, green, fresh				✓	✓	✓
1-Hexanol	Alcohol	Green/fat	Green, herbal	✓	✓	✓	✓	✓	✓
1-Nonanol	Alcohol	Green/fat	Fat, green	✓	✓	✓			
4-Methyl-2-pentanol	Alcohol	Green/fat	Oil, green, wine	✓			✓		
cis-3-Hexen-1-ol	Alcohol	Green/fat	Grass, leaf	✓		✓	✓	✓	✓
Furfuryl alcohol	Alcohol	Roasted/caramelized	Caramel		✓				
2-Heptanol	Alcohol	Vegetal	Mushroom, herbal	✓	✓	✓			
Phenylacetaldehyde	Aldehyde	Floral	Floral, honey, rose		✓				
Decanal	Aldehyde	Green/fat	Soap, orange peel	✓	✓	✓	✓	✓	✓
Nonanal	Aldehyde	Green/fat	Fat, citrus, green	✓	✓	✓	✓	✓	✓
Octanal	Aldehyde	Green/fat	Fat, soap, green	✓	✓	✓		✓	✓
4-Methylbenzaldehyde	Aldehyde	Roasted/caramelized	Almond, caramel					✓	✓
5-Methylfurfural	Aldehyde	Roasted/caramelized	Bread, almond					✓	
Benzaldehyde	Aldehyde	Roasted/caramelized	Almond, caramel	✓	✓	✓			
Furfural	Aldehyde	Roasted/caramelized	Almond, caramel		✓	✓		✓	✓
Methionol	Alkyl sulfide	Vegetal	Cooked potato	✓	✓	✓	✓	✓	✓

Table 7 (Cont.)

Compound[a]	Compound class	Aroma category	Aroma descriptors[b]	2017			2018		
				No oak	American oak	French oak	No oak	American oak	French oak
Benzothiazole	Benzothiazole	Chemical	Gasoline, rubber	✓	✓	✓			
Octanoic acid, 3-methylbutyl ester	Carboxylic acid	Fruity	Fruit, pineapple	✓	✓	✓			
Decanoic acid	Carboxylic acid	Green/fat	Fat, soap	✓	✓	✓	✓	✓	✓
2-Methylbutyric acid	Carboxylic acid	Unpleasant	Cheese, sweat	✓	✓	✓			
Butyric acid	Carboxylic acid	Unpleasant	Cheese, sweat	✓	✓	✓	✓	✓	
Hexanoic acid	Carboxylic acid	Unpleasant	Sweat, cheese	✓	✓	✓	✓	✓	✓
Isovaleric acid	Carboxylic acid	Unpleasant	Sweat, cheese	✓			✓		
Octanoic acid	Carboxylic acid	Unpleasant	Sweat, cheese, fat	✓	✓	✓	✓	✓	✓
2-Phenylethyl acetate	Ester	Floral	Honey, floral, rose				✓	✓	✓
2-Methylbutyl acetate	Ester	Fruity	Fermented fruit, banana, rum	✓	✓	✓	✓	✓	✓
Diethyl succinate	Ester	Fruity	Wine, fruit, watermelon	✓	✓	✓	✓	✓	✓
Ethyl 2-furoate	Ester	Fruity	Fruit, floral				✓	✓	✓
Ethyl 2-hexenoate	Ester	Fruity	Fruit	✓	✓	✓	✓	✓	✓
Ethyl 2-methylbutyrate	Ester	Fruity	Apple, strawberry	✓	✓	✓	✓	✓	✓
Ethyl 3-hydroxybutyrate	Ester	Fruity	Grape, coconut, marshmallow	✓	✓	✓	✓	✓	✓
Ethyl butanoate	Ester	Fruity	Apple, strawberry, bubblegum	✓	✓	✓	✓	✓	✓
Ethyl decanoate	Ester	Fruity	Grape	✓	✓	✓	✓	✓	✓
Ethyl dodecanoate	Ester	Fruity	Mango, leaf	✓	✓	✓	✓	✓	✓
Ethyl heptanoate	Ester	Fruity	Fruit	✓	✓	✓	✓	✓	✓
Ethyl hexanoate	Ester	Fruity	Apple peel, strawberry, anise	✓	✓	✓	✓	✓	✓
Ethyl isobutyrate	Ester	Fruity	Strawberry	✓	✓	✓	✓	✓	✓
Ethyl isovalerate	Ester	Fruity	Anise, apple, black currant	✓	✓	✓	✓	✓	✓
Ethyl nonanoate	Ester	Fruity	Tropical fruit, rose	✓	✓	✓	✓	✓	✓

Table 7 (Cont.)

Compound[a]	Compound class	Aroma category	Aroma descriptors[b]	2017			2018		
				No oak	American oak	French oak	No oak	American oak	French oak
Ethyl octanoate	Ester	Fruity	Fruit, floral	✓	✓	✓	✓	✓	✓
Ethyl pentanoate	Ester	Fruity	Fruit, yeast				✓	✓	✓
Hexyl acetate	Ester	Fruity	Fruit, herb, wine	✓	✓	✓	✓	✓	✓
Isoamyl acetate	Ester	Fruity	Banana, pear	✓	✓	✓	✓	✓	✓
Isobutyl acetate	Ester	Fruity	Apple, banana	✓	✓	✓	✓	✓	✓
Isopentyl hexanoate	Ester	Fruity	Fruit				✓	✓	✓
Isopentyl octanoate	Ester	Fruity	Fruit, pineapple				✓	✓	✓
Methyl decanoate	Ester	Fruity	Wine, fruit				✓	✓	✓
Methyl hexanoate	Ester	Fruity	Fruit, fresh, paint thinner	✓	✓	✓	✓	✓	✓
Dibutyl ether	Ether	Chemical	Ethereal				✓	✓	✓
2,5-Diethyltetrahydrofuran	Furan	Roasted/caramelized	Caramel			✓			
2,3-Butanediol	Glycol	Fruity	Fruit, onion	✓	✓	✓	✓	✓	✓
2,3-Hexanedione	Ketone	Green/fat	Butter, cream, caramel				✓	✓	✓
6-Methyl-5-hepten-2-one	Ketone	Vegetal	Mushroom, earthy	✓					
Oak lactone	Lactone	Oaked	Coconut, floral		✓				
Citronellol	Terpene	Floral	Rose, citrus, clove	✓	✓	✓	✓	✓	
Linalool	Terpene	Floral	Floral, lavender, Earl Grey tea	✓	✓	✓	✓	✓	✓
β-damascenone	Terpene	Fruity	Apple, rose, honey				✓	✓	✓
alpha-Terpineol	Terpene	Herbal/spicy	Anise, mint, toothpaste	✓			✓	✓	
p-Cymene	Terpene	Herbal/spicy	Herbal, spicy	✓	✓	✓			

[a] Compounds were identified by comparison of mass spectra with NIST14 (National Institute of Standards and Technology, Gaithersburg, MD, USA), Flavors and Fragrances of Natural and Synthetic Compounds (FFNSC3, John Wiley & Sons, Inc., Hoboken, NJ, USA), and Adam's Essential Oils (Adams 2007) mass spectral libraries and comparison of calculated Kovats retention indices (Kováts 1958) with previously reported values .

[b] Aroma descriptors obtained from the Flavornet (Acree and Arn 2004) and Pherobase (Sayed 2003) databases.

[c] A ✓ indicates positive identification.

Table 8. Summary of principal components analysis on volatile aroma compound categories in 2017 and 2018 wines at 0-months storage at 15°C produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (Clarksville, AR).

		2017			
		Component 1 (37.7%)[a]	**Component 2 (18.5%)**	**Component 3 (17.2%)**	**Component 4 (10.3%)**
Positive loadings[b]	Aroma categories[c]	Green/fat Unpleasant Vegetal Floral Fruity	Chemical	Roasted/caramelized	Oaked
	Key samples	Tannin, no oak	No tannin, no oak No tannin, American oak	No tannin, American oak Tannin, American oak No tannin, French oak Tannin, French oak	No tannin, American oak Tannin, American oak
Negative loadings[d]	Aroma categories	---	---	Herbal/spicy	---
	Key samples	No tannin, American oak Tannin, American oak	Tannin, no oak Tannin, French oak	No tannin, no oak Tannin, no oak	No tannin, no oak No tannin, French oak Tannin, French oak
		2018			
		Component 1 (45.5%)	**Component 2 (16.4%)**	**Component 3 (14.7%)**	**Component 4 (8.7%)**
Positive loadings	Aroma categories	Green/fat Fruity Unpleasant Chemical Floral Vegetal	Herbal/spicy	Roasted/caramelized	---
	Key samples	No tannin, no oak No tannin, American oak No tannin, French oak	No tannin, no oak Tannin, no oak No tannin, American oak	No tannin, American oak Tannin, American oak	No tannin, American oak Tannin, American oak
Negative loadings	Aroma categories	---	---	---	---
	Key samples	Tannin, no oak Tannin, American oak Tannin, French oak	No tannin, French oak Tannin, French oak	No tannin, no oak Tannin, no oak	No tannin, no oak Tannin, French oak

[a] Percent of variation in data explained by each component.

[b] Loading values >0.6 were considered positive loadings for aroma categories on each component.

[c] Aroma categories represent the sum of the total ion chromatogram (TIC) peak areas of positively identified compounds within each category (Table 7).

[d] Loading values <-0.6 were considered negative loadings for aroma categories on each component.

Figures

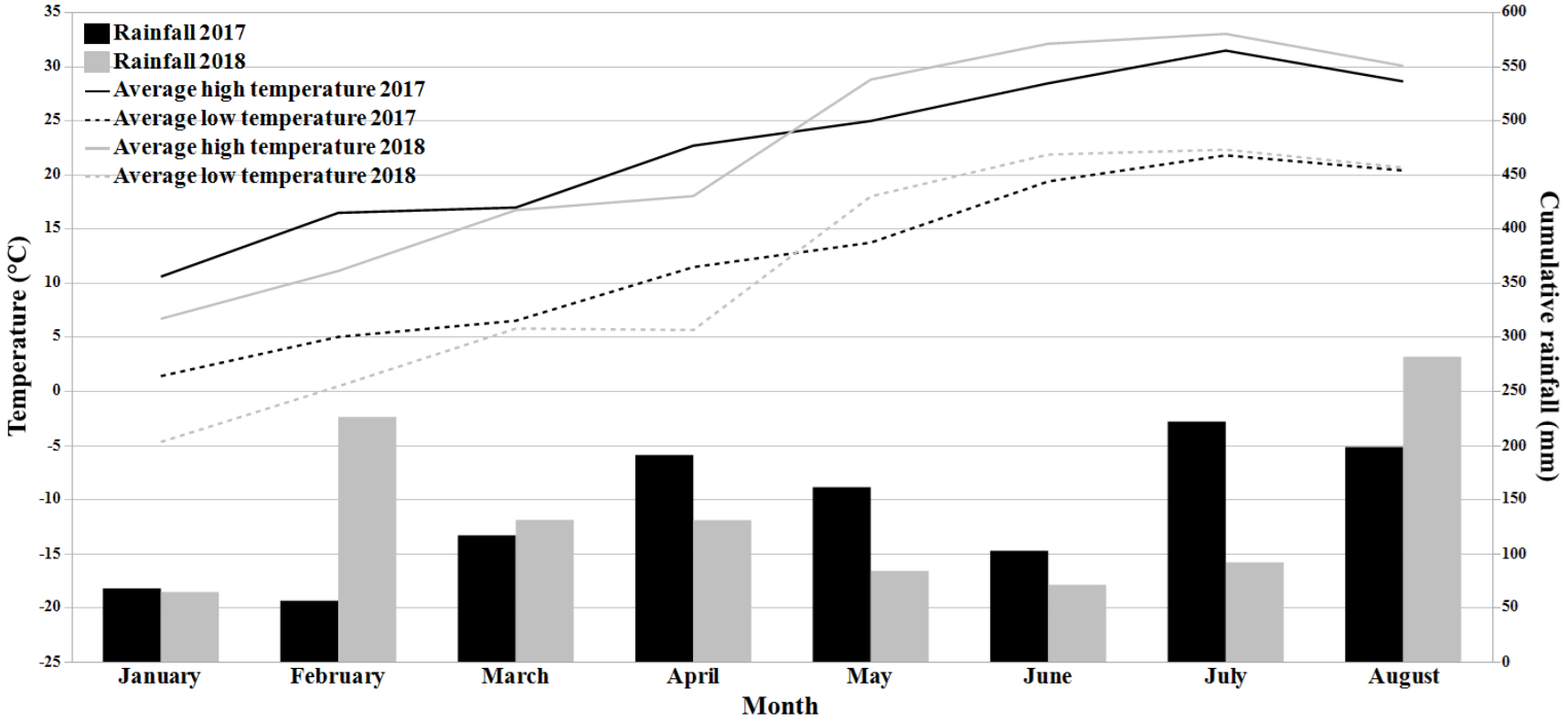


Figure 1. Average monthly high and low temperatures and cumulative rainfall[a] from January-August 2017 and 2018 at the University of Arkansas System Division of Agriculture Fruit Research Station (Clarksville, AR).
[a] Data was gathered from a USDA weather station in Clarksville, Arkansas.

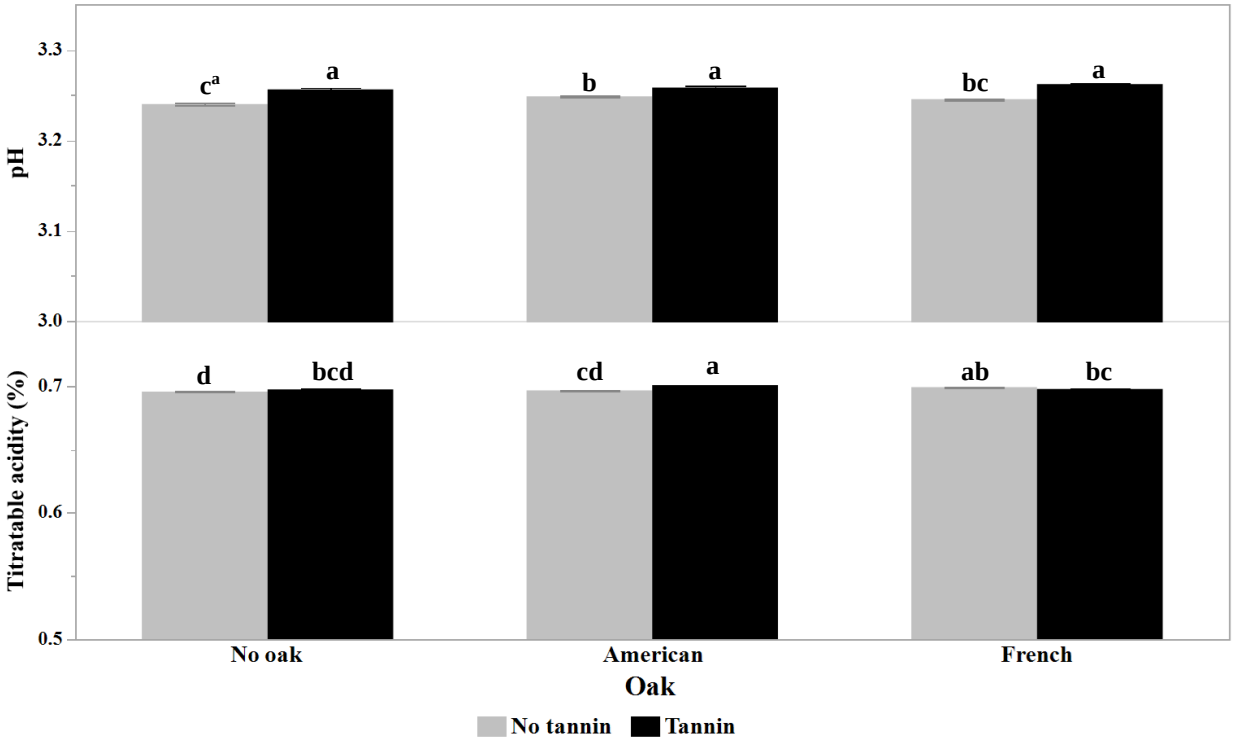


Figure 2. Effect of Tannin and Oak on pH and titratable acidity at 0-months storage at 15°C of wines produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (2018).
[a] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters for each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

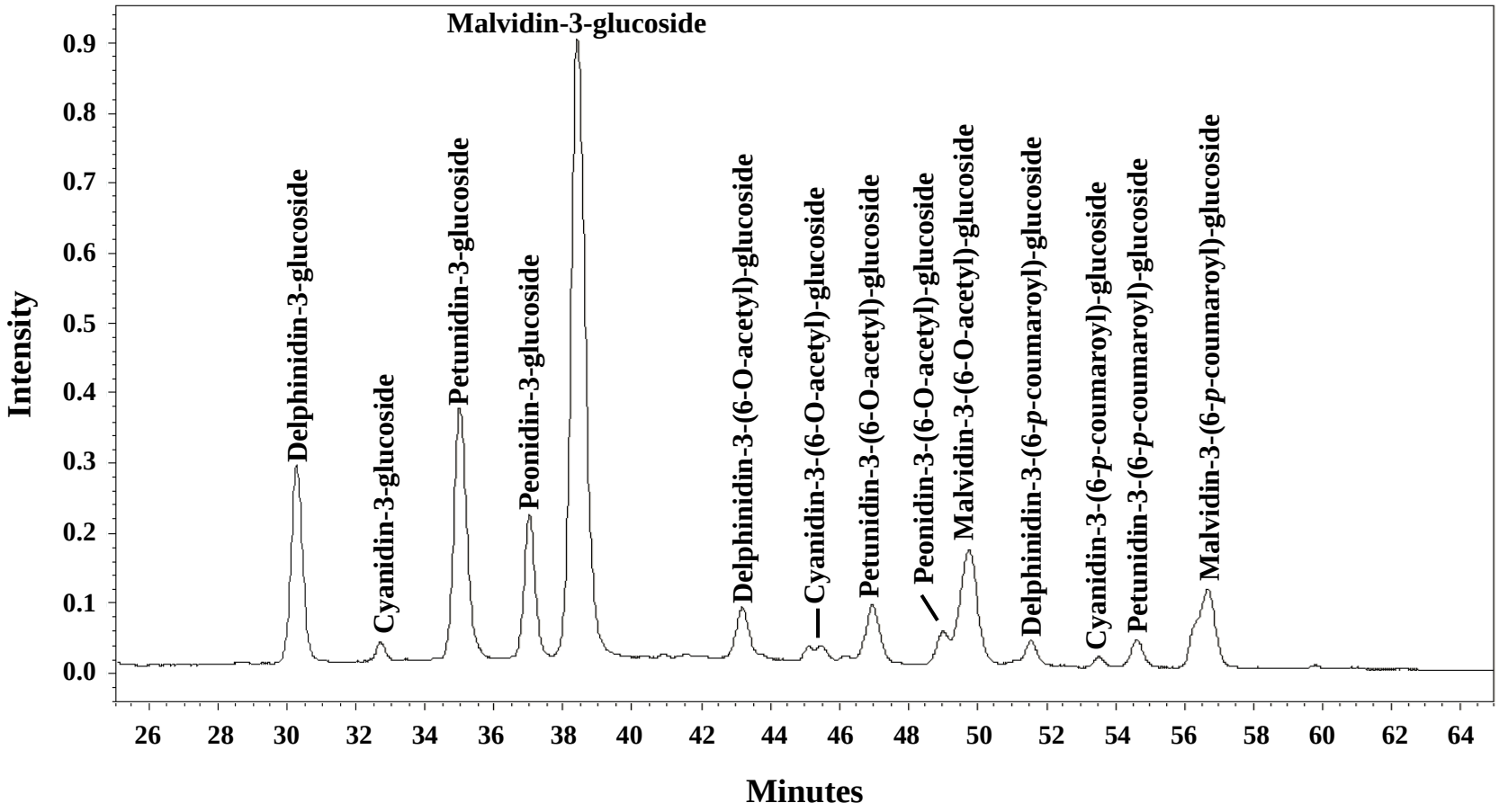


Figure 3. High performance liquid chromatography (HPLC) chromatogram for anthocyanins positively identified in wines produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station.

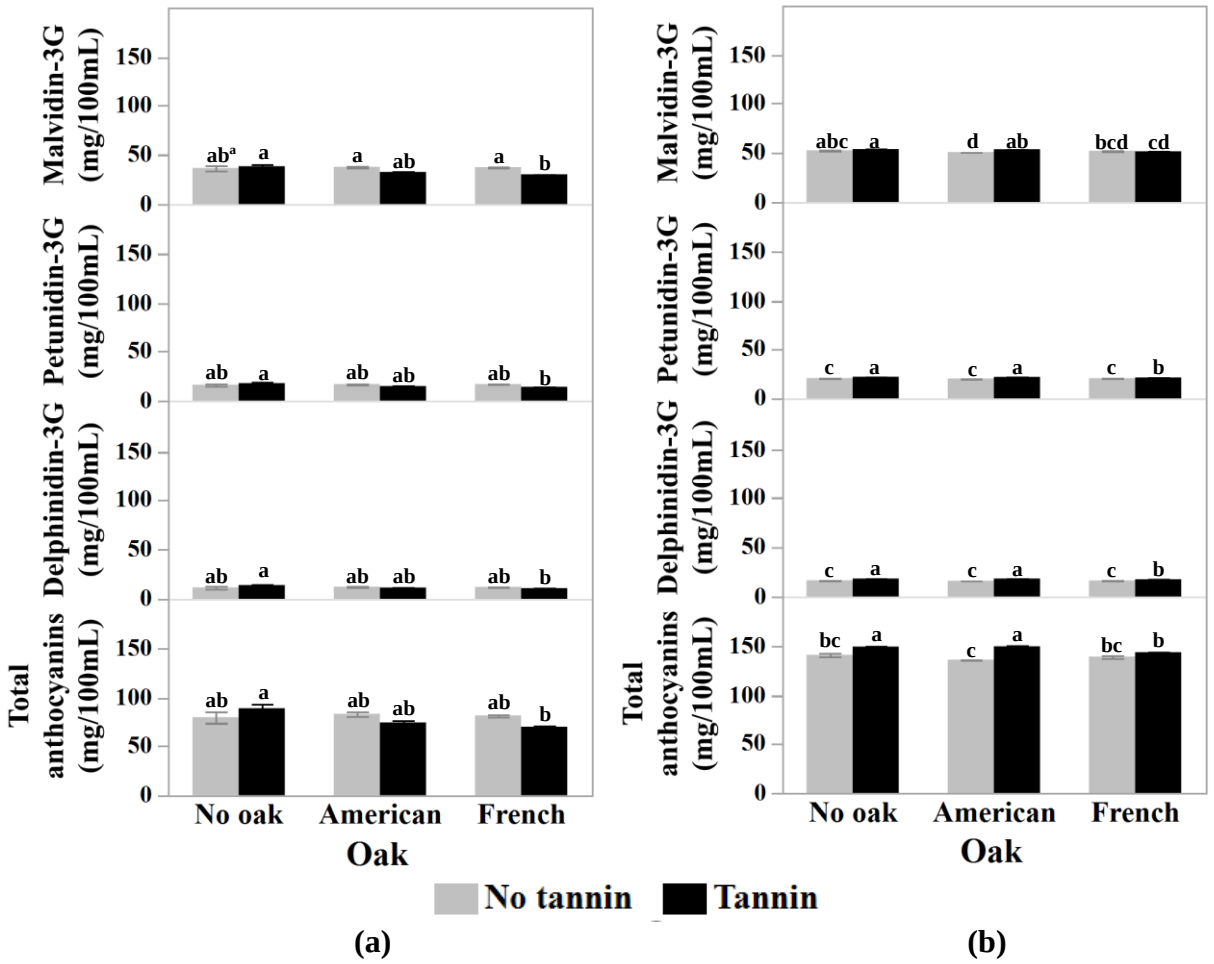


Figure 4. Effect of Tannin and Oak on malvidin-3-glucoside (malvidin-3G), petunidin-3-glucoside (petunidin-3G), delphinidin-3-glucoside (delphinidin-3G), and total anthocyanins at 0-months storage at 15°C of 2017 (a) and 2018 (b) wines produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station. [a] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters for each attribute and year are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

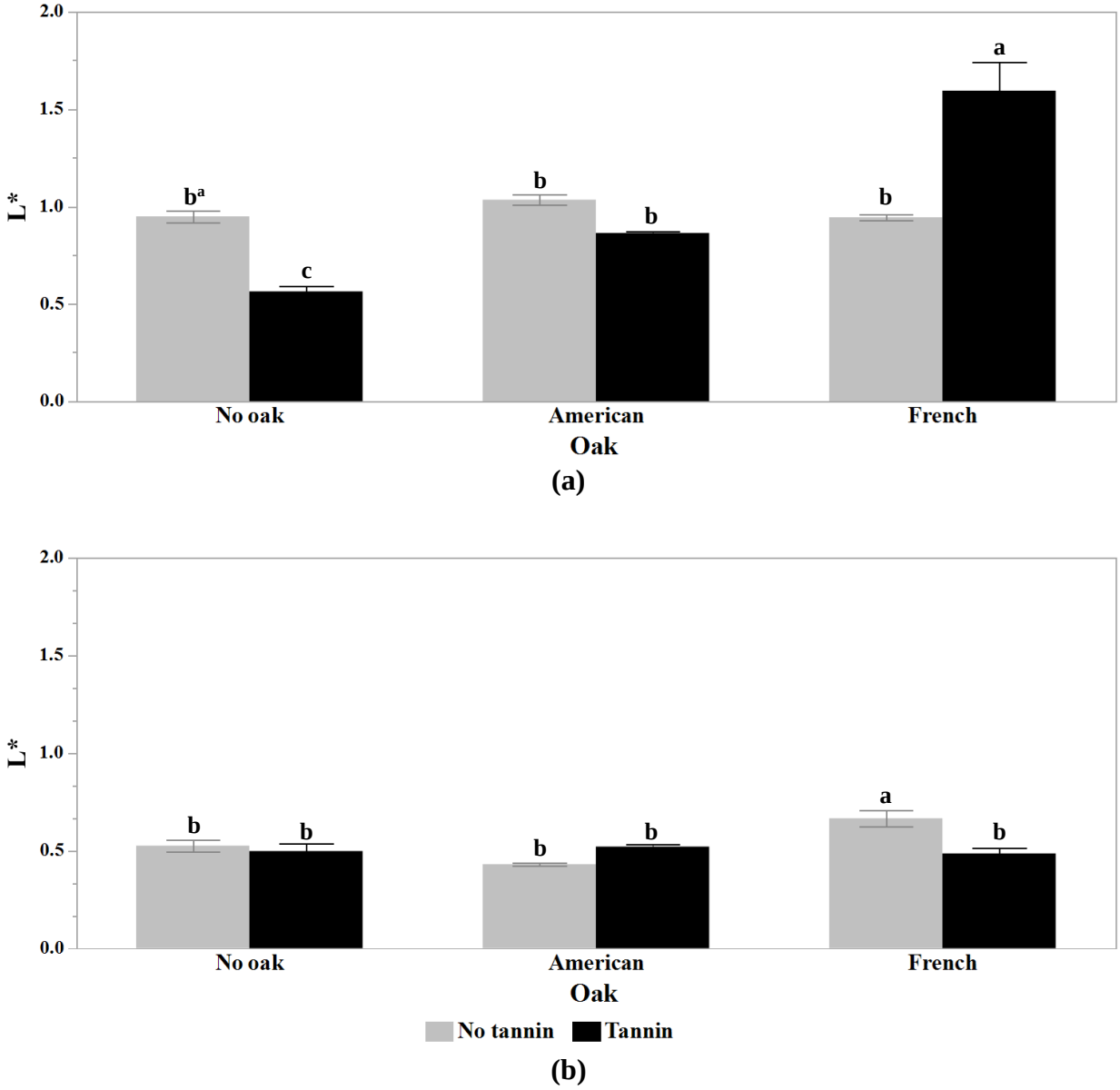
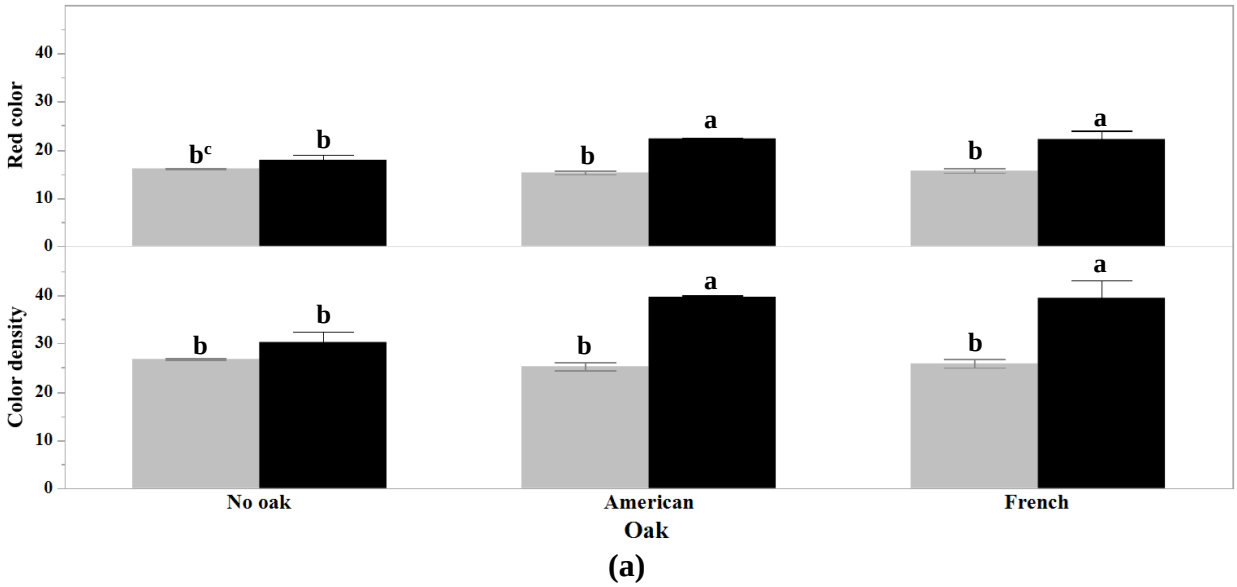
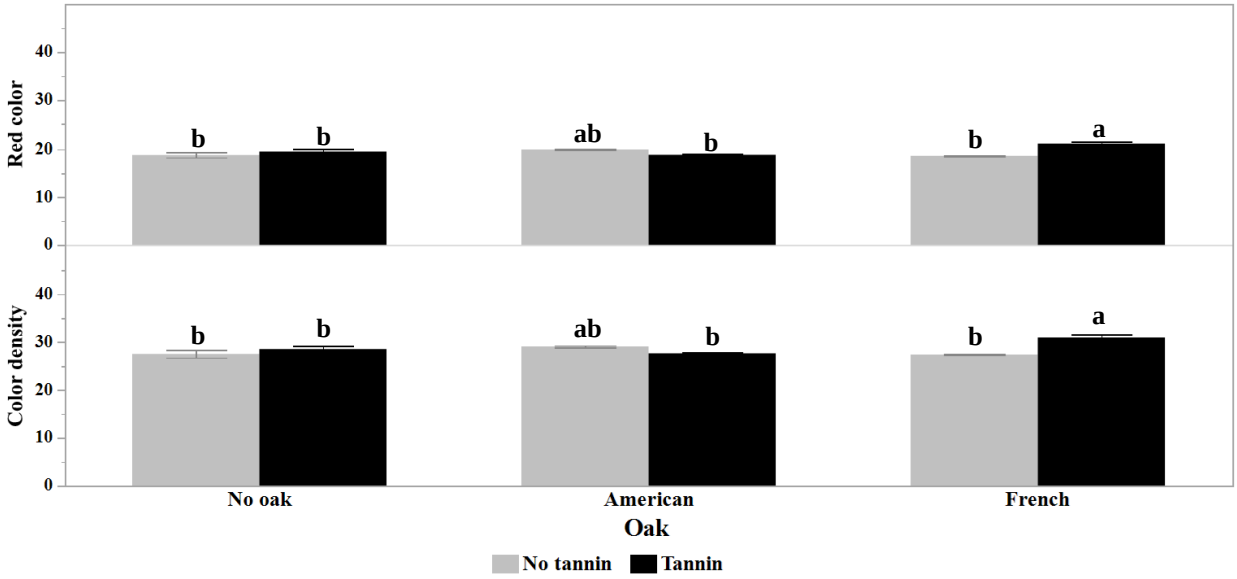


Figure 5. Effect of Tannin and Oak on L* at 0-months storage at 15°C of 2017 (a) and 2018 (b) wines produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station.

[a] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters for each year are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.



(a)



(b)

Figure 6. Effect of Tannin and Oak on red color[a] and color density[b] at 0-months storage at 15°C of 2017 (a) and 2018 (b) wines produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station.

[a] Red color was calculated as absorbance of wine at 520 nm.

[b] Color density was calculated as absorbance 520 nm + absorbance 420 nm.

[c] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters for each attribute and year are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

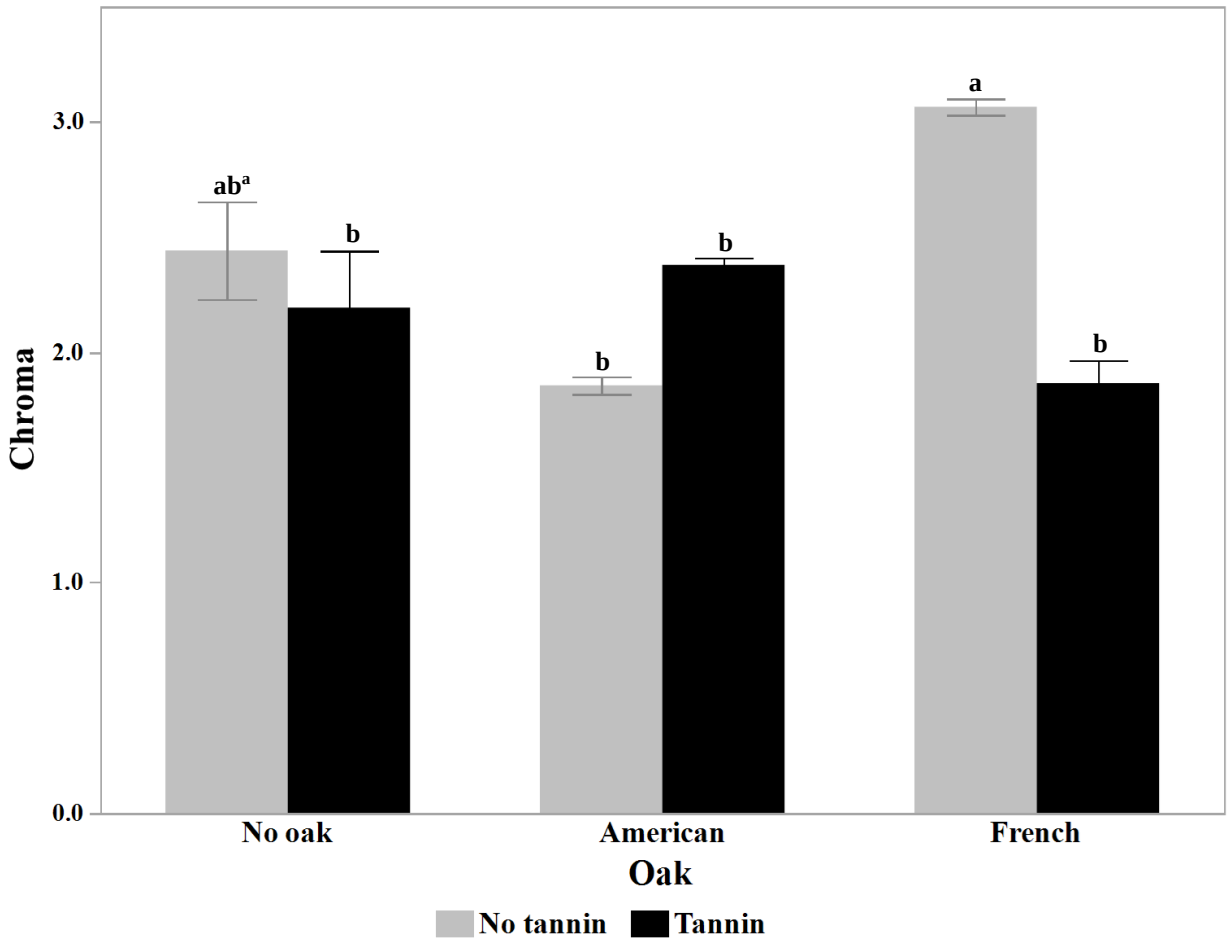


Figure 7. Effect of Tannin and Oak on chroma at 0-months storage at 15°C of wines produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (2018).
[a] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

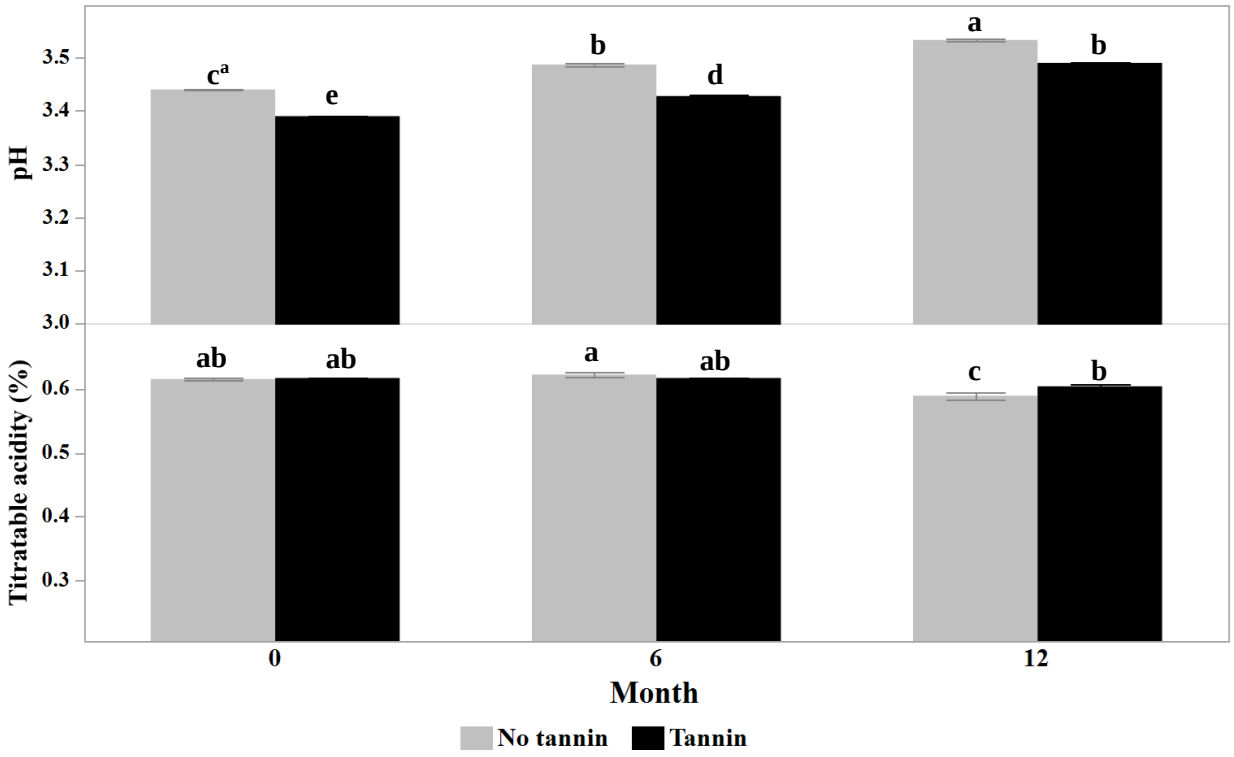


Figure 8. Effect of Tannin and Storage on pH and titratable acidity during storage (0, 6, and 12 months at 15°C) of 2017 wines produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (2017).
[a] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters for each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

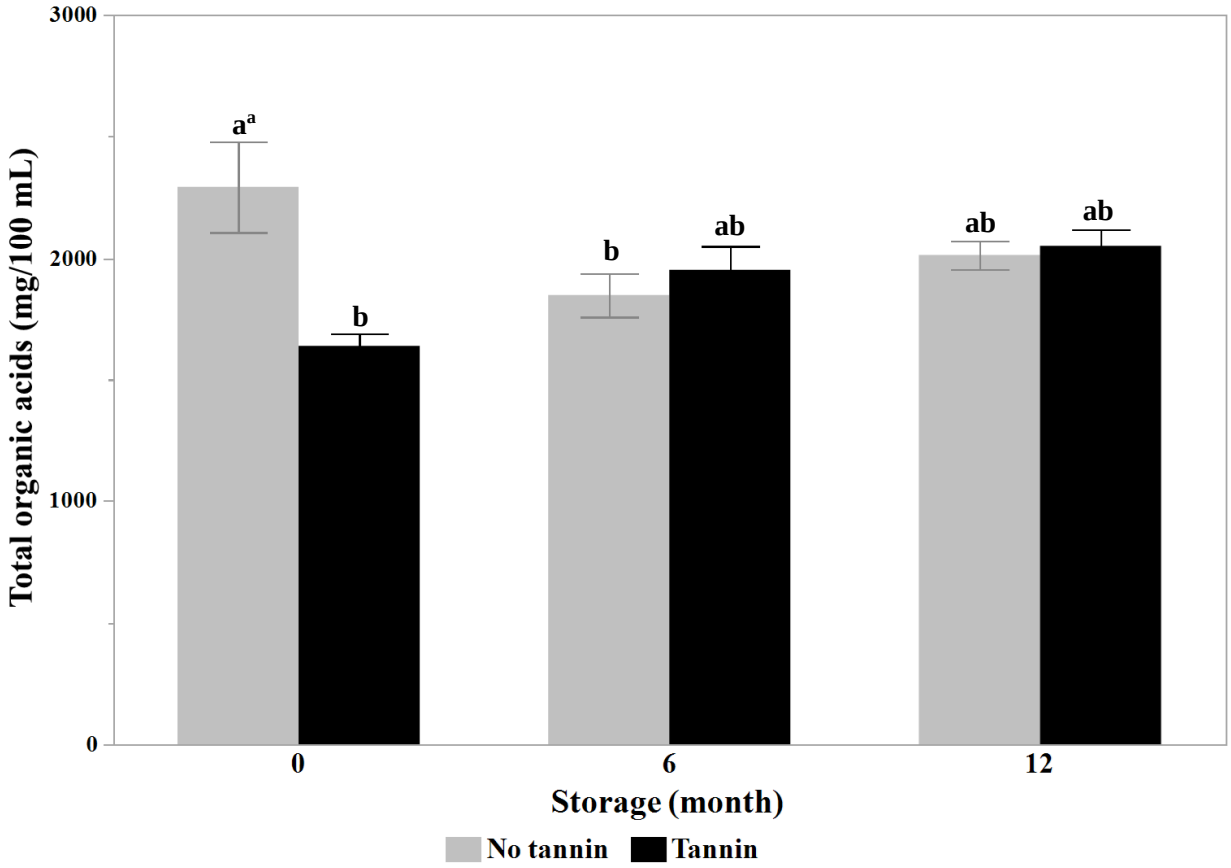


Figure 9. Effect of Tannin and Storage on total organic acids during storage (0, 6, and 12 months at 15°C) of wines produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (2017).
[a] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

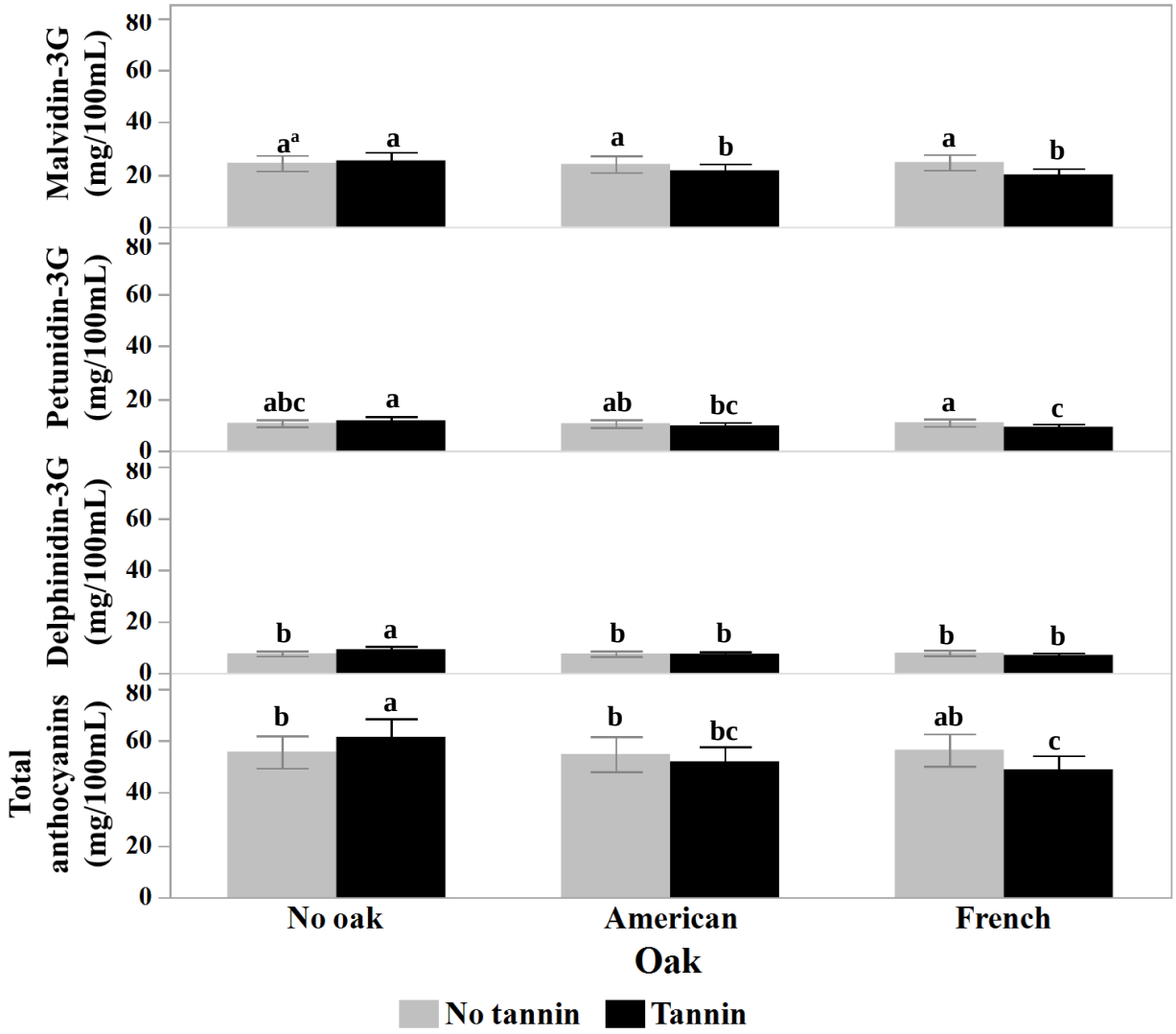


Figure 10. Effect of Tannin and Oak on malvidin-3-glucoside (malvidin-3G), petunidin-3-glucoside (petunidin-3G), delphinidin-3-glucoside (delphinidin-3G), and total anthocyanins during storage (0, 6, and 12 months at 15°C) of wines produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (2017).
[a] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters for each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

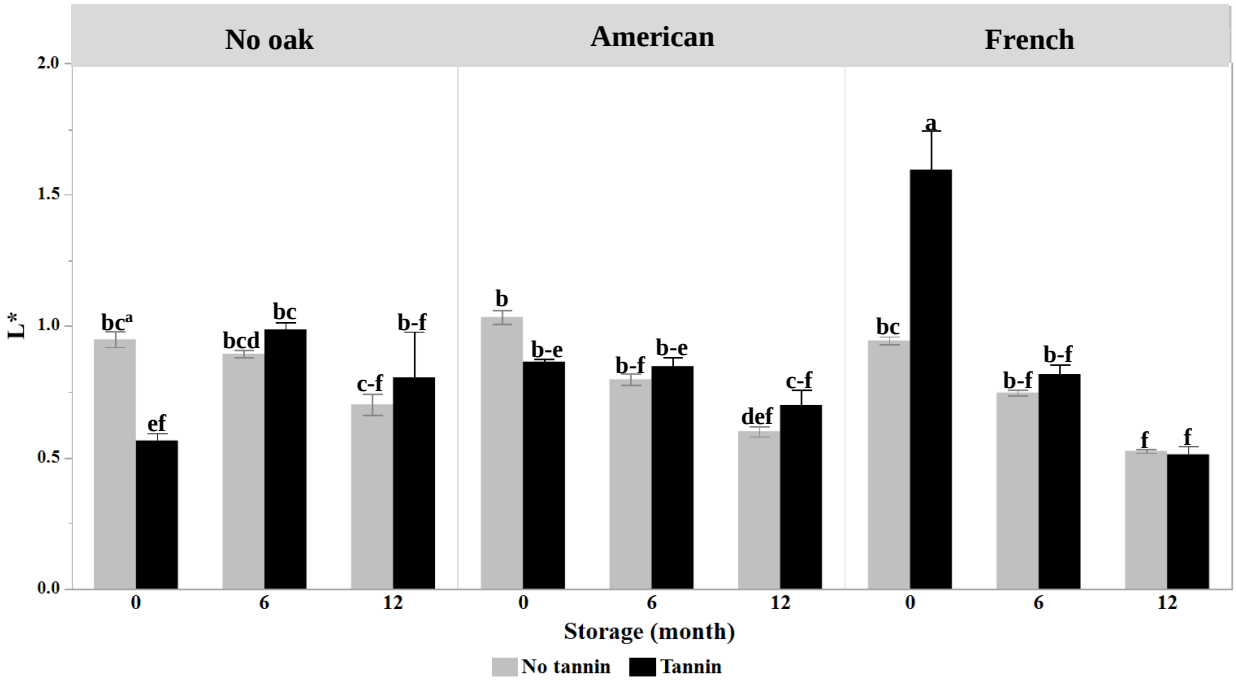


Figure 11. Effect of Tannin, Oak, and Storage on L* during storage (0, 6, and 12 months at 15°C) of wines produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (2017).
[a] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

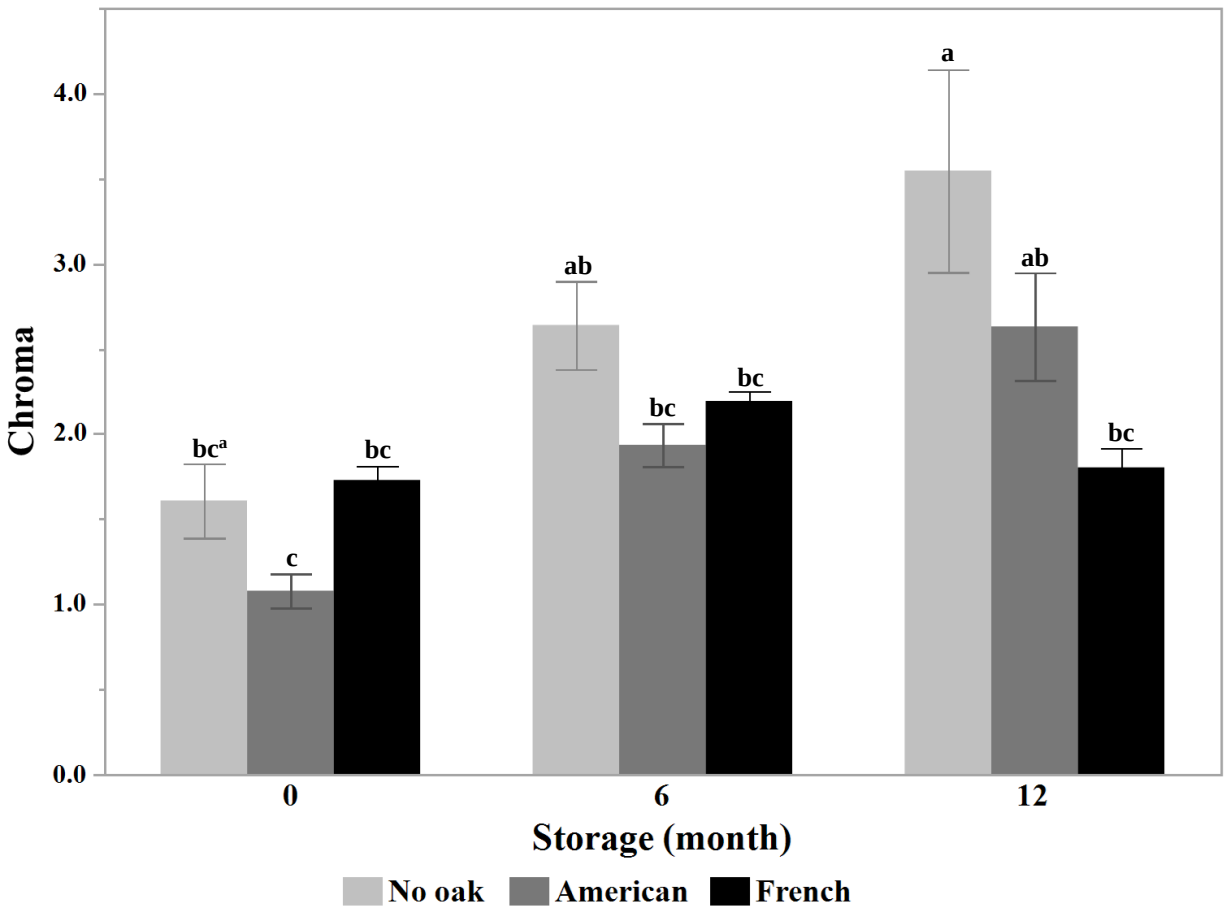


Figure 12. Effect of Oak and Storage on chroma during storage (0, 6, and 12 months at 15°C) of wines produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (2017).
[a] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

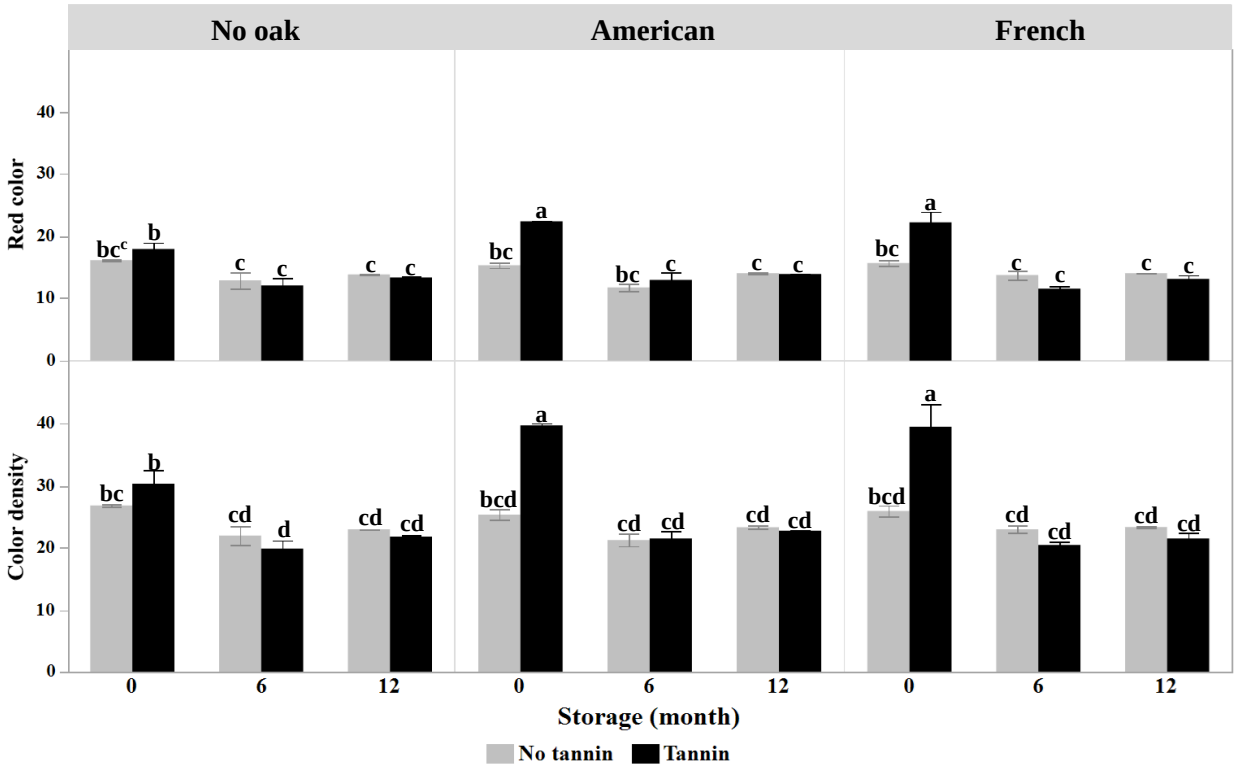


Figure 13. Effect of Tannin, Oak, and Storage on red color[a] and color density[b] during storage (0, 6, and 12 months at 15°C) of 2017 wines produced from Enchantment grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (Clarksville, AR).
[a] Red color was calculated as absorbance of wine at 520 nm.
[b] Color density was calculated as absorbance 520 nm + absorbance 420 nm.
[c] Each standard error bar was constructed using 1 standard error from the mean. Means with different letters for each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test.

CHAPTER V

Screening of University of Arkansas System Division of Agriculture grapes for white wine production

Abstract

Grapevines (*Vitis* spp.) are one of the most widely-planted horticultural crops worldwide, and *V. vinifera* is the most commercially-important species of wine grapes. However, *V. vinifera* grapevines are vulnerable to pests, diseases, and extreme temperatures and are difficult to grow in many regions of the United States, including Arkansas. Opportunity, A-2359, and A-2574 are *Vitis* hybrid white wine genotypes (cultivars and breeding selections) from the University of Arkansas System Division of Agriculture (UA System) wine grape breeding program with *V. vinifera* cultivars in their parentage. These genotypes have berries with unique, aromatic flavors and have shown potential for wine production, yielding wines with fruity, floral, and spicy characteristics. The objective of this study was to evaluate the composition, color, aroma, and sensory attributes of wines produced from the UA System white wine grape genotypes. Opportunity, A-2359, and A-2574 grapes were harvested in August-September 2015, 2017, and 2018 from the UA System Fruit Research Station (Clarksville, AR). Wines were produced at the UA System Department of Food Science in 2015, 2017, and 2018 and wines were bottled and stored at 15°C. Wines were analyzed at 3-years storage (2015 wines), 1-year storage (2017 wines), and 0-months storage (2018 wines) for composition, color, and aroma attributes and at 2-years storage (2015 wines) and 0-months storage (2017 wines) for sensory attributes. The pH (3.1-3.4), titratable acidity (0.5-0.8%), and other composition attributes of all wines were similar to typical ranges for dry white table wines, even after 3-years storage. Although 2015 wines at 3-

years storage had slightly higher brown color than 2017 or 2018 wines, the brown color of all wines was very low. Aroma compounds identified in wines included green/unripe, herbal, and spicy alcohols, floral and fruity esters, and floral, herbal, and spicy terpenes. The esters were the largest class of aroma compounds in all wines, and A-2359 wines contained a larger variety of terpene compounds than Opportunity or A-2574 wines. Younger wines were associated with higher overall aroma impacts, whereas more aged wines were associated with weaker aromas. A-2359 wines were more associated with floral, herbal, and spicy aroma compounds than other wines. The liking of wine appearance, aroma, flavor, sweetness, acidity, and overall impression were evaluated by an industry sensory panel (26 panelists). The sensory attribute ratings for all wines were generally positive. The aroma, flavor, and overall impression for 2017 A-2359 wines were rated higher than other wines. In general, panelists rated the aroma, flavor, and overall impression of 2017 wines more favorably than 2015 wines, indicating that panelists preferred the younger wines. The aroma/flavor of Opportunity wines was described as spicy, green apple, stone fruit, and citrus. The aroma/flavor of A-2359 wines was described as floral, grapefruit, stone fruit, and Muscat-like. The aroma/flavor of A-2574 wines was described as spicy, green apple, rose, and stone fruit. Therefore, the UA System white wine grape genotypes produced wines with stable composition and color and unique and pleasant aroma and flavor characteristics and could provide new opportunities for grape growers and wine makers in Arkansas and the mid-South United States.

Acknowledgements

We would like to thank the staff at the University of Arkansas System Division of Agriculture Fruit Research Station (Clarksville, AR) for their assistance with this project.

Introduction

Grapevines (*Vitis* spp.) are one of the most widely-planted horticultural crops in the world. In the United States, 95% of grape and wine production occurs in California, Washington, New York, Pennsylvania, and Oregon, but production is focused mostly on *V. vinifera*, which is the most popular species of grapevines (Creasy and Creasy 2009, OIV 2000, TTB 2015, USDA NASS 2019). *V. vinifera* grapevines are highly vulnerable to pests, diseases, and extreme temperatures and are difficult to grow in much of the United States, including Arkansas. The high cost of maintaining *V. vinifera* grapevines in non-ideal climates offsets the profit from producing these wines.

Hybrids (a cross of two or more *Vitis* species) and native species, such as *V. rotundifolia*, are better-adapted to surviving stressors that devastate *V. vinifera* grapes (Reisch et al. 2012). Despite the challenges, grape and wine production contribute significantly to the Arkansas economy. In 2010, the Arkansas grape and wine industry was responsible for 1,700 jobs and over $42 million in wages, and wine-related tourism generated $21 million in revenue (Frank 2010). Grapes grown in Arkansas include mostly native species and hybrids.

The University of Arkansas System Division of Agriculture (UA System) has a Fruit Breeding Program established in 1964 and located at the Fruit Research Station in Clarksville, AR. The program focuses on development of fruit cultivars for Arkansas production of fresh-market fruits and has released about 70 cultivars. This program has released many cultivars of blackberries, peaches and nectarines, table and juice grapes, and blueberries.

In addition, the Fruit Breeding Program began breeding wine grapes over 40 years ago, with a goal to develop new hybrid cultivars that grow well in Arkansas, have desirable flavor attributes, and are suitable for winemaking. The UA System Food Science Department has

worked collaboratively with the Fruit Breeding Program for decades to evaluate 100-150 wine grape genotypes for wine production, with about 20 of these genotypes extensively evaluated. In 2016, the first wine grape cultivars, Enchantment (red-wine cultivar) and Opportunity (white-wine cultivar), were released from the UA System. Two other white-wine advanced breeding selections, A-2359 and A-2574, are being evaluated for potential release and will be named if released. These genotypes (cultivars and advanced selections) are *Vitis* hybrids that show potential for regions that have limited productivity of wine grape cultivars.

Opportunity (formerly A-2245) was crossed in 1987 and selected (chosen from seedlings from the crosses that had potential) in 1991. The female parent of Opportunity is Cayuga White, a New York Seyval Blanc (*V. vinifera*) x Schuyler (*V. vinifera*, *V. labrusca*, and *V. rupestris*) hybrid. The male parent of Opportunity, A-1754, resulted from a cross of *V. vinifera* cultivars Semillon, a French wine grape, and Rkatsiteli, a wine grape from the Eastern European country of Georgia (Clark et al. 2018, Robinson et al. 2012).

A-2359 and A-2574 were selected in 1992 and 1995, respectively, and have hybrid and *V. vinifera* parents. A-2359 has Muscat characteristics (aromatic berries with floral and spicy aromas) and A-2574 has Gewürztraminer characteristics (pink-skinned berries with spicy, floral, and lychee aromas). Specific information about the parentage of these genotypes will be revealed if released and patented.

In vineyard evaluations at the UA System Fruit Research Station from 1998-2015, Opportunity vines had yields of 10.9 kg/vine, cluster weight of 234 g, and berry weight of 2.7 g, A-2359 vines had yields of 9.1 kg/vine, cluster weight of 171 g, and berry weight of 2.3 g, and A-2574 vines had yields of 8.2 kg/vine, cluster weight of 184 g, and berry weight of 2.1 g. All genotypes displayed good commercial yields for wine grapes in Arkansas, hardiness for growth

in the Arkansas climate, the potential to withstand typical disease pressures of the region, and good composition for wine production. Opportunity and A-2359 produce green-skinned grapes, while A-2574 produces pink-skinned grapes. The average harvest date for Opportunity was 30 August, for A-2359 was 15 August, and for A-2574 was 19 August. The composition of Arkansas white-wine genotypes at harvest was comparable to other white-wine cultivars grown in the mid-South United States. From 1994-2015, Opportunity grapes had 17.3% soluble solids (SS), 3.5 pH, and 0.5% titratable acidity (TA) at harvest, A-2359 had 18.6% SS, 3.4 pH, and 0.6% TA, and A-2574 had 20.2% SS, 3.3 pH, and 0.6% TA (Clark et al. 2018; Threlfall and Clark, unpublished data).

Small-scale wine production was done at the UA System Department of Food Science. All wines had commercially acceptable compositions at bottling with unique aromas and flavors. The aroma/flavor of Opportunity wine was described as spicy and Semillon-like with the bouquet of Cayuga White. A-2359 had aromas and flavors typical of Muscat varieties, and A-2574 had Gewürztraminer-like characteristics and showed potential for the production of late-harvest wines (Clark et al. 2018; Threlfall and Clark, unpublished data).

Although Opportunity, A-2359, and A-2574 grapes and wine have been preliminarily evaluated over the last 20 years, further exploration of winemaking potential and the unique flavors and aromas of these wines would be of interest. Given the potential that these wine grapes have shown for grape growers and wine makers in the mid-South United States, the objective of this study was to evaluate the composition, color, aroma, and sensory attributes of wines produced from the UA System white wine grape genotypes. The information presented in this chapter will be used to support the effort for potential release of the new genotypes and to

provide more data on Opportunity. Since the Fruit Breeding Program is no longer breeding wine grapes, these would be the last wine grapes released by the U of A System.

Materials and Methods

Grape harvest

Opportunity, A-2359, and A-2574 grapes were grown at the UA System Fruit Research Station in Clarksville, AR (USDA hardiness zone 7b). The soil type was Linker fine sandy loam (fine-loamy, siliceous, semi active, thermic Typic Hapludult). The grapes were grown on a high-wire bilateral cordon system on own-rooted, variable-age vines. The grapes were hand harvested in August-September 2015, 2017, and 2018 (Table 1). Harvest date was determined based on ideal composition attributes for white wine grapes, as well as past harvest data, weather, and quality of the fruit. Average daily temperature and rainfall for January-September 2015, 2017, and 2018 were recorded in Clarksville, AR (Figure 1). Approximately 26-72 kg of grapes were used for wine production. The grapes were taken to the UA System Food Science Department in Fayetteville, AR and stored at 4°C overnight for wine production the following day.

Wine production

Wines were produced from Opportunity, A-2359, and A-2574 grapes according to a traditional white-wine style. There was only one wine produced for each genotype and year in this study (no true replicates). Each batch of grapes was passed twice through a crusher/destemmer and 30 mg/L sulfur dioxide (SO_2) as potassium metabisulfite (KBMS) was added at crush. Musts (juice, skins, seeds, and pulp after crushing) were immediately pressed with a 70-L Enoagricola Rossi Hydropress (Calzolaro, Italy) using three 10-minute press cycles and a pressure of 207 kPa. The juices were collected into 22.7 L glass carboys, sealed with

rubber corks, and cold-settled overnight at 2°C to allow any sediment to settle to the bottom of the carboy. The juice was racked (wine removed from the sediment) the following day into a new carboy. The composition of the juice/wine was evaluated prior to, during, and at the end of fermentation, and adjustments were made to the juice/wine to ensure a complete fermentation. The free SO_2 levels of the juice/wine were evaluated and adjusted as needed. SS, pH, and TA of juice were evaluated prior to fermentation. The SS (expressed as %) of juice was determined using a Bausch & Lomb Abbe Mark II refractometer (Scientific Instruments, Keene, NH). The pH and TA were measured using a Metrohm 862 Compact Titrosampler (Metrohm AG, Herisau, Switzerland) fitted with a pH meter.

The initial compositions of the juices for 2015, 2017, and 2018 wine production are shown in Table 1. Soluble solid levels of the juices were adjusted to 20-22% using table sugar (sucrose), and the TA of the juices was adjusted to 0.8-0.9% to reduce the pH of the juice < 3.6 for fermentation. Juices were inoculated with Lalvin QA23® wine yeast (Lallemand, Inc., Montreal, Canada) at a rate of 0.26 g/L juice and carboys were fitted with fermentation locks filled with SO_2 solution to allow release of carbon dioxide and limit oxygen exposure. Wines were fermented at 15°C for approximately four months, and then held at 2°C for an additional four months for cold-stabilization. Wines were racked several times during fermentation. After fermentation completion, the free SO_2 content of wines was determined using the aeration-oxidation method (Iland et al. 1993) and adjusted to 60 mg/L.

Wines were bottled into 750-mL glass bottles, sealed with plastisol-lined screw caps, and stored at 15°C until analysis. The ethanol content of all wines was 11.7-14.3% (v/v) at bottling, measured by high performance liquid chromatography (HPLC) (Walker et al. 2003). The composition, color, and aroma attributes of the wines were evaluated in 2019. The 2015 wines

were analyzed at 3-years storage at 15°C, the 2017 wines were analyzed at 1-year storage, and the 2018 wines were analyzed at 0-months storage. The 2018 wines were stored at 15°C for one week prior to month-0 analysis. The sensory attributes of the 2015 and 2017 wines were evaluated in 2018. The 2015 wines were analyzed at 2-years storage and the 2017 wines were analyzed at 0-months storage. For analysis of composition, color, and aroma attributes, three 750-mL bottles were taken from each genotype and year for analysis. For analysis of sensory attributes, two 750-mL bottles from each genotype and year were combined.

Composition attributes analysis

The composition attributes analysis of the wines included pH, TA, glycerol, ethanol, residual sugars, and organic acids. Opportunity, A-2359, and A-2574 wines were analyzed at 3-years storage (2015 wines), 1-year storage (2017 wines), and 0-months storage (2018 wines) at 15°C for composition attributes. Analysis was done on each wine sample (genotype and year), and samples were measured in analytical duplicates.

pH. The pH of wines was measured using a Metrohm 862 Compact Titrosampler fitted with a pH meter. The probe was left in the samples for two minutes to equilibrate before recording the pH value. Wine was degassed prior to analysis.

Titratable acidity (TA). The TA of wines were expressed as % w/v (g/100 mL) tartaric acid and measured using a Metrohm 862 Compact Titrosampler. Six grams of sample was added to 50 mL degassed, deionized water and titrated with 0.1 N sodium hydroxide to an endpoint of pH 8.2. Wine was degassed prior to analysis.

Glycerol, ethanol, residual sugars, and organic acids. The glycerol, ethanol, residual sugars, and organic acids in wines were identified and quantified according to the HPLC procedure of Walker et al. (2003). Samples were passed through a 0.45 μm polytetrafluoroethylene (PTFE)

syringe filter (Varian, Inc., Palo Alto, CA) before injection onto an HPLC system consisting of a Waters 515 HPLC pump, a Waters 717 plus autosampler, and a Waters 410 differential refractometer detector connected in series with a Waters 996 photodiode array (PDA) detector (Waters Corporation, Milford, MA). Analytes were separated with a Bio-Rad HPLC Organic Acids Analysis Aminex HPX-87H ion exclusion column (300 x 7.8 mm) connected in series with a Bio-Rad HPLC column for fermentation monitoring (150 x 7.8 mm; Bio-Rad Laboratories, Hercules, CA). A Bio-Rad Micro-Guard Cation-H refill cartridge (30 x 4.5 mm) was used as a guard column. Columns were maintained at a temperature of 65 ± 0.1°C by a temperature control unit. The isocratic mobile phase consisted of pH 2.28 aqueous sulfuric acid at a flow rate of 0.45 mL/min. Injection volumes of both 10 μL (for analysis of organic acids and sugars) and 5 μL (for ethanol and glycerol) were used to avoid overloading the detector. The total run time per sample was 60 minutes.

Citric, tartaric, malic, lactic, and succinic acids were detected at 210 nm by the PDA detector, and glucose, fructose, ethanol, and glycerol were detected at 410 nm by the differential refractometer detector. Analytes in samples were identified and quantified using external calibration curves based on peak area estimation with baseline integration. Results were expressed as milligrams analyte per 100 mL wine for organic acids and residual sugars, grams per liter wine for glycerol, and % v/v (alcohol by volume, ABV) for ethanol. Total residual sugars was calculated as the sum of glucose and fructose. Total organic acids was calculated as the sum of citric, tartaric, malic, lactic, and succinic acids.

Color attributes analysis

The color attributes analysis of the wines included brown color. Opportunity, A-2359, and A-2574 wines were analyzed at 3-years storage (2015 wines), 1-year storage (2017 wines),

and 0-months storage (2018 wines) at 15°C for color attributes. Analysis was done on each wine sample (genotype and year), and samples were measured in analytical duplicates.

Brown color. Brown color of wines was measured spectrophotometrically as absorbance at 420 nm (Iland et al. 1993). Absorbance values were measured using a Hewlett-Packard 8452A Diode Array spectrophotometer equipped with UV-Visible ChemStation software (Agilent Technologies, Inc., Santa Clara, CA). Samples were measured against a blank sample of deionized water and a 1-cm cell was used for all spectrophotometer measurements.

Aroma attributes analysis

The volatile aroma profiles analysis of Opportunity, A-2359, and A-2574 wines was conducted at Graz University of Technology (Graz, Austria) Institute of Analytical Chemistry and Food Chemistry. Wines were packaged in 20-mL clear glass vials, sealed with a polypropylene cap with a polytetrafluoroethylene-line silicon septum, wrapped with Parafilm® flexible film (Bemis Company, Inc., Neenah, WI), and shipped to Graz University of Technology for analysis. Wines were analyzed at 3-years storage (2015 wines), 1-year storage (2017 wines), and 0-months storage (2018 wines) at 15°C for aroma attributes. Analysis was done on each wine sample (genotype and year), and samples were measured in analytical triplicates.

Determination of volatile aroma profiles. To identify the volatile aroma compounds in wines, volatile compounds were extracted from 1 mL of wine in a 10-mL glass vial using solid-phase microextraction (SPME) with a 2-cm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber for 30 minutes at 40°C. A gas chromatography-mass spectrometry (GC-MS) system equipped with a Shimadzu GC 2010 (Shimadzu Corporation, Kyoto, Japan), Shimadzu QP 2010 MS, and a PAL HTX autosampler (CTC Analytics AG, Zwingen,

Switzerland) was used to separate and identify volatile compounds. Samples were extracted/injected in analytical triplicate. Volatiles were separated on a nonpolar Restek Rxi 5MS column (30 m x 0.25 mm x 1 μm; Restek, Bellefonte, PA) 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 were recorded in the scan mode (*m/z* 35-350) with a 9.8 minute solvent cut time and a detector voltage relative to the tuning result.

Data was analyzed using the Shimadzu GCMS Postrun Analysis software. Compounds were identified using comparison of mass spectra with NIST14 (National Institute of Standards and Technology, Gaithersburg, MD), Flavors and Fragrances of Natural and Synthetic Compounds (FFNSC3, John Wiley & Sons, Inc., Hoboken, NJ), and Adam's Essential Oils (Adams 2007) mass spectral libraries and comparison of calculated Kovats retention indices (Kováts 1958) with values reported in the Flavornet (Acree and Arn 2004) and Pherobase (Sayed 2003) databases. A matching library result and a retention index 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.

Sensory attributes evaluation

An industry sensory panel for 2015 and 2017 wines was conducted at the UA System Department of Food Science during a seminar in May 2018 for grape growers and wine makers in the mid-South United States. The sensory attributes evaluation included liking of wine appearance, aroma, flavor, sweetness, and overall impression. Opportunity, A-2359, and A-2574 wines were evaluated at 2-years storage (2015 wines) and 0-months storage (2017 wines) at 15°C for sensory attributes. For sensory evaluation, two bottles of each wine were combined.

Industry sensory panel. The industry sensory panel was conducted at the UA System Department of Food Science (Institutional Review Board protocol # 05-11-193; Figure 2). In total, 26 panelists evaluated wines for liking of wine appearance, aroma, flavor, sweetness, acidity, and overall impression, and provided comments on wine appearance, aroma, flavor, and overall impression. Each panelist evaluated approximately 30-mL of wine, and each wine was evaluated one time. The wines were served monadically (one at a time) at 15°C in wine glasses, and all panelists evaluated wines in the same order. Panelists were instructed to cleanse their palates with water between samples. Expectorant cups were provided. The panelists used a nine-point hedonic scale (1 = dislike extremely; 9 = like extremely) to indicate their liking of wine appearance, aroma, flavor, sweetness, acidity, and overall impression. After evaluating each attribute, panelists were instructed to provide comments about wine appearance, aroma, flavor, and overall impression. An example of a ballot presented to industry sensory panelists is shown in Figure 3.

Design and statistical analysis

After about four months of fermentation and cold-stabilization, Opportunity, A-2359, and A-2574 wines were bottled in May 2016 (2015 wines), 2018 (2017 wines), and 2019 (2018 wines) and stored at 15°C. The composition, color, and aroma attributes of the wines were evaluated in 2019. The 2015 wines were analyzed at 3-years storage at 15°C, the 2017 wines were analyzed at 1-year storage at 15°C, and the 2018 wines were analyzed at 0-months storage at 15°C. The 2018 wines were stored at 15°C for one week prior to month 0 analysis. The sensory attributes of the 2015 and 2017 wines were evaluated in 2018. The 2015 wines were evaluated at 2-years storage at 15°C and the 2017 wines were evaluated at 0-months storage at 15°C. For analysis of composition, color, and aroma attributes, three 750-mL bottles were taken

from each genotype and year and treated as individual experimental units (replicates). For evaluation of sensory attributes, two 750-mL bottles from each genotype and year were combined. Statistical analyses were conducted using JMP® Pro statistical software (version 15.0.0, SAS Institute, Cary, NC). Additional information of the statistical analyses is provided below.

Composition and color attributes. For wines at 3-years storage (2015 wines), 1-year storage (2017 wines), and 0-months storage (2018 wines), means were obtained for each attribute within each year and genotype. As this was a screening study and there were no true replicates, further statistical analysis was not conducted. Figures were created in JMP®.

Aroma attributes. Peak areas (TIC) for each positively identified compound in wines at 3-years storage (2015 wines), 1-year storage (2017 wines), and 0-months storage (2018 wines) were used as a semi-quantitative measure for multivariate statistics. Each compound was assigned a general aroma group based on aroma descriptors reported in the Flavornet (Acree and Arn 2004) and Pherobase (Sayed 2003) databases. The areas of compounds within each group were summed to create general "aroma category" variables. This was done so that the model did not overfit to noise, which occurs when the number of parameters is greater than the number of variables. A principal components analysis (PCA), based on the aroma categories, was used to explore the relationship between genotypes and years and volatile aroma profiles.

Sensory attributes. For the industry sensory evaluation at 2-years storage (2015 wines) and 0-months storage (2017 wines), nine-point hedonic scales were 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). Means were obtained for each attribute within each year and genotype. As this was a

screening study, further statistical analysis was not conducted. Figures were created in Microsoft Excel® (version 16, Microsoft Corporation, Redmond, WA).

Results and Discussion

The 2015, 2017, and 2018 wine grape production seasons at the Fruit Research Station were relatively mild in terms of temperature and rain (Figure 1). Due to scheduling conflicts, 2016 wines were produced by a commercial winemaker, but the data was not obtained. The high and low temperatures in all years were similar from January-September. Rainfall varied among the years from April (bud emergence) to harvest in August-September. In all years, grapes were harvested early- to mid-August, with the exception of 2015 Opportunity grapes, which were harvested on 11 September (Table 1). In August of 2015, 2017, and 2018, the average daily high temperature was 30.2°C, 28.6°C, and 30.0°C, respectively. In September of 2015, the average daily high temperature was 28.9°C. In August of 2015, 2017, and 2018, there was 127.3 mm, 198.4 mm, and 281.9 mm, respectively, of cumulative monthly rainfall. In September of 2015, there was 47.2 mm of cumulative monthly rainfall.

The composition of wine grapes at harvest varied among genotypes and years (Table 1). In general, grapes had low SS typical of Arkansas-grown wine grapes (Morris et al. 1984). Parameters of 19.5-23.0% SS, <3.4 pH, and >0.70% TA have been established as ideal for California white wine grapes (Amerine et al. 1979). However, wine grapes from warm climate regions tend to have low sugar levels, which can impact wine quality (Coombe et al. 1980, Fanizza 1982). With the exception of 2018 A-2574 grapes (20.5% SS), sugar additions were needed to increase the SS to 20-22% prior to fermentation. Tartaric acid addition was needed in some instances to decrease the pH <3.6 for wine production.

The UA System white wine grape genotypes and advanced selections displayed SS, pH, and TA values similar to those found by others for *V. vinifera* and hybrid grapes grown in the region. Morris et al. (1984) and Striegler and Morris (1984) reported 14.9-23.4% SS, 3.36-4.32 pH, and 0.35-0.98% TA for various *V. vinifera* and hybrid white wine grape cultivars in Arkansas. It was of note that A-2574 grapes consistently had the highest SS among years and acceptable acid levels. Morris et al. (1987) determined that Gewürztraminer grapes have unacceptable compositions under Arkansas growing conditions. Therefore, A-2574, which has Gewürztraminer characteristics, shows potential as an alternative for the Arkansas and mid-South grape and wine industry.

After about four months of fermentation at 15°C and four months of cold-stabilization at 2°C, Opportunity, A-2359, and A-2574 wines were bottled in May 2016 (2015 wines), 2018 (2017 wines), and 2019 (2018 wines) and stored at 15°C. The composition, color, and aroma attributes of wines were evaluated at 3-years (2015 wines), 1-year (2017 wines), and 0-months (2018 wines) storage at 15°C. The sensory attributes of wines were evaluated at 2-years (2015 wines) and 0-months (2017 wines) storage at 15°C.

Analysis of composition attributes (2015, 2017, and 2018)

At bottling in 2015, 2017, and 2018, Opportunity, A-2359, and A-2574 wines had acceptable compositions with pH and TAs within the typical ranges of 3.1-3.5 pH and 0.5-1.0% TA for a dry white table wine (Waterhouse et al. 2016).Opportunity wines had 3.11-3.52 pH and 0.64-0.72% TA, A-2359 wines had 3.26-3.44 pH and 0.57-0.90% TA, and A-2574 wines had 3.26-3.46 pH and 0.58-0.92% TA across all years (Table 1).

Opportunity, A-2359, and A-2574 wines were analyzed at 3-years storage (2015 wines), 1-year storage (2017 wines), and 0-months storage (2018 wines) at 15°C for pH, TA, glycerol,

ethanol, fructose, total residual sugars, tartaric acid, malic acid, citric acid, succinic acid, lactic acid, and total organic acids. The pH and TA values of all wines were within typical ranges of 3.0-3.5 pH and 0.5-0.8% TA for dry white table wines (Table 2). Even after 3-years storage, 2015 Opportunity, A-2359, and A-2574 wines maintained acceptable pH (3.19, 3.09, and 3.09, respectively) and TA (0.53, 0.73, and 0.78%, respectively). Main et al. (2002) reported 3.3-3.8 pH and 0.7-0.8% TA for Arkansas Chardonel (interspecific hybrid white wine grape) wines, and the numbers in the present study were consistent with this finding.

The glycerol contents of all wines (7.27-11.91 g/L) were similar to the typical range of 7-10 g/L for a dry white table wine. The slightly elevated glycerol concentrations of 2017 (10.51 g/L) and 2018 (10.78 g/L) A-2574 wines were consistent with its potential as a late-harvest wine, as botrytized late-harvest wines tend to have higher glycerol concentrations. The detection threshold of glycerol in wine is 5.2-7.5 g/L, and concentrations above this give wine a slight sweetness and body (Liu and Davis 1994, Sarrazin et al. 2007). In general, the ethanol content of all wines (11.71-14.31% v/v) was similar to the typical range of 9-13% (v/v) for dry table wines. Exceptions to this were 2017 A-2359 (13.77%) and A-2574 (14.31%) wines. Ethanol contents of 13-15% are not uncommon in dry table wines, although such wines may have a more perceptible alcoholic pungency (Liu and Davis 1994, Waterhouse et al. 2016).

Fructose concentrations (38.91-414.10 mg/100 mL) were at least nine-times greater than glucose concentrations (0.00-8.46 mg/100 mL) in all wines. This was because yeast preferentially ferment glucose, decreasing its concentration throughout fermentation (Waterhouse et al. 2016). Although glucose levels of all wines were lower than the typical range of 50-100 mg/100 mL for dry table wines, fructose levels were similar to the typical range of 20-400 mg/100 mL for dry table wines (Liu and Davis 1994). The detection threshold of fructose in

wine is 180-240 mg/100 mL (Hufnagel and Hofmann 2008, Noble and Bursick 1984). The only wine in the present study that had a fructose concentration above the detection threshold was 2015 Opportunity wine (414.10 mg/100 mL), and therefore this wine could have had a perceptible sweetness. Total residual sugar levels of all wines were similar (38.91-91.71 mg/100 mL), with the exception of 2015 Opportunity wine (414.10 mg/100 mL).

All wines in the present study had tartaric acid concentrations (27.74-106.86 mg/100 mL) below the typical range of 200-600 mg/100 mL for dry table wines. Low acid levels are characteristic of wine grapes from warm climate regions (Coombe et al. 1980, Fanizza 1982). However, pH and TA, which are the most important measures of acidity for determining wine stability, were within acceptable ranges. The typical malic acid concentration for dry table wines is 200-700 mg/100 mL. While 2015 wines (234.49-335.28 mg/100 mL) had malic acid concentrations within this range, 2017 and 2018 wines (62.73-111.31 mg/100 mL) were below the typical range for dry white table wines. Unlike tartaric and malic acids, citric (135.70-429.51 mg/100 mL) and succinic (75.21-658.74 mg/100 mL) acid levels were higher than the typical ranges of 10-70 mg/100 mL citric acid and 50-100 mg/100 mL succinic acid for dry table wines. Citric acid is synthesized in grape berries during ripening and can be produced during fermentation. Like tartaric, malic, and lactic acids, citric acid gives wines sourness and astringency. Succinic acid is the primary acid produced during alcoholic fermentation and gives wines bitterness and sourness. The lactic acid concentration of all wines (6.47-76.31 mg/100 mL) was within the typical range of 0-300 mg/100 mL for dry table wines (Da Conceicao Neta et al. 2007, Fowles 1992, Sowalsky and Noble 1998). Total organic acid levels were 498.23-1,350.65 mg/100 mL across all genotypes and years. A-2574 wines (1,080.66-1,350.65 mg/100 mL) had

higher total organic acid concentrations than Opportunity (498.23-847.39 mg/100 mL) and A-2359 (532.94-666.94 mg/100 mL) wines across years.

Analysis of color attributes (2015, 2017, and 2018)

Opportunity, A-2359, and A-2574 wines were analyzed at 3-years storage (2015 wines), 1-year storage (2017 wines), and 0-months storage (2018 wines) at 15°C for brown color. The brown color measured in white wines in the present study (0.04-0.11) was similar to the range of 0.09-0.11 reported by Sims et al. (1990) for a Florida hybrid white wine immediately after fermentation (Figure 4). Even the 2015 wines, which were analyzed at 3-years storage, had brown colors of only 0.08-0.11. Browning can occur during storage of white wines due to oxidation of phenolic compounds. This process occurs slowly at wine pH but is accelerated by increases in pH, the presence of metals such as iron or copper, and oxygen exposure (Fernandez-Zurbano et al. 1995, Oszmianski et al. 1996, Simpson 1982). In general, the older (2015) wines had higher brown color.

Within each year, A-2574 wines had a slightly higher brown color than Opportunity or A-2359 wines. This was likely because A-2574 is a pink-skinned grape and thus produces wines with more color than the pale-yellow color of typical white wines. Within each genotype, the brown color of 2015 wines at 3-years storage was higher than that of the 2018 wines at 0-months storage. However, the brown color of all wines was very low. In combination with the stability of wine composition attributes during storage, the preservation of wine color quality indicated that Opportunity, A-2359, and A-2574 wines had potential for maintaining quality up to three years of bottle storage.

Analysis of aroma attributes (2015, 2017, and 2018)

Opportunity, A-2359, and A-2574 wines were analyzed at 3-years storage (2015 wines), 1-year storage (2017 wines), and 0-months storage (2018 wines) at 15°C for volatile aroma compound profiles. Across all years, there were 66 volatile aroma compounds positively identified in Opportunity wines, 82 volatile aroma compounds identified in A-2359 wines, and 66 volatile aroma compounds identified in A-2574 wines. Table 3 shows the compounds identified in wines, their compound class, the aroma category each was grouped into, more detailed aroma descriptors, and whether or not the compound was identified in wines within each genotype and year. Compounds included chemical, floral, green/fat (waxy, oily, rancid), and herbal/spicy alcohols, green/fat and roasted/caramelized alcohols, green/fat alkanes, chemical benzothiazoles, green/fat and unpleasant carboxylic acids, floral, fruity, green/fat, and herbal/spicy esters, chemical and herbal/spicy ethers, fruity glycols, fruity, green/fat, and vegetal ketones, herbal/spicy oxanes, floral pyrans, and chemical, floral, and herbal/spicy terpenes. The esters were the largest class of compounds in all wines. Esters are characteristic byproducts of alcoholic fermentation and are critical for the aroma of most wines (Waterhouse et al. 2016).

A-2359 wines contained a larger variety of terpenes than Opportunity or A-2574 wines. Terpenes are important for the aroma of aromatic wines such as Muscat (Macaulay and Morris 1993, Park et al. 1991), Riesling (Reynolds et al. 1996b), and Gewürztraminer (Ong and Acree 1999, Reynolds et al. 1996a) and give wines characteristic floral, herbal, and spicy aromas. In preliminary evaluations, A-2359 grapes and wine were found to have Muscat-like floral aromas. Terpenes identified in 2015, 2017, and 2018 A-2359 wines included *cis*-linalool oxide (floral aroma), D-limonene (citrus aroma), and α-terpineol (anise, mint aroma). Linalool (floral, lavender, Earl Grey tea aroma) is a characteristic odor compound of Muscat wines, and it was

identified in 2017 and 2018 A-2359 wines. In general, terpene aromas decrease during storage as monoterpene alcohols, such as linalool, are oxidized to terpene oxides, like *cis*-linalool oxide. Terpene oxides have higher odor detection thresholds than terpene alcohols (Rapp and Mandery 1986, Simpson 1979).

PCA was used to reduce dimensionality of the data and to elucidate relationships between aroma categories and genotypes and years. Examining the PCA results, distinctions could be made among genotypes and years. Three components explained over 85% of the variation in the data (Table 4). PC1 (37.4%) had positive loadings for green/fat, fruity, floral, unpleasant, and herbal/spicy aroma categories. Therefore, it was determined that PC1 represented high amounts of aroma compounds in general and was correlated with overall aroma impact. Opportunity 2017 and 2018 wines, A-2359 2015 and 2018 wines, and A-2574 2018 wine had positive loadings on PC1, whereas Opportunity 2015, A-2359 2017, and A-2574 2015 and 2017 wines had negative loadings on PC1. With the exception of the A-2359 2015, the wines that loaded positively on PC1 were the younger 2017 and 2018 wines, whereas the wines that loaded negatively were the more aged 2015 and 2017 wines. Therefore, younger wines were associated with a higher overall aroma impact, and more aged wines were associated with weaker aromas. Chisholm et al. (1995) saw a decrease in the fruity aroma of Vidal blanc (white hybrid grape) wine during storage. Wines from white hybrid grapes are typically consumed 1-2 years after bottling, when their fruity and floral aromas reach a peak. Muscat-type wines in particular do not improve with bottle aging, as their characteristic terpene aromas can dissipate over time (Chisholm et al. 1995).

PC2 (29.0%) had positive loadings for roasted/caramelized, vegetal, and chemical aroma categories, and A-2359 2015 and A-2574 2017 wines. Opportunity 2017, Opportunity 2018, A-2359 2018, and A-2574 2018 wines had negative loadings on PC2. Therefore, A-2359 2015 and

A-2574 2017 wines could be associated with higher amounts of roasted/caramelized, vegetal, and chemical aromas. PC3 (23.1%) had positive loadings for Chemical and unpleasant aroma categories and Opportunity 2017 and 2018 wines and all A-2574 wines. PC3 had negative loadings for herbal/spicy and floral aroma categories and all A-2359 wines. Therefore, A-2359 wines were more associated with floral, herbal, and spicy aromas than Opportunity or A-2574 wines. This was consistent with the larger variety of terpene compounds seen in A-2359 wines, and the Muscat-character perceived in preliminary sensory evaluations.

Evaluation of sensory attributes (2015 and 2017)

Opportunity, A-2359, and A-2574 wines were evaluated at 2-years storage (2015 wines) and 0-months storage (2017 wines) for sensory attributes by an industry sensory panel. Twenty-six panelists from the mid-South United States grape/wine industry evaluated wines during a May 2018 seminar for liking of wine appearance, aroma, flavor, sweetness, acidity, and overall impression using a nine-point hedonic scale (1 = dislike extremely; 9 = like extremely). After evaluating each attribute, panelists were instructed to provide comments about wine appearance, aroma, flavor, and overall impression. On average, the appearance, aroma, flavor, and overall impression of the wines were scored “like moderately” and the sweetness and acidity of the wines were scored “like slightly” (Figure 5). This indicated an overall positive reaction to Opportunity, A-2359, and A-2574 sensory attributes.

The appearance liking ratings of all wines were similar, and appearance was rated “like moderately” on average. This was consistent with the low brown color seen in all wines. The appearance of Opportunity wines was described as slightly yellow, pale melon, clear, brilliant, very light, straw-colored, and pale (Table 5). The appearance of A-2359 wines was described as clean, clear, bright, golden, light, and slightly green. The appearance of A-2574 wines was

described as golden and clear. The golden color of wines from pink-skinned A-2574 grapes was likely related to the higher brown color ratings.

A-2359 wines from 2017 had higher aroma liking scores than the other wines. The 2017 A-2359 wine aroma was scored “like very much”, the aroma of 2015 A-2574 wine was scored “like slightly”, and the aroma of all other wines was scored “like moderately”. It is possible that the higher aroma liking ratings for 2017 A-2359 wine were due to the higher amounts of floral, herbal, and spicy aroma compounds as indicated by PCA. When comparing the aroma descriptors used for 2015 and 2017 wines, fewer descriptors were used for 2015 wines than 2017 wines. In general, 2017 wines were described as fruitier, more floral, and overall more pleasing than 2015 wines. The fruity and floral aromas of white wines can dissipate during storage (Chisholm et al. 1995). This could explain the difference in aroma descriptors between the 2015 wines, which were analyzed at 2-years storage, and 2017 wines, which were analyzed at 0-months storage. The aroma of 2015 Opportunity wines was described as soft, delicate, and spicy, and the aroma of 2017 Opportunity wines was described as pleasant, slightly floral, fruity, Muscat, apple, peach, citrus, spicy, grassy, stone fruit, and guava. The aroma descriptors used for Opportunity wines in the present study were similar to those found by Schmidtke et al. (2013) and Siebert et al. (2018), who reported stone fruit, grassy, citrus, honey, slightly floral, and hay/straw aromas in Semillon wine (a wine grape in the parentage of Opportunity). The aroma of 2015 A-2359 wines was described as faint, citrus, Riesling-like, and light. This indicated that the characteristic Muscat character of A-2359 grapes/wine was not present in the 2015 wine. The aroma of 2017 A-2359 wines was described as floral, beautiful, very pleasant, citrus, peach, Muscat-like, and honeysuckle. These aroma descriptors were closer to the characteristic floral, fruity, herbal, and spicy aromas of Muscat wines. The aroma of 2015 A-2574 wines was

described as soft, pleasant, spicy, and faint, and the aroma of 2017 A-2574 wines was described as fruity, green apple, bell pepper, soft, and hay/straw. These descriptors were consistent with the apple, pear, apricot, grapefruit, spice, rose, and floral aromas found in Traminette (a hybrid with Gewürztraminer parentage) by Skinkis et al. (2010).

Similar to aroma ratings, the flavor of 2017 A-2359 wines was rated the highest ("like moderately") and the flavor of 2015 A-2574 wines was rated the lowest ("like slightly"). Also similar to the aroma evaluations, there were fewer descriptors used for the flavor of 2015 wines compared to 2017 wines. The flavor of 2015 Opportunity wines was described as pleasant and light, and the flavor of 2017 Opportunity wines was described as crisp, citrus, tree fruit, peach, clean, green apple, good mouthfeel, long finish, green, citrus pith, and refreshing. The flavor of 2015 A-2359 wines was described as refreshing, grapefruit, pleasant, and gentle, and the flavor of 2017 A-2359 wines was described as pleasant, confident, fruity, peach, stone fruit, clean, and good mouthfeel. The flavor of 2015 A-2574 wines was described as very pleasant, citrus, fruit, and clean, and the flavor of 2017 A-2574 wines was described as stone fruit, peach, clean, crisp, good finish, and nice tannins. These results suggest that both the retronasal aromatic quality and the mouthfeel/finish of the 2017 wines were preferable to the 2015 wines.

There were not obvious differences among the wines for sweetness and acidity liking. Wines were scored "like slightly" to "like moderately" for these attributes. The A-2359 wines from 2015 and 2017 had slightly higher sweetness and acidity liking ratings than other wines. This could have been due to higher amounts of fruity/floral aroma compounds in these wines that masked the acidity and made wines taste sweeter (Lawless and Heymann 2010). In general, wines were fairly acidic and very dry (low residual sugars). To reduce the perceived acidity of wines, winemakers could commercially finish wines by adding small amounts of sugar prior to

bottling to balance sourness and mouthfeel. However, wines in the present study were not commercially finished.

The overall impression liking of 2017 A-2359 wines was scored “like very much”, whereas the other wines were scored “like slightly” to “like moderately”. The better overall impression of 2017 A-2359 wine was likely correlated with higher ratings for aroma, flavor, sweetness and acidity. The overall impression of 2015 Opportunity wines was described as slightly smoky, slightly floral, clean, and clear, and the overall impression of 2017 Opportunity wines was described as nice acid, fresh, pleasant aftertaste, and floral. The overall impression of 2015 A-2359 wines was described as citrus, grapefruit, and very pleasant, and the overall impression of 2017 A-2359 wines was described as bright, very pleasant, and Muscat-like. The overall impression of 2015 A-2574 wines was described as slightly floral and delicate, and the overall impression of 2017 A-2574 wines was described as fruity and stone fruit. Therefore, Opportunity, A-2359, and A-2574 grapes produced wines with unique and pleasant aroma and flavor characteristics and could provide new opportunities for grape growers and wine makers in Arkansas and the mid-South United States.

Conclusions

In 2015, 2017, and 2018, Opportunity, A-2359, and A-2574 grapes had typical sugar levels for Arkansas-grown wine grapes at harvest but were less than 20% SS. However, wines had acceptable compositions at bottling within typical ranges for dry white table wines and maintained acceptable pH and TA after 3-years storage. The brown color of all wines was very low, even for the wines stored for three years. Therefore, in combination with the stability of

wine composition attributes, the preservation of wine color quality indicated that Opportunity, A-2359, and A-2574 wines had potential for maintaining quality up to three years of bottle storage.

Fruity esters were the largest class of volatile aroma compounds identified in Opportunity, A-2359, and A-2574 wines. Younger wines were associated with higher amounts of aroma compounds, which indicated that younger wines had higher aroma impacts than wines that had been aged for 2-3 years. Wines from A-2359 grapes were associated with higher amounts of floral, herbal, and spicy aromas than Opportunity or A-2574 wines, indicating that A-2359 grapes produced wines with characteristic Muscat aromas.

The sensory attributes of Opportunity, A-2359, and A-2574 wines were scored generally positive. The aroma and flavor liking ratings were higher for A-2359 wines than other wines, and the aroma and flavor of A-2359 wine were described as floral, Muscat-like, citrus, stone fruit, and honeysuckle. The aroma and flavor of Opportunity wines were described as green apple, spicy, stone fruit, and green, and the aroma and flavor of A-2574 wines were described as spicy, stone fruit, and citrus. In general, the aroma, retronasal aromatic, and mouthfeel/finish of 2017 wines were described in more positive terms than that of the 2015 wines, indicating that the younger wines had preferable sensory characteristics.

Opportunity, A-2359, and A-2574 wines had compositions and colors that were stable during storage, although some degradation of sensory quality was seen in the older wines. Therefore, the UA System white wine grape cultivars and advanced selections produced wines with unique and pleasant aroma and flavor characteristics and could provide new opportunities for grape growers and wine makers in Arkansas and the mid-South United States.

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Tables

Table 1. Initial composition of Opportunity, A-2359, and A-2574 juices after pressing in 2015, 2017, and 2018 and composition of wines at bottling (University of Arkansas System Division of Agriculture Fruit Research Station, Clarksville, AR).

		Juice at press			Wine at bottling	
Genotype	**Harvest date**	**Soluble solids (%)**	**pH**	**Titratable acidity (%)**	**pH**	**Titratable acidity (%)**
Opportunity	11 September 2015	16.2	3.63	0.39	3.52	0.64
	17 August 2017	14.0	2.98	0.80	3.11	0.72
	8 August 2018	16.4	3.47	0.72	3.42	0.66
A-2359	19 August 2015	18.2	3.19	0.52	3.44	0.90
	17 August 2017	14.9	3.06	0.73	3.26	0.58
	8 August 2018	16.4	3.41	0.62	3.32	0.57
A-2574	19 August 2015	19.5	3.45	0.58	3.46	0.92
	17 August 2017	16.5	3.01	0.63	3.33	0.58
	7 August 2018	20.5	3.29	0.78	3.26	0.74

Table 2. Composition attributes of Opportunity, A-2359, and A-2574 wines at 3-years storage (2015 wines), 1-year storage (2017 wines), and 0-months storage (2018 wines) produced from grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (Clarksville, AR).

Wine	pH	Titratable acidity (%)	Glycerol (g/L)	Ethanol (% v/v)	Glucose (mg/100 mL)	Fructose (mg/100 mL)	Total residual sugars (mg/100 mL)	Tartaric acid (mg/100 mL)	Malic acid (mg/100 mL)	Citric acid (mg/100 mL)	Succinic acid (mg/100 mL)	Lactic acid (mg/100 mL)	Total organic acids (mg/100 mL)
Opportunity													
2015	3.19	0.53	7.27	12.60	4.79	414.10	418.89	46.36	234.49	135.70	75.21	6.47	498.23
2017	3.24	0.69	8.58	11.86	2.89	88.82	91.71	57.06	89.38	418.98	179.35	19.19	763.95
2018	3.42	0.66	11.91	12.45	4.27	70.30	74.57	71.16	62.73	429.51	264.39	19.61	847.39
A-2359													
2015	3.09	0.73	9.01	11.99	6.30	51.46	57.76	29.01	236.52	317.44	75.67	8.30	666.94
2017	3.37	0.55	10.18	13.77	4.42	41.43	45.85	27.74	74.99	289.51	129.13	11.57	532.94
2018	3.32	0.57	11.51	13.37	0.00	38.91	38.91	35.03	76.47	205.51	232.57	76.31	625.90
A-2574													
2015	3.09	0.78	9.67	12.00	6.48	54.10	60.58	60.66	335.28	287.07	658.74	8.90	1350.65
2017	3.35	0.55	10.51	14.31	8.46	81.54	90.00	52.74	94.40	264.86	654.88	13.77	1080.66
2018	3.26	0.74	10.78	11.71	3.37	51.62	54.99	106.86	111.31	396.29	613.22	11.80	1239.47

Table 3. Volatile aroma compounds identified in Opportunity, A-2359, and A-2574 wines at 3-years storage (2015 wines), 1-year storage (2017 wines), and 0-months storage (2018 wines) produced from grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (Clarksville, AR).

Compound[a]	Compound class	Aroma category	Aroma descriptors[b]	Opportunity			A-2359			A-2574		
				2015	2017	2018	2015	2017	2018	2015	2017	2018
Octanol	Alcohol	Chemical	Chemical, metal	✓[c]	✓	✓	✓	✓		✓	✓	✓
2-Ethylhexanol	Alcohol	Floral	Rose, citrus	✓	✓	✓	✓	✓	✓	✓	✓	✓
2-Phenylethanol	Alcohol	Floral	Honey, rose	✓	✓	✓	✓	✓	✓	✓	✓	✓
3,7-Dimethyloctan-1-ol	Alcohol	Floral	Floral, rose	✓								
1-Pentanol	Alcohol	Fruity	Balsamic, fruit					✓				
1-Undecanol	Alcohol	Fruity	Mandarin					✓			✓	
3-Ethoxy-1-propanol	Alcohol	Fruity	Fruit		✓	✓	✓	✓		✓	✓	✓
3-Methyl-1-pentanol	Alcohol	Fruity	Wine, cognac	✓	✓	✓	✓	✓		✓	✓	✓
1-Decanol	Alcohol	Green/fat	Fat	✓	✓	✓	✓	✓	✓	✓	✓	✓
1-Dodecanol	Alcohol	Green/fat	Fat, wax	✓	✓	✓	✓	✓	✓	✓	✓	✓
1-Hexanol	Alcohol	Green/fat	Green, herbal	✓	✓	✓	✓	✓	✓	✓	✓	✓
1-Tetradecanol	Alcohol	Green/fat	Coconut, oil					✓				
4-Methyl-2-pentanol	Alcohol	Green/fat	Oil, green, wine		✓	✓	✓	✓	✓		✓	✓
cis-3-Hexen-1-ol	Alcohol	Green/fat	Grass, leaf	✓	✓	✓	✓	✓	✓	✓	✓	✓
Dimetol	Alcohol	Herbal/spicy	Herbal					✓		✓		
Phenylacetaldehyde	Aldehyde	Floral	Floral, honey, rose	✓								
Tridecanal	Aldehyde	Floral	Floral, citrus				✓					
2-Methylbenzaldehyde	Aldehyde	Fruity	Fruit							✓	✓	✓
2-Heptenal	Aldehyde	Green/fat	Green				✓					
Decanal	Aldehyde	Green/fat	Soap, orange peel	✓	✓	✓	✓	✓	✓	✓	✓	✓
Dodecanal	Aldehyde	Green/fat	Fat, citrus, wax	✓		✓		✓	✓			✓
Heptanal	Aldehyde	Green/fat	Fat, citrus, green						✓	✓	✓	
Nonanal	Aldehyde	Green/fat	Fat, citrus, green	✓	✓					✓	✓	✓
Octanal	Aldehyde	Green/fat	Fat, soap, green				✓			✓	✓	
4-Methylbenzaldehyde	Aldehyde	Roasted/caramelized	Almond, caramel		✓	✓						
Benzaldehyde	Aldehyde	Roasted/caramelized	Almond, caramel				✓	✓			✓	

Table 3 (Cont.)

Compound[a]	Compound class	Aroma category	Aroma descriptors[b]	Opportunity			A-2359			A-2574		
				2015	2017	2018	2015	2017	2018	2015	2017	2018
Furfural	Aldehyde	Roasted/caramelized	Almond, caramel	✓			✓			✓	✓	
Heptadecane	Alkane	Green/fat	Alkane, fusel						✓			
Hexadecane	Alkane	Green/fat	Alkane, fusel					✓				
Pentadecane	Alkane	Green/fat	Alkane, green					✓				
Tetradecane	Alkane	Green/fat	Alkane, herbal		✓	✓						
Tridecane	Alkane	Green/fat	Alkane, fusel			✓			✓			✓
Benzothiazole	Benzothiazole	Chemical	Gasoline, rubber	✓	✓	✓	✓			✓	✓	✓
Decanoic acid	Carboxylic acid	Green/fat	Fat, soap	✓	✓	✓	✓	✓	✓	✓	✓	✓
2-Methylbutyric acid	Carboxylic acid	Unpleasant	Cheese, sweat				✓			✓	✓	✓
Butyric acid	Carboxylic acid	Unpleasant	Cheese, sweat	✓		✓	✓	✓	✓	✓	✓	✓
Hexanoic acid	Carboxylic acid	Unpleasant	Sweat, cheese	✓	✓	✓		✓	✓	✓	✓	✓
Isovaleric acid	Carboxylic acid	Unpleasant	Sweat, cheese	✓	✓	✓		✓	✓	✓	✓	✓
Octanoic acid	Carboxylic acid	Unpleasant	Sweat, cheese, fat	✓	✓	✓	✓	✓	✓	✓	✓	✓
2-Phenylethyl acetate	Ester	Floral	Honey, floral, rose	✓	✓	✓		✓	✓		✓	✓
2-Hexenyl acetate	Ester	Fruity	Fruit, green			✓						✓
2-Methylbutyl acetate	Ester	Fruity	Fermented fruit, banana, rum	✓	✓	✓	✓	✓	✓	✓	✓	✓
Diethyl malonate	Ester	Fruity	Apple				✓					
Diethyl succinate	Ester	Fruity	Wine, fruit, watermelon	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ethyl 2-furoate	Ester	Fruity	Fruit, floral			✓	✓	✓		✓	✓	✓
Ethyl 2-hexenoate	Ester	Fruity	Fruit	✓	✓	✓		✓	✓	✓	✓	✓
Ethyl 2-methylbutyrate	Ester	Fruity	Apple, strawberry	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ethyl 3-hexenoate	Ester	Fruity	Pineapple	✓	✓	✓						
Ethyl 3-hydroxybutyrate	Ester	Fruity	Grape, coconut, marshmallow	✓	✓	✓		✓		✓	✓	✓
Ethyl butanoate	Ester	Fruity	Apple, strawberry, bubblegum	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ethyl decanoate	Ester	Fruity	Grape	✓	✓	✓	✓	✓	✓	✓	✓	✓

Table 3 (Cont.)

Compound[a]	Compound class	Aroma category	Aroma descriptors[b]	Opportunity			A-2359			A-2574		
				2015	2017	2018	2015	2017	2018	2015	2017	2018
Ethyl dodecanoate	Ester	Fruity	Mango, leaf	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ethyl heptanoate	Ester	Fruity	Fruit	✓	✓			✓			✓	
Ethyl hexanoate	Ester	Fruity	Apple peel, strawberry, anise	✓	✓		✓	✓	✓	✓	✓	✓
Ethyl isobutyrate	Ester	Fruity	Strawberry	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ethyl isovalerate	Ester	Fruity	Anise, apple, black currant	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ethyl nonanoate	Ester	Fruity	Tropical fruit, rose	✓	✓	✓	✓	✓		✓	✓	✓
Ethyl octanoate	Ester	Fruity	Fruit, floral	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ethyl pentanoate	Ester	Fruity	Fruit, yeast	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ethyl phenylacetate	Ester	Fruity	Fruit	✓	✓							
Hexyl acetate	Ester	Fruity	Fruit, herb, wine	✓	✓	✓		✓	✓	✓	✓	✓
Isoamyl acetate	Ester	Fruity	Banana, pear	✓	✓	✓	✓	✓	✓	✓	✓	✓
Isobutyl acetate	Ester	Fruity	Apple, banana	✓	✓	✓	✓	✓	✓	✓	✓	✓
Isobutyl hexanoate	Ester	Fruity	Fruit, tropical					✓		✓	✓	✓
Isopentyl hexanoate	Ester	Fruity	Fruit		✓	✓	✓	✓	✓	✓	✓	✓
Isopentyl octanoate	Ester	Fruity	Fruit, pineapple	✓	✓	✓	✓	✓	✓	✓	✓	✓
Methyl decanoate	Ester	Fruity	Wine, fruit		✓	✓		✓	✓		✓	✓
Methyl hexanoate	Ester	Fruity	Fruit, fresh, paint thinner	✓	✓		✓	✓	✓	✓	✓	✓
Propyl octanoate	Ester	Fruity	Fruit, wine, brandy		✓	✓	✓	✓		✓	✓	✓
3-Hexenyl acetate	Ester	Green/fat	Green, banana		✓	✓		✓	✓			✓
cis-3-Hexenyl isobutyrate	Ester	Green/fat	Green, cut grass				✓					
Pentyl acetate	Ester	Herbal/spicy	Herbal			✓						✓
Dibutyl ether	Ether	Chemical	Ethereal	✓								
Dill ether	Ether	Herbal/spicy	Dill				✓					
2,3-Butanediol	Glycol	Fruity	Fruit, onion	✓	✓	✓	✓	✓	✓	✓	✓	✓
2-Decanone	Ketone	Fruity	Citrus, orange	✓								
2-Nonanone	Ketone	Green/fat	Hot milk, soap, fat		✓			✓				✓

Table 3 (Cont.)

Compound[a]	Compound class	Aroma category	Aroma descriptors[b]	Opportunity			A-2359			A-2574		
				2015	2017	2018	2015	2017	2018	2015	2017	2018
2-Octanone	Ketone	Green/fat	Soap, fat, green	✓	✓	✓	✓	✓	✓	✓	✓	✓
6-Methyl-5-hepten-2-one	Ketone	Vegetal	Mushroom, earthy				✓					
Linaloyl oxide	Oxane	Herbal/spicy	Herbal, camphor, rosemary					✓	✓			
Nerol oxide	Pyran	Floral	Floral				✓	✓	✓			
γ-Terpinene	Terpene	Chemical	Gasoline, turpentine						✓			
cis-Linalool oxide	Terpene	Floral	Floral			✓	✓	✓	✓			
Citronellol	Terpene	Floral	Rose, citrus, clove			✓		✓	✓		✓	✓
Geraniol	Terpene	Floral	Rose, geranium, citrus					✓	✓			
Linalool	Terpene	Floral	Floral, lavender, Earl Grey tea		✓	✓		✓	✓		✓	✓
trans-Linalool oxide	Terpene	Floral	Floral				✓	✓				
α-Terpinene	Terpene	Fruity	Lemon					✓	✓			
D-Limonene	Terpene	Fruity	Citrus, orange			✓	✓	✓	✓	✓	✓	✓
β-Damascenone	Terpene	Fruity	Apple, rose, honey	✓			✓	✓		✓		
α-Terpineol	Terpene	Herbal/spicy	Anise, mint, toothpaste		✓	✓	✓	✓	✓	✓	✓	✓
β-Ocimene	Terpene	Herbal/spicy	Herbal					✓	✓			
Eucalyptol	Terpene	Herbal/spicy	Mint, licorice, pine				✓					
Myrcene	Terpene	Herbal/spicy	Balsamic, must, spice					✓	✓			
p-Cymene	Terpene	Herbal/spicy	Herbal, spice								✓	✓

[a] Compounds were identified by comparison of mass spectra with NIST14 (National Institute of Standards and Technology, Gaithersburg, MD, USA), Flavors and Fragrances of Natural and Synthetic Compounds (FFNSC3, John Wiley & Sons, Inc., Hoboken, NJ, USA), and Adam's Essential Oils (Adams 2007) mass spectral libraries and comparison of calculated Kovats retention indices (Kováts 1958) with previously reported values .

[b] Aroma descriptors obtained from the Flavornet (Acree and Arn 2004) and Pherobase (Sayed 2003) databases.

[c] A ✓ indicates positive identification.

Table 4. Summary of principal components analysis on volatile aroma compound categories for Opportunity, A-2359, and A-2574 wines at 3-years storage (2015 wines), 1-year storage (2017 wines), and 0-months storage (2018 wines) produced from grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (Clarksville, AR).

		Component 1 (37.4%)[a]	**Component 2 (29.0%)**	**Component 3 (23.1%)**
Positive loadings[b]	Aroma categories[c]	Green/fat Fruity Floral Unpleasant Herbal/spicy	Roasted/caramelized Vegetal Chemical	Chemical Unpleasant
	Wines	Opportunity 2017 Opportunity 2018 A-2359 2015 A-2359 2018 A-2475 2018	A-2359 2015 A-2574 2017	Opportunity 2017 Opportunity 2018 A-2574 2015 A-2574 2017 A-2574 2018
Negative loadings[d]	Aroma categories	---	---	Herbal/spicy Floral
	Wines	Opportunity 2015 A-2359 2017 A-2574 2015 A-2574 2017	Opportunity 2017 Opportunity 2018 A-2359 2018 A-2574 2018	A-2359 2015 A-2359 2017 A-2359 2018

[a] Percent of variation in data explained by each component.
[b] Loading values >0.5 were considered positive loadings for aroma categories on each component.
[c] Aroma categories represent the sum of the total ion chromatogram (TIC) peak areas of positively identified compounds within each category (Table 3).
[d] Loading values <-0.5 were considered negative loadings for aroma categories on each component.

Table 5. Aroma, flavor, and overall impression descriptors from an industry sensory panel (26 panelists) for Opportunity, A-2359, and A-2574 wines at 2-years storage (2015 wines) and 0-months storage (2017 wines) produced from grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (Clarksville, AR).

Genotype	Year	Appearance	Aroma	Flavor	Overall impression
Opportunity	2015	Slightly yellow Pale melon Clear Brilliant Very light	Soft Delicate Spicy	Pleasant Light	Slightly smoky Slightly floral Clean Clear
	2017	Straw-colored Brilliant Clear Pale	Pleasant Slightly floral Fruity Muscat Apple Peach Citrus Spicy Grassy Stone fruit Guava	Crisp Citrus Tree fruit Peach Clean Green apple Good mouthfeel Long finish Green Citrus pith Refreshing	Nice acid Fresh Pleasant aftertaste Floral
A-2359	2015	Clean Clear Bright Golden	Faint Citrus Riesling-like Light	Refreshing Grapefruit Pleasant Gentle	Citrus Grapefruit Very pleasant
	2017	Light Slightly green Gold-colored Clear	Floral Beautiful Citrus Good nose Peach Muscat-like Honeysuckle	Pleasant Confident Fruity Peach Stone fruit Clean Good mouthfeel	Bright Very pleasant Muscat-like
A-2574	2015	Golden Clear	Soft Pleasant Spicy Faint	Very pleasant Citrus Fruity Clear	Slightly floral Delicate
	2017	Golden Clear	Fruity Green apple Bell pepper Soft Hay/straw	Stone fruit Peach Clean Crisp Good finish Nice tannins	Fruity Stone fruit

Figures

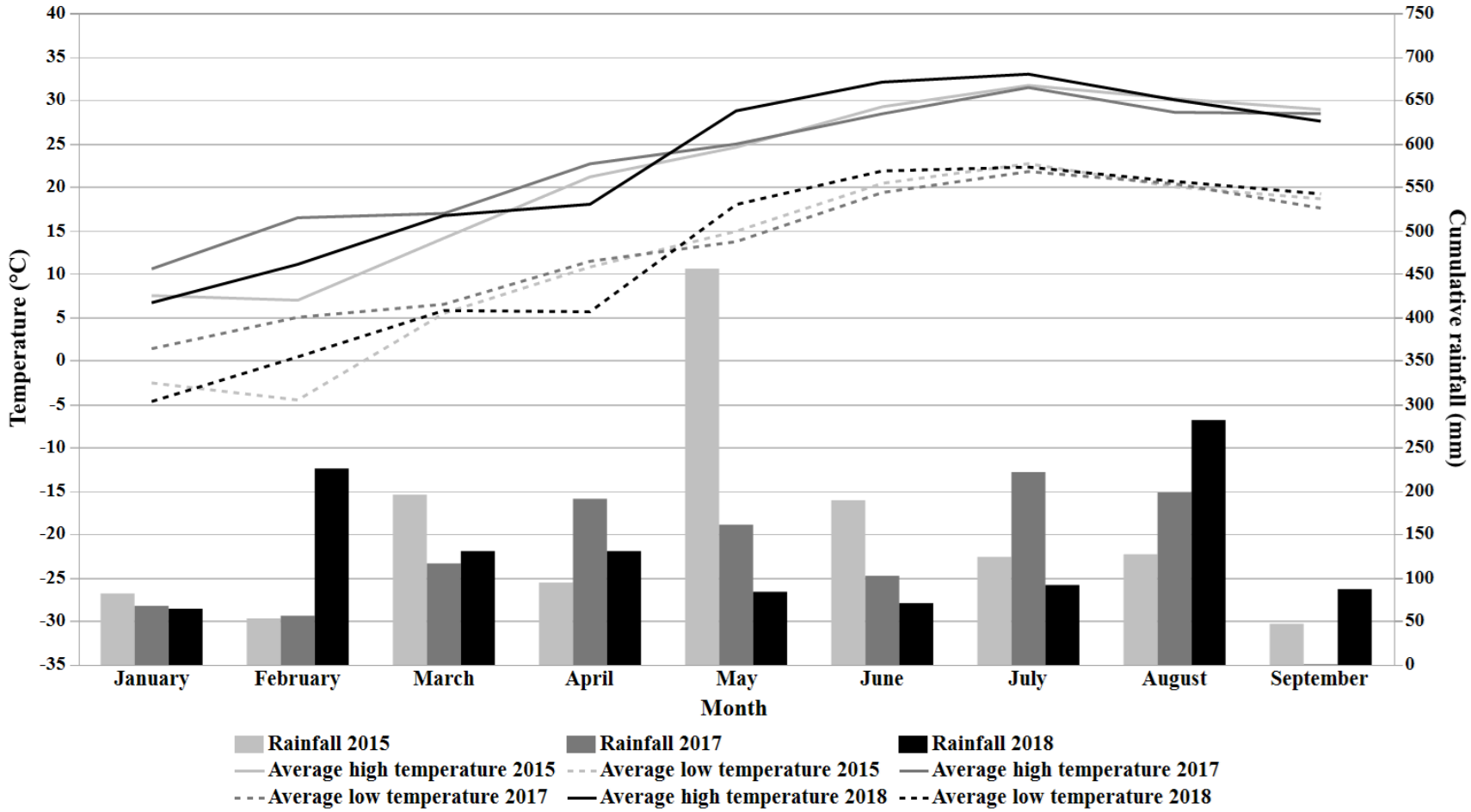

Figure 1. Average monthly high and low temperatures and cumulative rainfall[a] from January-September 2015, 2017, and 2018 at the University of Arkansas System Division of Agriculture Fruit Research Station (Clarksville, AR).

[a] Data was gathered from a USDA weather station in Clarksville, Arkansas.

UNIVERSITY OF ARKANSAS

Office of Research Compliance
Institutional Review Board

July 16, 2018

MEMORANDUM

TO:	Renee Threlfall Jean-François Meullenet
FROM:	Ro Windwalker IRB Coordinator
RE:	EXEMPT PROJECT CONTINUATION
IRB Protocol #:	05-11-193
Protocol Title:	*Evaluation of Grapes, Juices, Wines and Other Grape Products from Enology and Viticulture Experiments*
Review Type:	☒ EXEMPT
New Approval Date:	07/16/2018

Your request to extend the referenced protocol has been approved by the IRB. We will no longer be requiring continuing reviews for exempt protocols.

If you wish to make any modifications in the approved protocol that may affect the level of risk to your participants, you must seek approval *prior to* implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 109 MLKG Building, 5-2208, or irb@uark.edu.

Figure 2. University of Arkansas Institutional Review Board (IRB) protocol approval notice for sensory analysis of Opportunity, A-2359, and A-2574 wines at 2-years storage (2015 wines) and 0-months storage (2017 wines) produced from grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (Clarksville, AR).

White wines tasting survey

How much do you like or dislike the **appearance** of the wine (circle your answer)?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Please provide comments about the **appearance** of the wine: _____

_____

How much do you like or dislike the **aroma** of the wine (circle your answer)?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Please provide comments about the **aroma** of the wine: _____

_____

How much do you like or dislike the **flavor** of the wine (circle your answer)?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Please provide comments about the **flavor** of the wine: _____

_____

How much do you like or dislike the **sweetness** of the wine (circle your answer)?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

How much do you like or dislike the **acidity** of the wine (circle your answer)?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

How much do you like or dislike the **overall impression** of the wine (circle your answer)?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Please provide comments about the **overall impression** of the wine: _____

_____

Figure 3. Ballot presented to panelists for industry sensory panel evaluation of Opportunity, A-2359, and A-2574 wines at 2-years storage (2015 wines) and 0-months storage (2017 wines) produced from grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (Clarksville, AR).

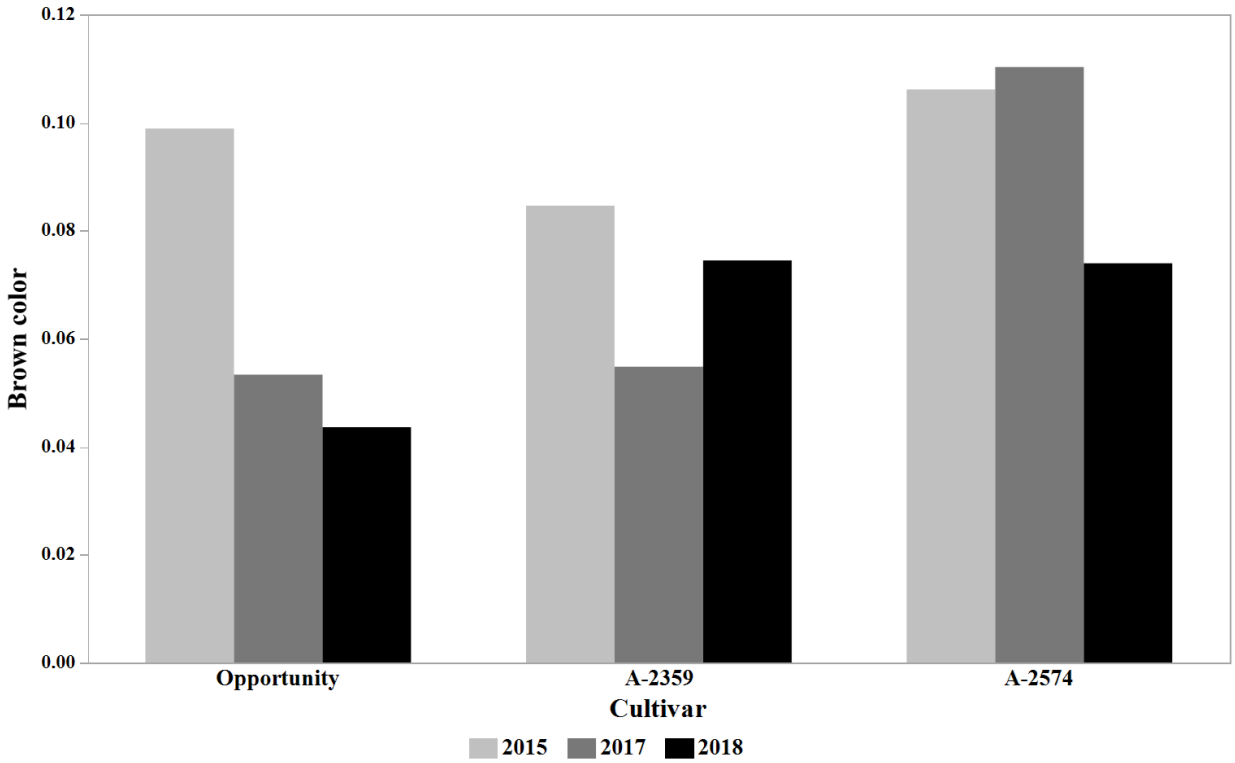

Figure 4. Brown color[a] of Opportunity, A-2359, and A-2574 wines at 3-years storage (2015 wines), 1-year storage (2017 wines), and 0-months storage (2018 wines) produced from grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (Clarksville, AR).

Figure 5. Radar plot for liking[a] of wine attributes from an industry sensory panel (26 panelists) for Opportunity, A-2359, and A-2574 wines at 2-years storage (2015 wines) and 0-months storage (2017 wines) produced from grapes grown at the University of Arkansas System Division of Agriculture Fruit Research Station (Clarksville, AR).
[a] Nine-point hedonic scale was converted to a numerical scale (1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, and 9=like extremely) for statistical analysis.

OVERALL CONCLUSIONS

Techniques to enhance the attributes of wines produced from grapes grown in Arkansas were evaluated. The impacts on grapes during ripening and at harvest and the resulting wine quality from application of a specific inactivated yeast to Chambourcin grapevines were evaluated. Application of a specific inactivated yeast to Chambourcin grapevines lead to grapes with better composition for winemaking and higher levels of red-colored anthocyanin compounds that could potentially be extracted at a higher rate during winemaking. Chambourcin wines produced from treated grapevines had higher anthocyanins, higher amounts of fruity ester aroma compounds, and potentially improved sensory attributes. These results demonstrated that specific inactivated yeast application could be used to improve the quality of Chambourcin grapes and wine. This was particularly significant as Chambourcin grows well in Arkansas and the mid-South United States, a region that struggles to produce high quality red-wine grapes.

In a study on Noble muscadine wines, variations in skin contact time and glycosidic enzyme addition impacted the evaluated attributes. Wines with higher skin contact times had more intense red color and spicy and dark-fruit aroma characteristics of red wines, whereas wines with no fermentation on the skins had lighter colors and fresh-fruit and candy-like aroma characteristics of muscadine juice. Enzyme addition decreased fruity aroma intensity and overall aroma liking of Noble muscadine wines, and wines without enzyme were perceived as having more pleasant red-fruit aromas. Muscadine grapes are one of the most widely-grown grapevine species in Arkansas and the Southeastern United States, as they are able to withstand climatic conditions unfavorable for other grapevines species. This study provided insight into how winemaking techniques can be used to alter the characteristics of Noble muscadine wines and could therefore be beneficial to the Arkansas grape and wine industry.

Wine grape cultivars and breeding selections from the UA System were evaluated for wine production potential. Enchantment grapes produced high-quality, deeply-red colored wines with aging potential and *V. vinifera*-like characteristics. Opportunity, A-2359, and A-2574 grapes produced aromatic wines with unique aromas/flavors that were positively perceived in sensory evaluations. All UA System cultivars and advanced selections present unique opportunities for grape growers and wine makers in Arkansas and the mid-South United States. These new hybrids show potential as alternatives to the native and hybrid species with less stable color and non-traditional aromas and to popular *V. vinifera* cultivars that are not able to withstand the climate of the region.

Overall, this research demonstrates the potential for various viticultural and enological techniques to enhance the attributes of Arkansas wines. It is our hope that these findings can contribute to the growth of the Arkansas grape and wine industry and can expand knowledge of how to produce high-quality grapes and wines in areas that are not suited for *V. vinifera* cultivation.