2025 R-25 Progress Report
Title: Investigating physicochemical, color, and sensory impacts from co-fermenting *Vitis rotundifolia (Noble)* and *Vitis vinifera* (Merlot) grapes

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Public Abstract

We will provide the public abstract when the final report is submitted in January.

Introduction

Muscadine grapes (*Vitis rotundifolia* Michx.) are a disease-resistant specialty crop native to the southeastern United States. There have been major advances in U.S. muscadine breeding efforts resulting in unique traits emerging for muscadine grapes used for commercial processing into juice and wine. While it is common for winemakers to blend wines after fermentation or prior to bottling to balance flavors and aromas, wines can also be improved using co-fermentation, a technique where the grapes or grape must (seeds, skins, juice, and pulp) are mixed after crushing the grapes and prior to fermentation. Grape must is typically fermented for red wine production to extract phenolic compounds, especially color pigments. García-

Carpintero et al. (2011) conducted a study blending different combinations of three red grape varieties from Spain and found that wines produced from co-fermentation produced aromatically richer wines compared to their monovarietal counterparts.

Another method to control color degradation in wine is the use of sulfur dioxide (SO_2) additions during winemaking to prevent browning and limit unwanted microbial growth, ultimately ensuring minimal oxygen exposure to the wines during aging. Sulfur dioxide exists in five forms in wine: molecular (SO_2), free bisulfite (HSO3-), sulfite (SO32), bound bisulfite, and total sulfur dioxide (Waterhouse et al. 2016). The antimicrobial protection of free SO_2 is dependent on its molecular form, which should be maintained ~0.8 mg/L in wine. As alcohols levels increase during fermentation, color attributes decrease due to the possible degradation of colored co-polymers of anthocyanins (Somers and Evans 1979).

Overall, factors affecting the rate of color change in wine include phenolic composition and concentration, pH, concentration of metals, storage conditions, oxygen exposure, and SO_2 levels (Zoecklein et al. 1995). While a higher percentage of free SO_2 exists in the molecular SO_2 form at lower pH, as pH increases, molecular SO_2 concentration decreases. Thus, maintaining wine pH at or below 3.6 translates to lower sulfur dioxide additions and better protection. Sims and Bates (1994) found that levels higher than 25 ppm free SO_2 bleached the color of the muscadine wines but decreased browning in high pH wines, and wines with lower pH had a greater loss of free, unpolymerized anthocyanins, resulting in greater polymerization. However, total SO_2, the combination of free and bound SO_2, has a legal limit of 350 mg/L (Alcohol Tobacco Tax and Trade Bureau 2023).

One of the challenges of growing *V. rotundifolia* grapes for winemaking is lower phenolic content compared to *V. vinifera* and hybrid grapes (crosses between different *Vitis* species). Initially, red muscadine wines have excellent color but can degrade quickly to an orange-brown color after bottling and during storage, paired with the presence of sedimentation in the bottle. In red wines produced from *V. vinifera* grapes, the main anthocyanins are in the monomeric form, presenting as the 3-*O*-glucosides of delphinidin (blue/violet), cyanidin (dark-red/pink), petunidin (dark purple), peonidin (yellow/pink), and malvidin (dark violet) (Câmara et al. 2022). Muscadine wines contain diglucoside anthocyanins (3,5 di-*O*-glucoside form), which brown more readily than monoglucoside anthocyanins found in *vinifera* wines. Overall, the lower degree of anthocyanin-tannin polymerization results in color instability in muscadine wines (Sims and Morris 1986). The main anthocyanin identified in the Noble wines were non-acylated 3,5 diglucosides, which are more susceptible to color degradation over time, compared to monoglucosides, due to their inability to form stable polymeric pigment complexes (Sims and Bates 1986, Mayfield 2020). Such chemical changes are considered quality defects in the final products, hampering a wider consumer appeal and subsequent growth of the muscadine juice and wine markets (Sims and Morris 1986, Lee and Talcott 2002).

This research from the University of Arkansas System Division of Agriculture (UA System) **investigated quality impacts from co-fermenting *Vitis rotundifolia* (Noble) and *Vitis vinifera* (Merlot) grapes** by evaluating physicochemical, color, and sensory attributes of the wine during storage. **This project is important because it will help establish the potential for improving color and flavor attributes of wines that have less stable color and flavor attributes than *vinifera* wines.** There is no known published research on co-fermentation of muscadine grapes with hybrid or *vinifera* grapes, thus it is of great interest to evaluate wines produced from such a technique at bottling and during storage.

Materials and Methods

Grape harvest

In 2024, 127 kg (279 lbs) of each variety of grapes were harvested from commercial growers. Machine-harvested Noble grapes were obtained from a commercial grower in Arkansas (USDA hardiness zone 7b). Hand-harvested Merlot grapes were obtained from a commercial grower in Washington (USDA hardiness zone 6a) and transported via refrigerated truck to Post Winery, Inc (Altus, AR) in disposable cardboard ½-ton (454 kg) bins. All grapes were transported to the UA System Food Science Department in Fayetteville, AR and refrigerated at 4°C for 24 hours until processing.

Wine Production

The grapes from each cultivar were weighed, crushed and destemmed, then frozen (-10°C) until blending and fermentation. There were five blending ratios based on weight of grapes (100% Noble, 75% Noble + 25% Merlot, 50% Noble + 50% Merlot, 25% Noble + 75% Merlot, 100% Merlot) in duplicate. The wines were processed in traditional red wine styles for wine production. For grape processing, Noble and Merlot grape batches were passed separately through a crusher/destemmer, and 30 mg/L SO_2 as potassium metabisulfite (KMBS) was added to the must at crush to reduce spoilage microorganisms and improve color stability. After crushing/destemming, Noble and Merlot individual batches were placed in 38-L plastic containers lined with 4-mm food-grade plastic bags, sealed, and frozen (-10°C) due to different harvest dates. Prior to fermentation, the batches were thawed at 2°C for 72 hours, then placed at room temperature (21°C) 24 hours before inoculation. The musts were placed in 60-L plastic containers with food-grade polyethylene liners for fermentation. Initial juice composition was analyzed and adjusted as needed. The musts were inoculated with D254 yeast (0.25 g/L) and GoFerm (0.30 g/L) yeast rehydration nutrient (0.26 g/L) and FermAid K (0.40 g/L), a fermentation nutrient (Lallemand, Montreal, Canada). The bags of must were sealed with tape to allow carbon dioxide to escape during fermentation. During fermentation, the must cap was punched down twice daily through the bag without exposing the must to air. The grapes were fermented at 15°C on the skins for three days. The must was pressed in a 70-L Enrossi bladder-type press at 4 bar pressure (Enoagricol Rossi, Calzolaro, Italy). The wines were collected into glass carboys with fermentation locks and fermented to dryness (≤0.3% residual sugar). During fermentation, the wines were racked once to clarify and remove spent yeast cells. At bottling, the wines were adjusted to three molecular SO_2 levels (0, 0.8, and 1.5 mg/L) then analyzed at bottling (0 days) and 6 months storage at 15°C. *Wines will be analyzed at 12-months storage at 15°C in January 2026.*

Composition attributes analysis

The composition attributes (soluble solids, pH, titratable acidity, ethanol, and free, bound, and total sulfur dioxide were evaluated at bottling (0-months storage) and 6 at 15°C. In addition, soluble solids, °Brix, pH, and titratable acidity were evaluated at harvest and during fermentation.

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

°Brix. °Brix was measured with an EasyDens portable density meter (Anton Paar, Austria) and expressed as degrees (°).

pH. The pH of juice, musts, and wines were measured using an APERA PH700 pH meter (Colombus, OH). Fermenting musts and wines were degassed prior to measurement.

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

Free, bound, and total sulfur dioxide. Free and bound sulfur dioxide levels of wines were determined using the aeration-oxidation method (Iland et al. 2021) expressed as mg/L. Total sulfur dioxide was calculated as the sum of free sulfur dioxide and bound sulfur dioxide.

Ethanol. Ethanol was measured with a DMA Density Meter 4501 paired with an Alcohol Meter Alcolyzer 3001 (Anton Paar, Austria). Ethanol was expressed as % (w/v).

Color attributes analyses

The color attributes (L*, hue angle, chroma, red color, brown color, and color density) of the wines were evaluated at bottling (0 months), and at 6-months storage at 15°C. Color of the wines were measured in analytical duplicate.

L*, hue angle, and chroma. Wine color analyses were 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).

Red color, brown color, and color density. Red color of wines were measured spectrophotometrically as absorbance at 520 nm, brown color at 420 nm, and color density as red color + brown color (Iland et al. 2021). Absorbance values were measured using a VWR Spectrophotometer UV-1600PC UV-VIS (VWR International, LLC, Radnow, PA). Wine samples were diluted with deionized water prior to analysis and measured against a blank sample of deionized water. A 1-cm cell was used for all spectrophotometer measurements.

Polymeric pigment. The wine polymeric pigment/color was measured using sodium metabisulfite added to bleach monomeric anthocyanins; anthocyanin-tannin complexes remain unbleached; absorbencies of samples were taken at 520 nm using a VWR Spectrophotometer UV-1600PC UV-VIS (VWR International, LLC, Radnow, PA) (Iland et al. 2021).

Phenolic attributes analysis

The phenolic attributes (total monomeric anthocyanins and total phenolics) of the wines were evaluated at bottling (0 months) and at 6-months storage at 15°C.

Total monomeric anthocyanins. The monomeric anthocyanin concentration was determined using the pH differential method of Giusti and Wrolstad (2001). This method is based on the reaction of anthocyanin pigments in their different forms at different pH values; specifically, the difference between the colored oxonium form at pH 1.0 and the colorless hemiketal form at pH 4.5. Samples were evaluated with a VWR Spectrophotometer UV-1600PC UV-VIS (VWR International, LLC, Radnow, PA). The absorbance of each diluted sample was measured in a 1-cm cell for all spectrophotometer measurements at 510 nm and at 700 nm (to correct for haze), against a blank cell filled with distilled water. For a pathlength of 1 cm, the absorbance of the diluted samples (A) was calculated as follows: $A = (A_{\text{ʎvis-max}} - A_{700})_{\text{pH1.0}} - (A_{\text{ʎvis-max}} - A_{700})_{\text{pH4.5}}$. The monomeric anthocyanin pigment concentration in the original sample was calculated using the following formula: Monomeric anthocyanin pigment (mg/liter) = $(A \times MW \times DF \times 1000)/(\varepsilon \times 1)$ where MW is the molecular weight DF is the dilution factor and ε is the molar absorptivity.

Total phenolics. The total phenolic concentration in the wine treatments was determined using the Folin-Ciocalteu assay (Slinkard and Singleton 1977), using gallic acid as the standard. The standard was made by mixing 10 mg gallic acid and 90 mL deionized water. Wine samples were diluted with deionized water prior to analysis and measured against a blank sample of deionized water. Serial dilutions were performed to provide the standard curve formula. A stock solution of Folin's reagent (sodium 1,2-naphthoquinone-4-sulfonate) was used and diluted to 0.2N with deionized water. A sodium carbonate (Na_2CO_3) stock solution was made by measuring 75 g of anhydrous Na_2CO_3 and bringing up to one liter with deionized water. 1,000 uL of 0.2N Folin's reagent and 800 uL of Na_2CO_3 (75g/L) was added to the diluted wine samples, standard, and blank in 1-cm cells. Samples were incubated for two hrs before reading at an absorbance of 760 nm using a VWR Spectrophotometer UV-1600PC UV-VIS (VWR International, LLC, Radnow, PA). Results were expressed as mg/L gallic acid equivalents (GAE).

Sensory attributes analysis

The sensory attributes of the wines were evaluated in March 2025 (2-months after bottling) at the Sensory Science Center at the UA System (https://aaes.uada.edu/technical-services/sensory-science-center/). Recruitment criteria included panelist age of at least 21 years old and a selection of at least "like a little" for red and white wine liking. Panelist selection was also based on consumption of muscadine wine of at least "once per month". For sensory analysis, the replications of each treatment were combined for a total of five samples presented to each panelist. Consumer panelists (n=64) assessed the appearance, astringency, aroma, and flavor of the five wines. The sample presentation order was randomized. Sample cups were labeled with three-digit codes, and each panelist was served 30 mL of wine at room temperature (21°C). Unsalted crackers and water were provided for palate cleansing between samples. Each consumer evaluated overall liking, overall flavor, appearance, aroma, and astringency on a 9-point verbal hedonic scale (1 = dislike extremely; 9 = like extremely) and astringency and appearance on a 5-point Just About Right (JAR) scale (1 = not nearly enough 3 = just about right; 5 = much too much). Consumers also selected Check-All-That-Apply (CATA) terms for aroma and flavor attributes. After evaluating each wine, panelists were asked to rank the wines (1=most preferred and 4= least preferred). Overall, 34% of panelists were female and 66% were male. In terms of age, 28% were 21-30, 28% were 31-40, 13% were 41-50, 6% were 51-60, and 9% were 61-70. Among panelists, consumption of red wine, ranging from daily to at least twice per month, was 95%, while it was 89% for white wine, and 75% for muscadine wine.

Statistical Analysis

Analysis of physicochemical, color, and sensory attributes were conducted using JMP® (version 18.0; SAS Institute Inc., Cary, NC). For the physiochemical and color attributes, the study was analyzed as a completely randomized design with five co-fermentation treatments x three storage times x two replications. For the sensory attributes, the replications of each wine treatment were combined so that 50% of each replication was used for analysis. There were five co-fermentation treatments in total. Analysis of variance was used to determine main effects and interactions. Tukey's Honestly Significant Difference (HSD) was used for mean separation at a p-value ≤ 0.05.

Results and Discussion:

Grape composition attributes at harvest

After harvest and crushing (grapes crushed and stems removed), the initial compositions (soluble solids, pH, and titratable acidity) of the co-fermentation treatments were evaluated **(Table 1).** 100% Merlot has soluble solids, pH, and titratable acidity of 22.3%, 3.92, and 0.26%, respectively. 100% Noble had soluble solids, pH, and titratable acidity of 17.3%, 3.20, and

0.53%, respectively. In general, as the percentage of Merlot decreased in the co-fermentation treatments, pH decreased, while titratable acidity increased. Except for the 100% Merlot treatment, as Merlot decreased in the co-fermentation treatments, soluble solids also decreased. Compared to all other co-fermentation treatments, the 100% Noble had the lowest soluble solids and pH (17.3% and 3.20, respectively). Compared to *V. vinifera* red cultivars, *V. rotundifolia* cultivars do not accumulate as much sugar during ripening and tend to have lower pH and higher titratable acidity values (Carroll and Marcy 1982, Boulton et al. 1996).

Wine composition, color, and phenolic attributes at bottling

At bottling, the wine composition (pH, titratable acidity, free, bound, and total SO_2, and ethanol) of the co-fermentation treatments were evaluated **(Table 2)**. As the percentage of Merlot decreased in the co-fermentation treatments, pH decreased while titratable acidity increased. Compared to all other co-fermentation treatments, the 100% Noble had the lowest pH (3.10). In terms of SO_2 in the wine, the free SO_2 levels of the 100% Merlot were higher (123.20 mg/L) than wines co-fermented with at least 50% Noble (ranging 16.13-28.99 mg/L free SO_2). The high free SO_2 for the 100% Merlot was expected since this wine had the highest pH (4.09). The higher the pH, the more sulfur is needed to achieve the correct desired molecular SO_2 for microbial stability (Waterhouse et al. 2016). As the percent of Merlot decreased and Noble increased in the treatments, the percent difference between sulfur in the free form versus the bound form for each treatment increased from 23.37% to 164.73%. This difference could be due to the higher number of hydroxyls groups and their aglycones present on ring B of diglucoside anthocyanins. Aglycones are more susceptible to oxidation thus binding with SO_2 added to the wine, offering a possible explanation for the higher bound SO_2 values in wines with increased proportions of Noble grapes (Wang et al. 1997, Lee and Talcott 2002, de Rosso et al. 2024). There was no difference in bound SO_2 (146.50 mg/L) or total SO_2 (194.62 mg/L) values between the co-fermentation treatments. The 100% Merlot wine (15.85%) had the highest ethanol, while 100% Noble wine (11.46%) had the lowest.

Wine co-fermentation treatment impacted all color and phenolic treatments **(Table 3)**. The 100% Merlot wine had the highest L* (6.78), hue angle (380.82°), and chroma (28.86), and the lowest red color (1.79), brown color (1.87), and color density (3.66). Polyphenolic color, defined as the concentration of SO_2 resistant pigments in the wines formed from anthocyanin-tannin complexes, was lower for both 100% Merlot and 100% Noble (0.69-0.71), compared to the wines co-fermented with a blend of Merlot and Noble grapes (0.78-0.83). A higher polyphenolic color value indicates the wine's color is more stable, especially with the addition of SO_2, which bleaches anthocyanins that are not yet polymerized with polyphenolic compounds. This higher polyphenolic color in wines with varying proportion of both Merlot and Noble versus the monovarietal treatments could be a function of the anthocyanin-tannin complexes formed from the synergistic effect of the two different varieties. In terms of phenolics, the 100% Noble had the highest total phenolics (2,242,13 mg gallic acid/L) and total monomeric anthocyanins (2,133.69 mg/L). Total phenolics and monomeric anthocyanins steadily decreased as the percentage of Merlot increased in the treatments.

Wine composition, color, and phenolic attributes during storage

Wine composition, color, and phenolic attributes were evaluated at 0- and 6-months storage at 15°C (**Tables 4 and 5**).

Titratable acidity and pH. The co-fermentation main effect was significant for titratable acidity and pH where co-fermentation treatments with more Noble had the highest titratable acidity and lowest pH (0.87% and 3.08, respectively). The SO_2 x Storage interaction was significant for pH.

Free, bound, and total SO_2. The Co-fermentation x SO_2, Co-fermentation x Storage, and SO_2 x Storage interactions were significant for free SO_2. For the Co-fermentation x SO_2 interaction, for wines with at least 50% Merlot, as molecular SO_2 levels increased from 0 mg/L to 0.8 mg/L to 1.5 mg/L, free SO_2 increased **(Figure 1)**. However, from 0.8 to 1.5 mg/L molecular SO_2, free SO_2 did not increase in wines with at least 75% Noble. This interaction shows how higher pH wines, in this case blends with pH ≥ 3.44, require a higher rate of SO_2 to achieve the desired molecular SO_2. For the Co-fermentation x Storage interaction, from 0-6 months and for wines with at least 75% Merlot, free SO_2 decreased **(Figure 2).** The pH in the 75% Merlot-25% Noble and 100% Merlot wines were 3.76 and 4.03, respectively. As wines pH increases anthocyanin concentration decreases and wines are more prone to oxidation, resulting in challenges in maintaining constant free SO_2 levels over time (Forino et al. 2020, Boulton et al. 1996). In this case, the addition of Noble grapes to a co-fermentation resulted in lower pH wines, keeping free SO_2 levels low and constant over storage time. For the SO_2 x Storage interaction, from 0-6 months storage, free SO_2 levels decreased for wines with at least 0.8 mg/L molecular SO_2 (data not shown).

The Co-fermentation x SO_2 x Storage interaction was significant for bound and total SO_2 **(Figure 3).** From 0 to 6 months storage, bound and total SO_2 decreased for most co-fermentation treatments, regardless of molecular SO_2 level. Bound SO_2 was highest at 1.5 mg/L SO_2 level for 100% Merlot (229.60 mg/L and 145.60 mg/L) at 0- and 6- months storage, respectively. Total SO_2 was highest (470.40 mg/L and 345.60 mg/L) for 100% Merlot at 0- and 6-months storage, respectively, at the 1.5 mg/L SO_2 level.

L*, hue angle, and chroma. The Co-fermentation x SO_2 interaction significantly impacted L*, hue angle, and chroma **(Figure 4).** As percent of Noble decreased and molecular SO_2 levels increased in the blends, L* increased and wines became lighter. Compared to the co-fermentation treatments with ≤ 50% Merlot, where L* values ranged 1.03 -1.39, there was a significant increase in L* values (4.02) in blends with ≥75% Merlot at the 1.5 mg/L SO_2. The 100% Merlot with 1.5 mg/L SO_2 had highest L* (11.84). Overall, SO_2 had more impact on L* for co-fermentation treatments with more Merlot.

Hue angle values increased from 0.0 to 0.8 mg/L SO_2 for 100% Merlot, however, for wines with ≥25% Noble, hue angle did not increase substantially with increased SO_2 additions. This indicates the strong impact Noble had on maintaining red hue of the wines with added SO_2. Chroma (saturation levels) decreased gradually as Noble increased in the blends. For wines with at least 75% Merlot, as SO_2 levels increased, chroma increased. 100% Merlot wines treated with ≥ 0.8 mg/L SO_2 had the highest chroma (36.33-37.96), compared to wines ≥ 25% Noble at the 0.0 mg/L SO_2 level (6.88), and wines ≥ 50% Noble, regardless of SO_2 levels (1.31-7.02). These chroma results indicate that increasing levels of Noble led to wines with lower chroma, or less color saturation, possibly due to the higher concentrations of brown pigments observed in co-fermentation treatments with greater proportions of Noble.

The Co-fermentation x Storage interaction was significant for L* and hue angle **(Figure 5).** L* increased from 0-6 months storage for 100% Merlot, with no change in L* for all other treatments across storage period. This trend highlights the susceptibility to color change for 100% Merlot wines during storage. Hue angle decreased for 100% Noble from 0-6 months, where hue went from red to more yellow/brown. The SO_2 x Storage interaction was significant for L* and chroma **(Figure 6).** Both lightness (L*) and color intensity (chroma) increased from 0 to 6 months storage at 1.5 mg/L SO_2. Within the 0-month storage period, L* and chroma increased as SO_2 concentration increased from 0 to 0.8 mg/L, while the same color attributes, from 0 mg/L to 1.5 mg/L SO_2. increased within the 6-month storage period, indicating the impact that storage has on degradation of wine color at increasing molecular SO_2 levels.

Red color, brown color, and color density. The Co-fermentation x SO_2 and Co-fermentation x Storage interactions were significant for red color, brown color, and color density. As SO_2 levels increased from 0 to 1.5 mg/L, red color, brown color, and color density decreased in all treatments except the 100% Noble wine, which remained the same regardless of SO_2 levels (average 8.78 red color, 5.13 brown color, and 13.92 color density) **(Figure 7).** 100% Merlot with ≥ 0.8 mg/L SO_2 had the lowest red color (average 3.10), brown color (average 1.64), and color density (average 1.46) of all the treatments. Within each SO_2 treatment, increasing the proportion of Noble in the blend led to steady increases in red color, brown color, and overall color density. From 0 to 6 months storage, color density and brown color decreased for wines with at least 75% Noble, while red color decreased for wines with at least 50% Noble **(Figure 8).** Regardless of storage point, red color, brown color, and color density increased with each 25% incremental increase in Noble. At 6 months storage, compared to all other co-fermentation treatments, 100% Noble had the highest red color (8.08), brown color (4.99), and color density (13.07).

Polymeric pigment, total phenolics and total monomeric anthocyanin pigments. The SO_2 treatment main effect was significant for total monomeric anthocyanins, which increased from 1104.91 mg/L to 1156.34 mg/L from 0.0 to 0.8 mg/L SO_2. The Co-fermentation x SO_2 and SO_2 x Storage interactions were significant for polymeric pigment. Polymeric pigment decreased for wines ≥ 75% Merlot as SO_2 levels increased from 0 to 1.5 mg/L (**Figure 9**). Regardless of SO_2 level, in general, wines with some proportion of Merlot and Noble had higher polymeric pigment (0.98-1.13) compared to the monovarietal 100% Merot and 100% Noble wines (0.81 – 1.01).

Regardless of SO_2 levels, from 0-to 6-months storage, polymeric pigment increased **(Figure 10)**. Among SO_2 levels, at 0-months storage, there was no difference in polymeric pigment (average 0.76). At 6-months storage, as SO_2 levels increased, polymeric decreased from 1.30 to 1.12.

The Co-fermentation x Storage interaction was significant for polymeric pigment, total phenolics and total monomeric anthocyanins **(Figure 11)**. From 0-6 months storage, polymeric pigment and total phenolics increased for all co-fermentation treatments, while monomeric anthocyanins decreased for treatments with at least 50% Noble, indicating that formation of anthocyanin-tannin complexes (polymeric pigments) were especially important in blends that with moderate amounts of Noble grapes. As wines age, monomeric anthocyanins tend to decrease and polymeric pigments form (Waterhouse et al. 2016). At 0-months storage, and among the co-fermentation treatments, polymeric pigment values were similar (0.69-0.83). However, at 6-months storage, polymeric pigment was highest (1.27-1.32) for wines with some proportion of Merlot and Noble grapes, compared to the monovarietal treatments (1.08 -1.09).

At 0-months storage, and among co-fermentation treatments, total phenolics and total monomeric anthocyanins steadily increased as the proportion of Noble increased, though there was no difference between 75% Noble and 100% Noble blends for total phenolics. At 6-months storage, and among co-fermentation treatments, the same attributes increased steadily, where 100% Noble had 2885.4 mg/L total phenolics and 1358.5 mg/L total monomeric anthocyanins compared to 100% Merlot (1640.5 mg/L and 218.7 mg/L, respectively).

Consumer Sensory

Untrained consumers (n = 64) evaluated liking attributes (appearance, aroma, astringency, overall flavor and overall liking) and JAR attributes (appearance and astringency) of co-fermented wines at the 0.8 mg/L molecular SO_2 level two months after bottling. Co-fermentation treatment impacted all liking attributes of the wines **(Table 6).** Regardless of attributes, the wine treatments had liking scores that ranged from 4 (dislike slightly) to 7 (like moderately). In general, 100% Merlot wines ranked lower than most of the other treatment wines for all attributes. For overall flavor and overall liking, 100% Merlot wines ranked lower (4.41 and 4.50, respectively) than 75% Merlot - 25% Noble wines (6.31 and 6.30, respectively) and 50% Merlot-50% Noble wines (5.66 and 5.69 respectively). 100% Merlot wines ranked lowest (5.08) in appearance compared to all other wine treatments, which showed no difference in appearance (average 7.15). For aroma, 100% Merlot wines ranked lower (5.47) compared to 75% Merlot - 25% Noble (6.61) and 50% Merlot-50% Noble wines (6.34). For astringency, 100% Merlot wines ranked lower (5.34) compared to 75% Merlot - 25% Noble wines (6.34).

Ideally in JAR evaluations, it is desired that at least 75% of participants consider an attribute JAR. For JAR attributes, a higher percentage of consumers scored the co-fermentation treatments with varying proportions of Noble and Merlot (75-78%) as JAR for appearance compared to their monovarietal counterparts (22% for 100% Merlot and 64% for Noble) **(Table 7).** Consumers (75%) indicated the appearance of the 100% Merlot was too light. For astringency, 61% of consumers considered the 75% Merlot-25% Noble wine as JAR. The astringency level of the 50% Merlot-50% Noble was faint to none for 34% of consumers while 31% of consumers consider astringency strong to overpowering for the 100% Merlot.

In ranking the wines from most to least preferred, 38 % of the consumers ranked the 75% Merlot-25% Noble wines first (most preferred), 30% ranked the 50% Merlot – 50% Noble wines second, 31% ranked the 100% Merlot wines third, 30% ranked the 25% Merlot –75% Noble wines fourth, and 42% ranked the 100% Merlot wines (least preferred) **(Figure 12).** In general, wines co-fermented with varying proportions of Merlot and Noble grapes had decreasing cumulative percentage scores from most preferred (81%) to least preferred (34%). This indicated consumer preference for wines produced with some proportion of Noble and Merlot grapes compared to the monovarietal wines in this study.

Lastly, each consumer was asked to provide a comment on aroma for each wine evaluated. A word-cloud for each treatment was generated to visualize the consumer sensory differences among the five co-fermentation treatments, based on frequency of the descriptors listed for aroma **(Figure 13).** All wines were predominantly described as fruity aroma by consumers. The top three aroma attributes for each treatment, from most to least, were: 100% Merlot: Fruity, faint, and solvent; 75% Merlot-25% Noble: fruity, grapey, and tied for earthy, chemical, musty/moldy; 50% Merlot-50% Noble: fruity, candied, and tied for earthy and grapey; 25% Merlot-75% Noble: fruity, floral, and candied; and 100% Noble: fruity, candied, and chemical. The 100% Merlot was the only wine where several consumers described the aroma as being too faint. Wines with at least 25% Noble grapes were described as having candied aromas, a contribution most likely from the furaneol (burnt sugar) and o-aminoacetophenone (sweet, grapey) volatile compounds that have been previously identified in muscadine grape juice (Baek et al. 2006, Bhattarai et al. 2023). Three of the five co-fermentation treatments were described as having a musty/moldy aroma (100% Merlot, 75%-Merlot-25% Noble, and 25% Merlot-75% Noble), though the 50% Merlot-50% Noble blend was not. It’s possible at the 50/50 blend ratio, the musty/moldy aromas are at different concentrations and are perceived as less offensive aromas, such as smoky/peppery and earthy, or are overpowered by other volatile compounds.

Conclusions

In this study, the quality impacts of co-fermenting *Vitis rotundifolia* (Noble) and *Vitis vinifera* (Merlot) grapes were evaluated during storage to better assess the potential for improving color and sensory attributes in wines produced, in part, from native grape varieties, which have traditionally exhibited less stable color and flavor characteristics than *vinifera* wines.

At bottling, wines co-fermented with increasing proportions of Noble grapes showed higher titratable acidity, lower pH, lower ethanol content, and lower free SO_2 levels. The addition of Noble grapes to the co-fermentation treatments resulted in lower-pH wines, which helped maintain free SO_2 at a consistent and protective level during storage. As the proportion of Merlot decreased and Noble increased, the difference between free and bound sulfur forms grew larger—possibly due to the greater number of hydroxyl groups and oxidation-prone aglycones associated with the diglucoside anthocyanins present in Noble.

During storage, free SO_2 levels decreased across all treatments, as expected. As the percentage of Noble decreased and molecular SO_2 increased in the blends, L* values rose, resulting in lighter-colored wines. Merlot wines were significantly lighter than all other treatments, with L* increasing from 0 to 6 months in 100% Merlot wines but remaining stable in all others. This pattern underscores the importance of Noble grapes in producing darker-colored wines, regardless of storage period or SO_2 level.

As SO_2 levels increased, red color, brown color, and color density decreased across all treatments, except 100% Noble. However, regardless of storage time, each 25% increase in the proportion of Noble resulted in higher red color, brown color, and color density. At 6 months storage, 100% Noble wines exhibited the highest values for all three color metrics. In general, wines containing both Merlot and Noble had higher polymeric pigment compared to the monovarietal 100% Merlot and 100% Noble wines, suggesting that co-fermentation may promote more stable color formation.

From 0 to 6 months of storage, polymeric pigments and total phenolics increased across all co-fermentation treatments, while monomeric anthocyanins decreased in blends containing at least 50% Noble. This trend indicates that the formation of anthocyanin–tannin complexes (polymeric pigments) was particularly important in blends with moderate proportions of Noble grapes.

Wines co-fermented with 75% or more Noble were rated higher in consumer sensory evaluation than most other treatments for overall flavor and overall liking. Appearance was also preferred in wines with mixed proportions of Noble and Merlot compared to the 100% monovarietal wines. Consumers described all treatments as fruity, but those containing at least 25% Noble were most frequently associated with candy-like aromas, while 100% Merlot wines were often described as having aromas that were “too faint.”

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Table 1. Initial composition of co-fermentation treatments of musts at harvest from Noble and Merlot grapes (2024).

Co-fermentation treatment	**Soluble solids (%)**	**pH**	**Titratable acidity (% tartaric)**
100% Merlot	22.3 b [a]	3.92 a	0.26 b
75% Merlot/25% Noble	24.2 a	3.86 ab	0.50 a
50% Merlot/50% Noble	22.8 ab	3.64 bc	0.53 a
25% Merlot/75% Noble	22.0 b	3.53 c	0.57 a
100% Noble	17.3 c	3.20 d	0.53 a
P-value	***0.0002***	***0.0004***	***0.0016***

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

Table 2. Composition at bottling (0-months storage) of wines produced from co-fermentation of Noble and Merlot grapes (2024).

Co-fermentation treatment	**pH**	**Titratable acidity (% tartaric)**	**Free sulfur dioxide (SO_2) (mg/L)**	**Bound SO_2 (mg/L)**	**Total SO_2 (mg/L)**	**Ethanol (%)**
100% Merlot	4.04 a [a]	0.47 e	123.20 a	155.80	279.00	15.85 a
75% Merlot-25% Noble	3.76 b	0.55 d	54.67 ab	154.05	208.72	14.86 b
50% Merlot-50% Noble	3.45 c	0.67 c	28.99 b	127.80	156.79	13.62 c
25% Merlot-75% Noble	3.29 d	0.76 b	17.60 b	128.05	145.65	12.55 d
100% Noble	3.10 e	0.88 a	16.13 b	166.80	182.93	11.46 e
P value	***<0.0001***	***<0.0001***	***0.0130***	*0.2267*	*0.0965*	***<0.0001***

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

Table 3. Color and phenolics at bottling (0-months storage) of wines produced from co-fermentation of Noble and Merlot grapes (2024).

Co-fermentation treatment	**L***	**Hue (°) angle**[a]	**Chroma**	**Red color**[b]	**Brown color**[b]	**Color density**[b]	**Polymeric color**[b]	**Total phenolics (mg gallic acid/L)**	**Total monomeric anthocyanins (mg/L)**
100% Merlot	6.78 a[c]	380.82 a	28.86 a	1.79 e	1.87 d	3.66 e	0.71 b	1020.30 e	221.51 e
75% Merlot-25% Noble	2.24 b	374.29 b	13.87 b	3.23 d	2.82 c	6.05 d	0.82 a	1471.82 d	806.01 d
50% Merlot-50% Noble	1.50 b	372.72 b	6.40 bc	5.53 c	3.87 b	9.40 c	0.83 a	1798.39 c	1390.65 c
25% Merlot-75% Noble	0.31 b	372.78 b	1.48 c	7.86 b	4.83 b	12.69 b	0.78 a	2065.45 b	1896.12 b
100% Noble	0.20 b	373.00 b	0.85 c	9.50 a	5.27 a	14.77 a	0.69 b	2242.13 a	2133.69 a
P value	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***

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

Table 4. Main effects and interactions of composition attributes for wines produced from co-fermentation of Noble and Merlot grapes with different molecular sulfur dioxide levels (SO_2) at bottling and stored for 6-months at 15°C (2024).

Effect	**pH**	**Titratable acidity (% tartaric)**	**Free SO_2 (mg/L)**	**Bound SO_2 (mg/L)**	**Total SO_2 (mg/L)**
Co-fermentation treatment (Co-F)					
100% Merlot	4.03 a [a]	0.47 e	110.97 a	132.97 a	243.94 a
75% Merlot-25% Noble	3.76 b	0.55 d	48.80 b	97.23 b	146.03 b
50% Merlot-50% Noble	3.44 c	0.68 c	24.76 c	73.60 c	98.36 c
25% Merlot-75% Noble	3.27 d	0.76 b	13.97 d	74.76 c	88.73 c
100% Noble	3.08 e	0.87 a	11.93 d	129.07 a	141.00 b
P value	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
Sulfur dioxide treatment (SO_2)					
0.0 mg/L	3.52	0.67	0.40 c	78.72 c	79.12 c
0.8 mg/L	3.51	0.67	41.10 b	107.29 b	148.39 b
1.5 mg/L	3.51	0.67	86.76 a	118.56 a	203.32 a
P value	*0.2719*	*0.9545*	***<0.0001***	***<0.0001***	***<0.0001***
Storage (S)					
Month 0	3.53 a	0.67	48.12 a	146.50 a	194.62 a
Month 6	3.50 b	0.66	36.06 b	56.55 b	92.60 b
P value	***<0.0001***	*0.4948*	***<0.0001***	***<0.0001***	***<0.0001***
Co-F x SO_2 (P value)	*0.0626*	*0.4986*	***<0.0001***	***<0.0001***	***<0.0001***
Co-F x S (P value)	*0.0700*	*0.4973*	***0.0045***	***<0.0001***	***<0.0001***
SO_2 x S (P value)	***0.0442***	*0.2522*	***<0.0001***	***<0.0001***	***<0.0001***
Co-F x SO_2 x S (P value)	*0.4547*	*0.9069*	*0.1175*	***0.0008***	***0.0012***

[a] Means with different letters for each attribute within a day are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test and students t-test for Storage (S).

Table 5. Main effects and interactions of color and phenolic attributes for wines produced from co-fermentation of Noble and Merlot grapes with different molecular sulfur dioxide levels (SO_2) at bottling and stored for 6-months at 15°C (2024).

Effect	**L***	**Hue angle (°)** [a]	**Chroma**	**Red color** [b]	**Brown color** [b]	**Color density** [b]	**Polymeric color** [b]	**Total phenolics (mg/L)**	**Total monomeric anthocyanins (mg/L)**
Co-fermentation treatment (Co-F)									
100% Merlot	8.03 a[a]	382.07 a	30.22 a	1.79 e	1.88 e	3.67 e	0.89 c	1189.42 e	220.11 e
75% Merlot-25% Noble	2.41 b	374.23 b	15.34 b	3.18 d	2.83 c	6.01 d	1.04 ab	1583.49 d	752.68 d
50% Merlot-50% Noble	1.18 c	372.93 b	6.31 c	5.26 c	3.88 c	9.14 c	1.08 a	2011.17 c	1233.27 c
25% Merlot-75% Noble	0.46 c	371.81 b	2.28 d	7.27 b	4.73 b	12.00 b	1.02 b	2320.70 b	1659.06 b
100% Noble	0.57 c	366.47 c	1.48 d	8.79 a	5.13 a	13.92 a	0.89 c	2563.79 a	1887.09 a
P value	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
Sulfur dioxide treatment (SO_2)									
0.0 mg/L	1.18 c	371.70 b	6.34 c	5.73 a	4.00 a	9.73 a	1.04 a	1932.56	1104.91 b
0.8 mg/L	2.79 b	373.71 a	11.88 b	5.22 b	3.63 b	8.85 b	0.98 b	1916.65	1156.34 a
1.5 mg/L	3.61 a	375.10 a	15.16 a	4.83 c	3.44 c	8.26 c	0.93 c	1951.93	1190.08 a
P value	***<0.0001***	***0.0008***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	*0.5241*	***0.0002***
Storage (S)									
Month 0	2.21 b	374.72 a	10.29 b	5.58 a	3.73 a	9.31 a	0.94	1708.82 b	1289.60 a
Month 6	2.85 a	372.28 b	11.96 a	4.93 b	3.65 b	8.58 b	0.96	2158.61 a	1011.29 b
P value	***0.0004***	***0.0008***	***0.0081***	***<0.0001***	***0.0009***	***<0.0001***	*0.1251*	***<0.0001***	***<0.0001***
Co-F x SO_2 (P value)	***<0.0001***	***<0.0001***	***<0.0001***	***0.0001***	***<0.0001***	***<0.0001***	***0.0187***	*0.2378*	*0.5220*
Co-F x S (P value)	***0.0057***	***<0.0001***	*0.4636*	***<0.0001***	***0.0002***	***<0.0001***	***0.0034***	***0.0021***	***<0.0001***
SO_2 x S (P value)	***0.0057***	*0.8303*	***0.0333***	*0.0768*	*0.6177*	*0.4078*	***<0.0001***	*0.2445*	*0.1293*
Co-F x SO_2 x S (P value)	*0.0878*	*0.7880*	*0.5406*	*0.4495*	*0.4385*	*0.4706*	*0.0557*	*0.1600*	*0.8704*

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

[b] Red color calculated as absorbance of wine at 520 nm, brown color at 420 nm, color density at 520 nm + 420 nm, and polymeric pigment anthocyanin-tannin complexes at 520 nm.

[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 6. Attributes evaluated by a consumer sensory panel using a nine-point hedonic scale[a] for wine at 2 months storage at 15°C produced from different co-fermentation treatments of Merlot grapes (Washington, 2024) and Noble grapes (Arkansas 2024).

Co-fermentation treatment	**Appearance**	**Aroma**	**Astringency**	**Overall flavor**	**Overall liking**
Consumer Panel					
100% Merlot	5.08 b[b]	5.47 b	5.34 b	4.41 c	4.50 c
75% Merlot:25% Noble	7.19 a	6.61 a	5.27 a	6.31 a	6.30 a
50% Merlot:50% Noble	7.25 a	6.34 a	5.80 ab	5.66 ab	5.69 ab
25% Merlot:75% Noble	7.14 a	6.31 ab	5.59 ab	5.22 bc	5.23 bc
100% Noble	7.00 a	6.08 ab	5.52 ab	4.86 bc	4.94 bc
P value	***<0.0001***	***0.0053***	***0.0439***	***<0.0001***	***<0.0001***

[a] Wines were evaluated by a consumer sensory panel (2-months storage by 64 consumer panelists) using a nine-point hedonic 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).

[b] Means with the different letters for each attribute within panel type are significantly different ($P<0.05$) using Tukey's Honest Significant Difference (HSD) test.

Table 7. Percent (%) of responses for consumer sensory analysis using a collapsed five-point just-about-right (JAR) scale [a] for wine at 2-months storage at 15°C produced produced from different co-fermentation treatments of Merlot grapes (Washington, 2024) and Noble grapes (Arkansas 2024).

[a] Wines were evaluated by a consumer panelist (2-months storage by 64 panelists) using a five-point JAR scale (1 = much to low; 2 = too low; 3 = JAR; 4 = too much; 5 = much too much) collapsed to Too low, JAR, and Too much.

	Appearance			**Astringency**		
Co-fermentation treatment	**Too light**	**JAR**	**Too dark**	**Faint**	**JAR**	**Strong**
Consumer Panel						
100% Merlot	75	22	3	28	41	31
75% Merlot:25% Noble	17	78	5	20	61	19
50% Merlot:50% Noble	8	75	17	34	44	22
25% Merlot:75% Noble	2	75	23	25	50	25
100% Noble	3	64	33	30	44	27

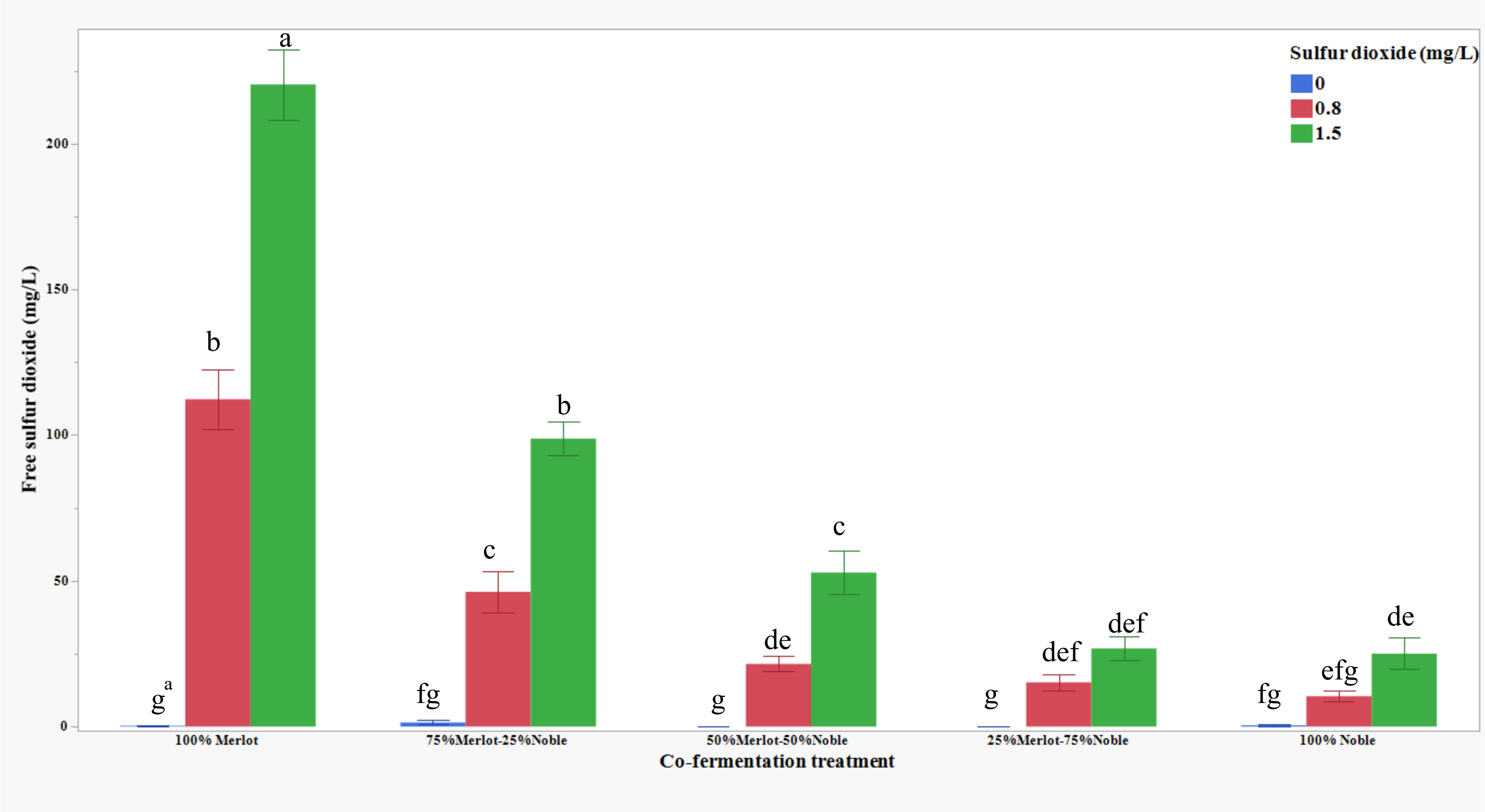


Figure 1. Effect of co-fermenation treatment and molecular sulfur dioxide levels on free sulfur dioxide levels in wines 2024.
[a]Means with different letters are significantly different ($p < 0.05$) according to Tukey's Honest Significance Difference (HSD) test.

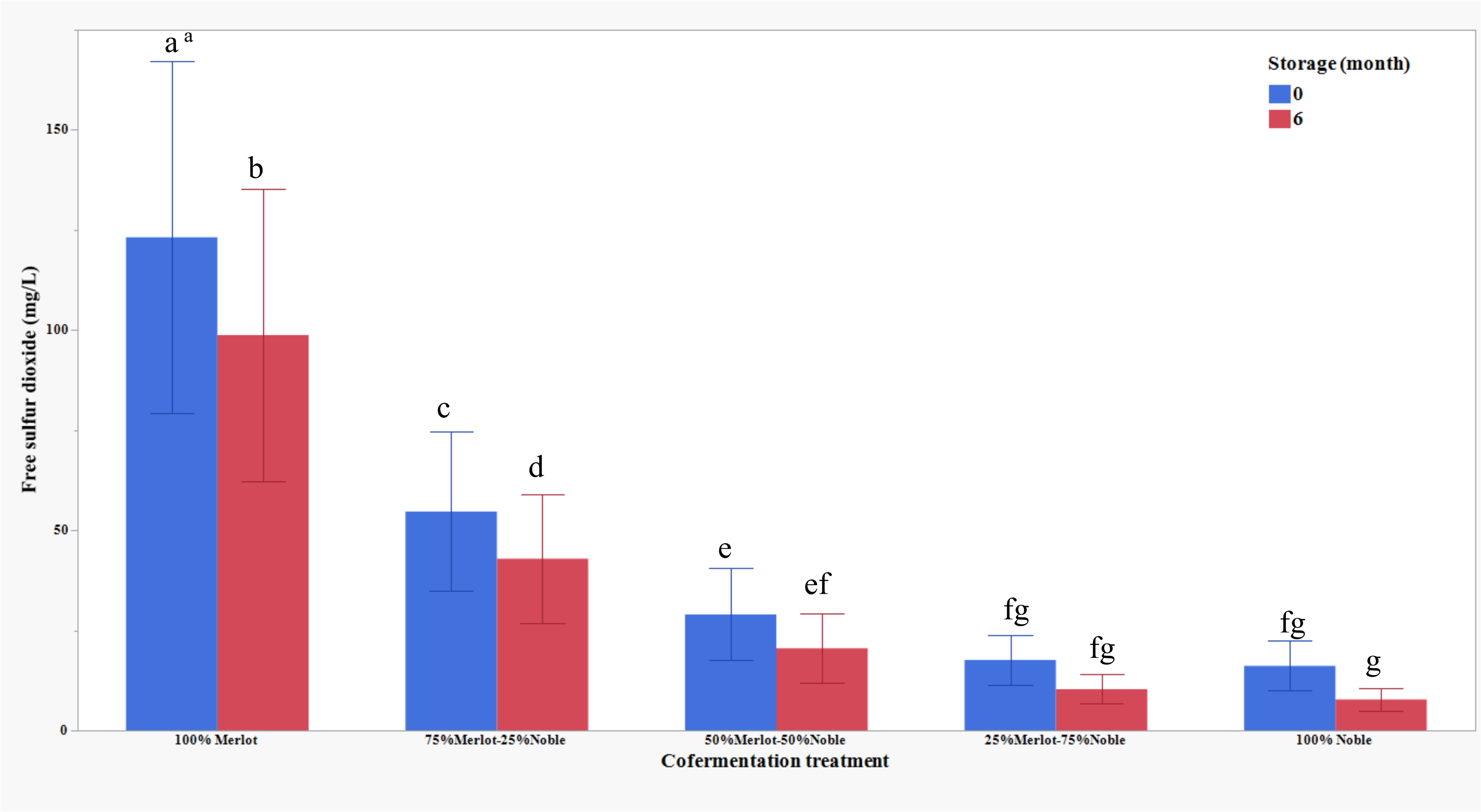


Figure 2. Effect of co-fermenation treatment and storage (0-and 6-months at 15°C) on free sulfur dioxide levels in wines in 2024. [a]Means with different letters are significantly different ($p < 0.05$) according to Tukey's Honest Significance Difference (HSD) test.

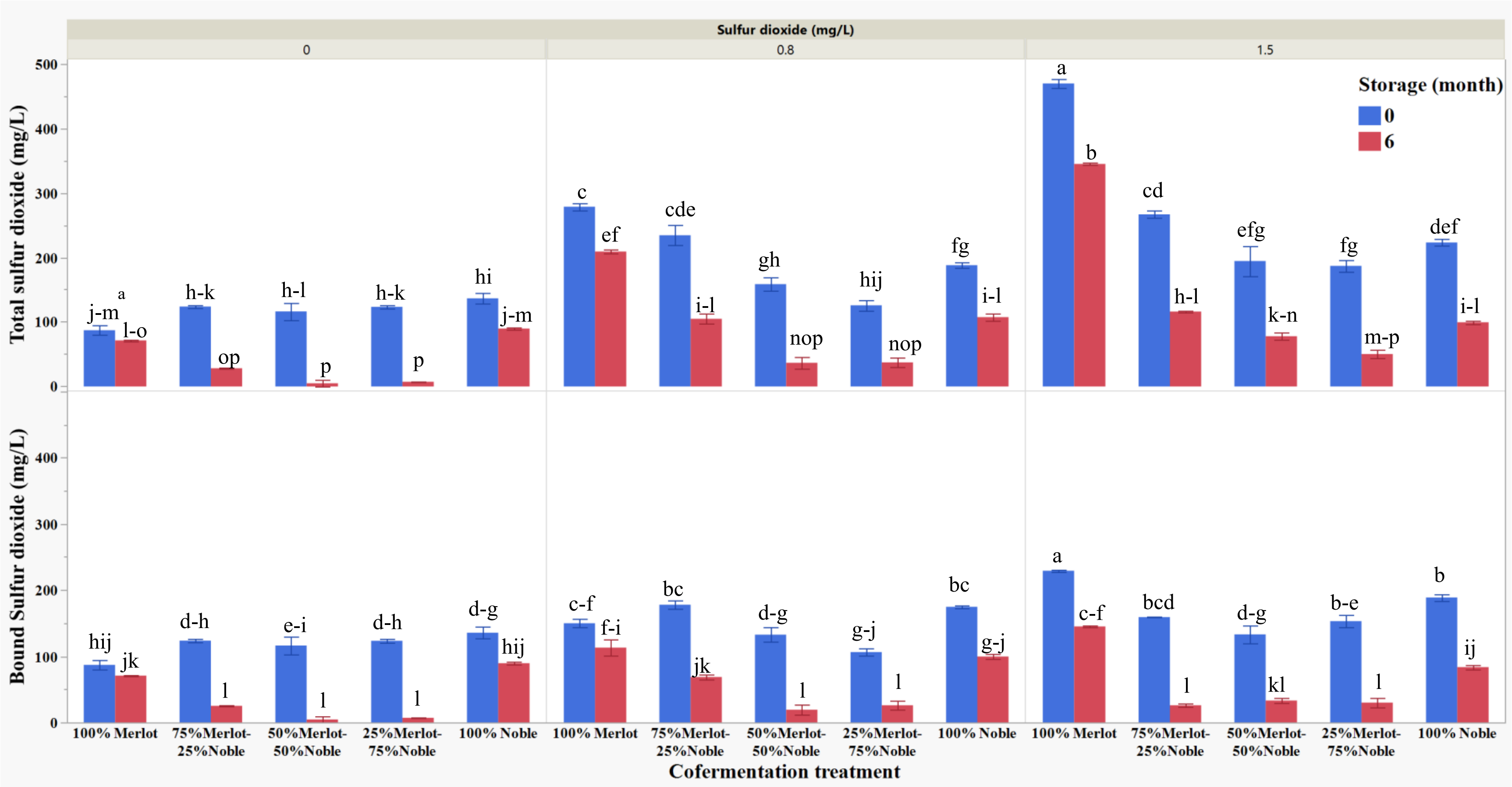


Figure 3. Effect of co-fermentation treatment, storage (0-and 6-months at 15°C), and molecular sulfur dioxide levels on free, bound, and total sulfur dioxide levels in wines in 2024.

[a] Means with different letters are significantly different ($p < 0.05$) according to Tukey's Honest Significance Difference (HSD) test.

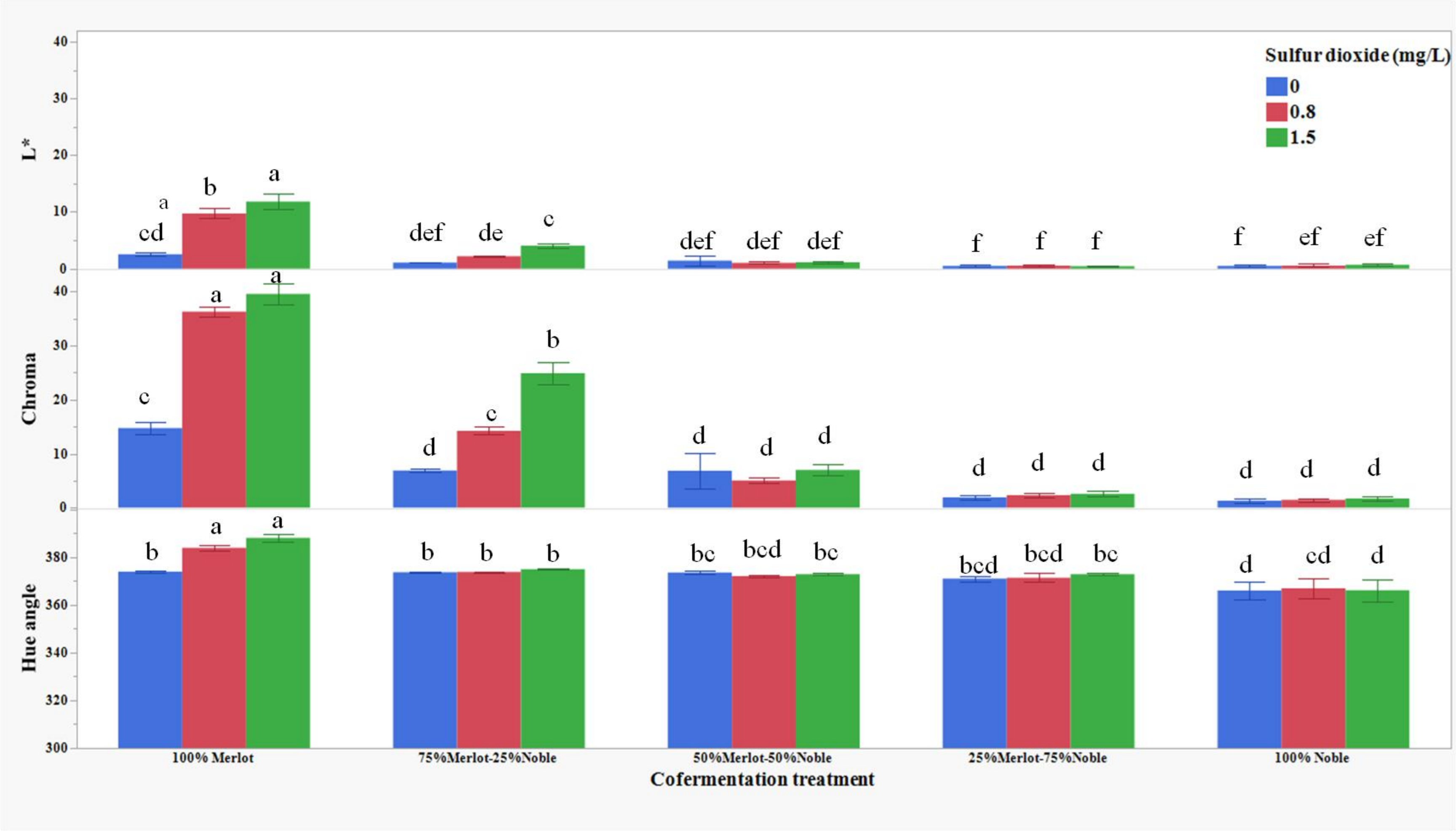


Figure 4. Effect of co-fermentation treatment and molecular sulfur dioxide on L*, hue angle, and chroma of wines in 2024.
[a]Means with different letters are significantly different ($p < 0.05$) according to Tukey's Honest Significance Difference (HSD) test.

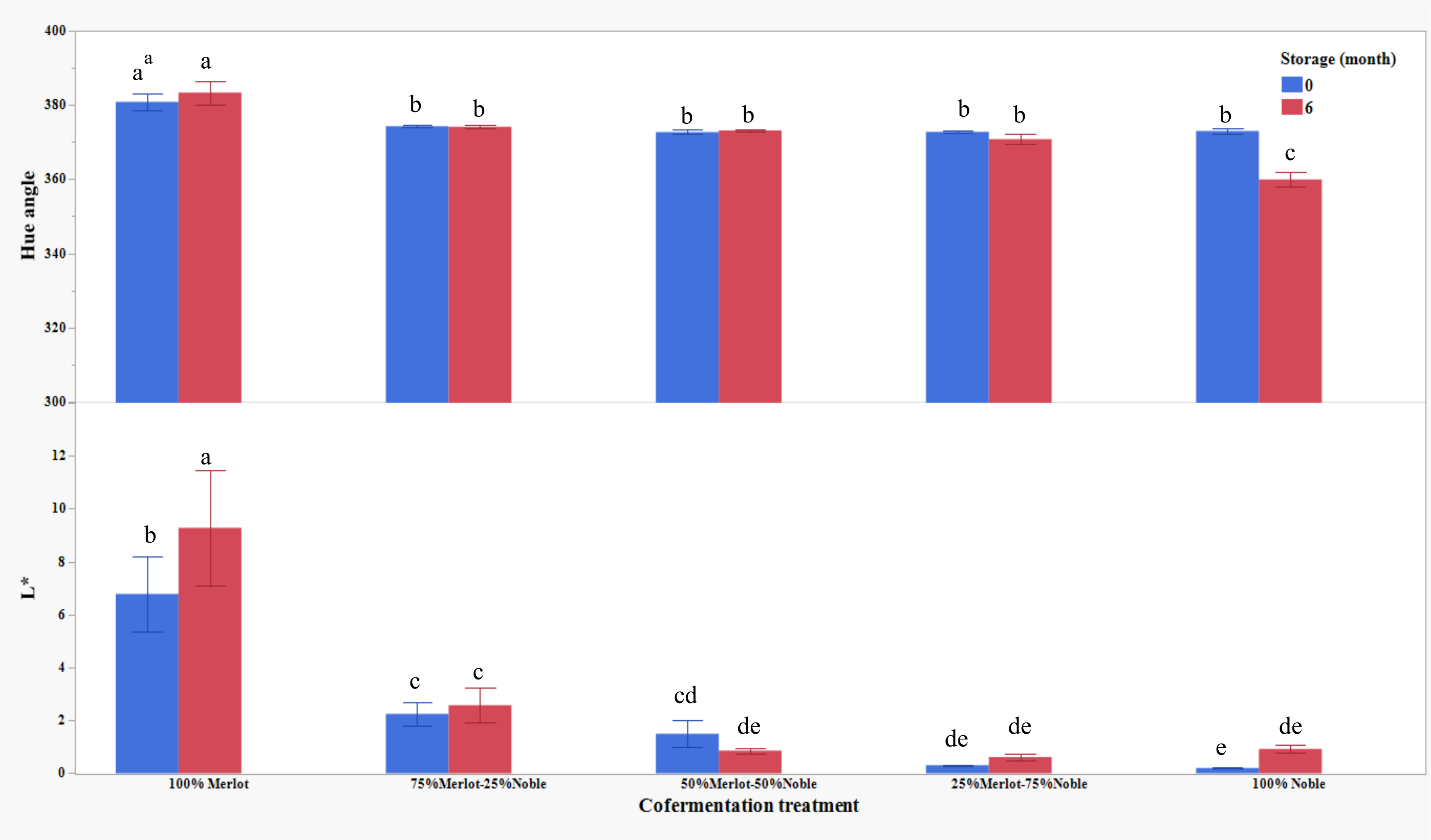


Figure 5. Effect of co-fermenation treatment and molecular sulfur dioxide on L* and hue angle of wines in 2024.
[a]Means with different letters are significantly different ($p < 0.05$) according to Tukey's Honest Significance Difference (HSD) test.

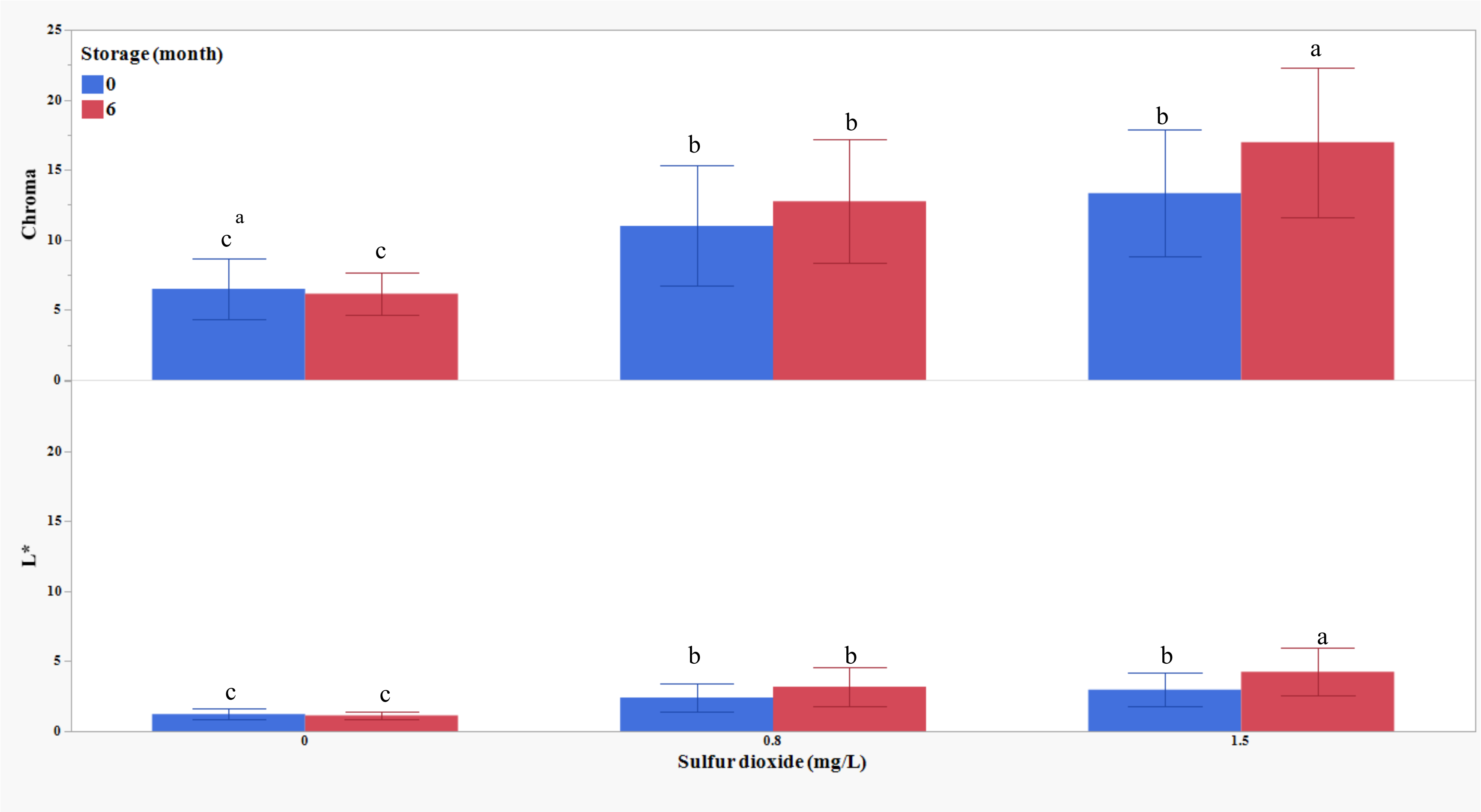


Figure 6. Effect of molecular sulfur dioxide and storage (0-and 6-months at 15°C) on L*and chroma of wines in 2024.
[a]Means with different letters are significantly different ($p < 0.05$) according to Tukey's Honest Significance Difference (HSD) test.

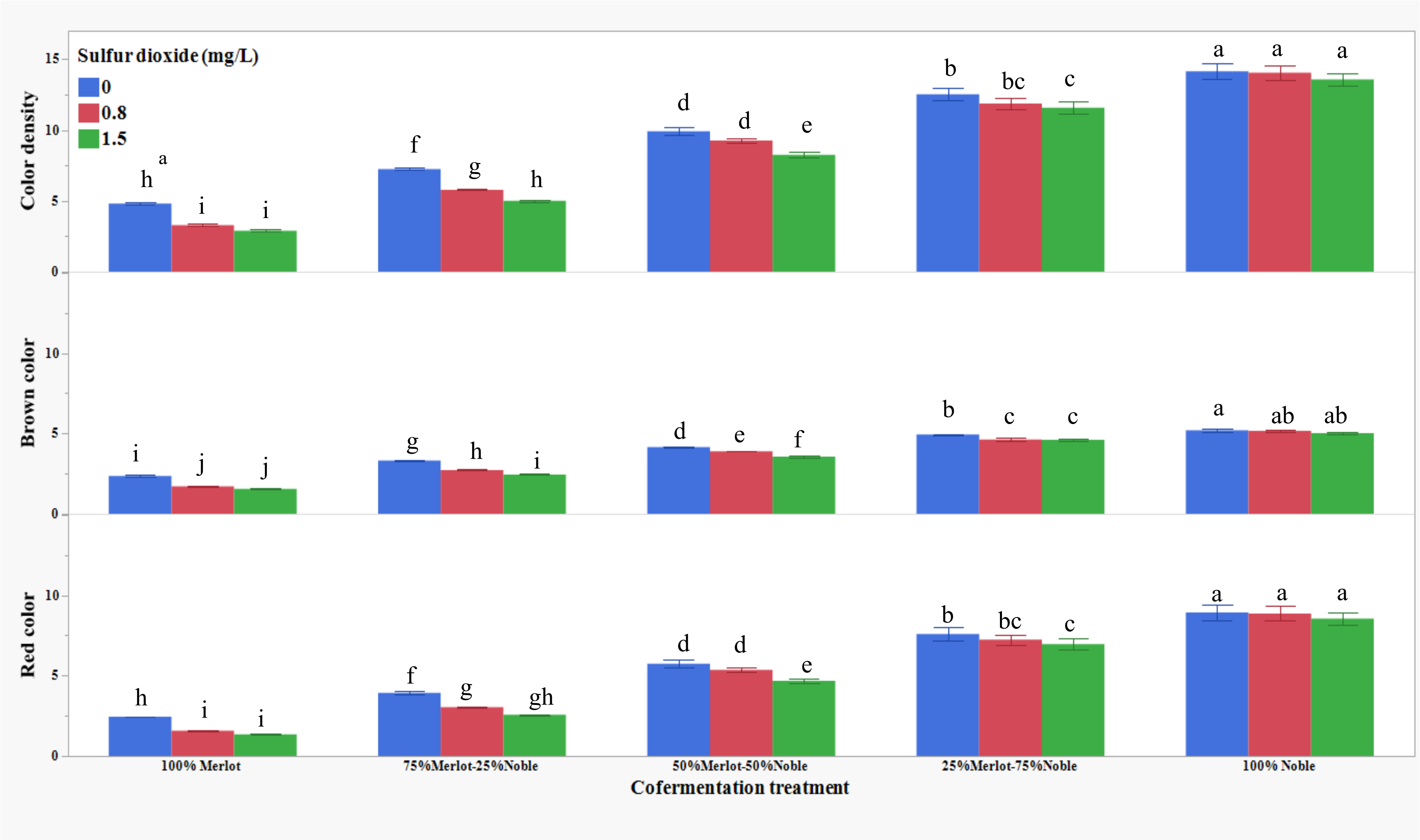


Figure 7. Effect of co-fermentation treatment and molecular sulfur dioxide on red color, brown color and color density of wines in 2024.

[a] Means with different letters are significantly different ($p < 0.05$) according to Tukey's Honest Significance Difference (HSD) test.

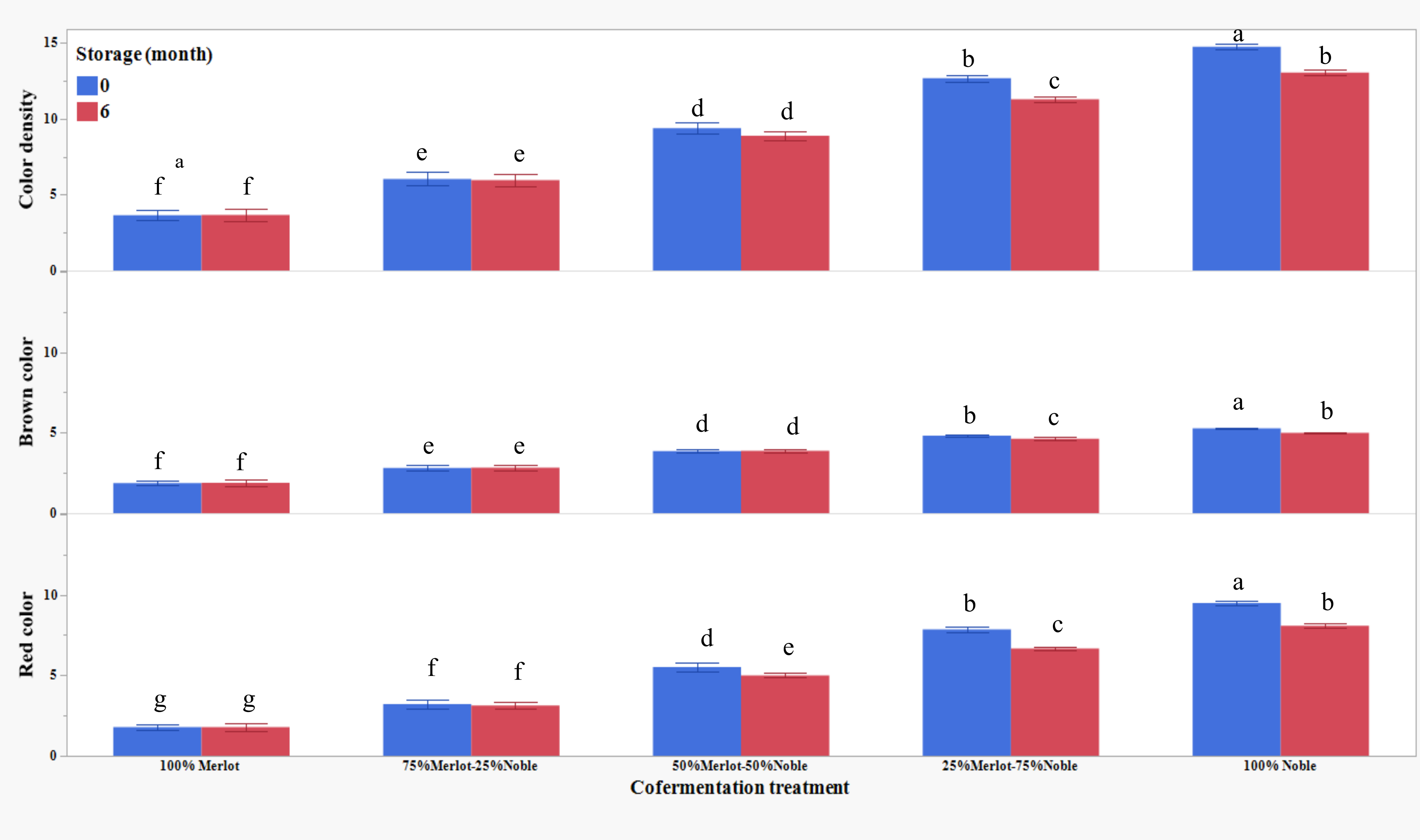


Figure 8. Effect of co-fermenation treatment and storage (0-and 6-months at 15°C) on red color, brown color and color density for wines in 2024.

[a]Means with different letters are significantly different ($p < 0.05$) according to Tukey's Honest Significance Difference (HSD) test.

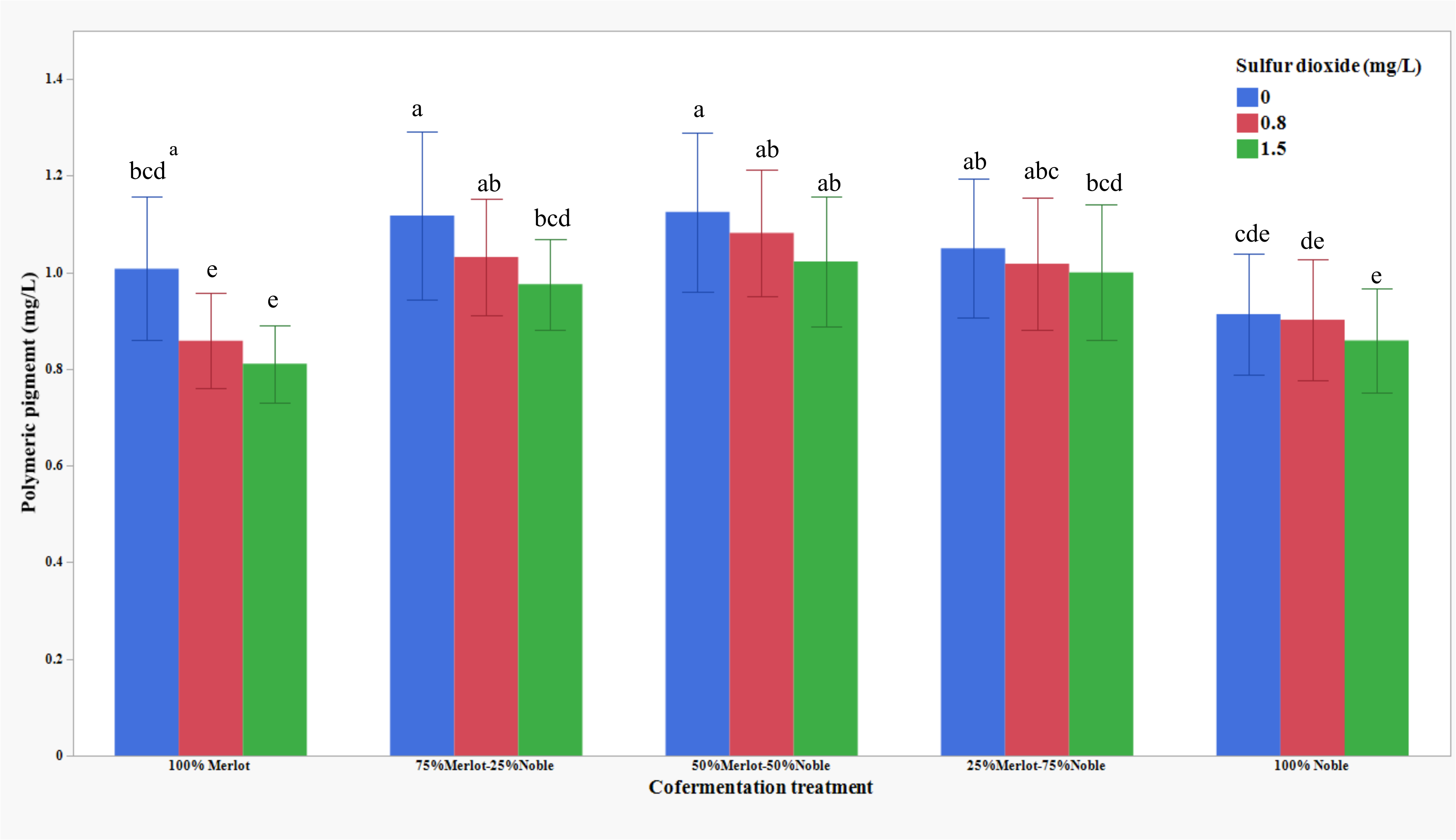


Figure 9. Effect of co-fermenation treatment and molecular sulfur dioxide on polymeric pigment for wines in 2024.
[a]Means with different letters are significantly different ($p < 0.05$) according to Tukey's Honest Significance Difference (HSD) test.

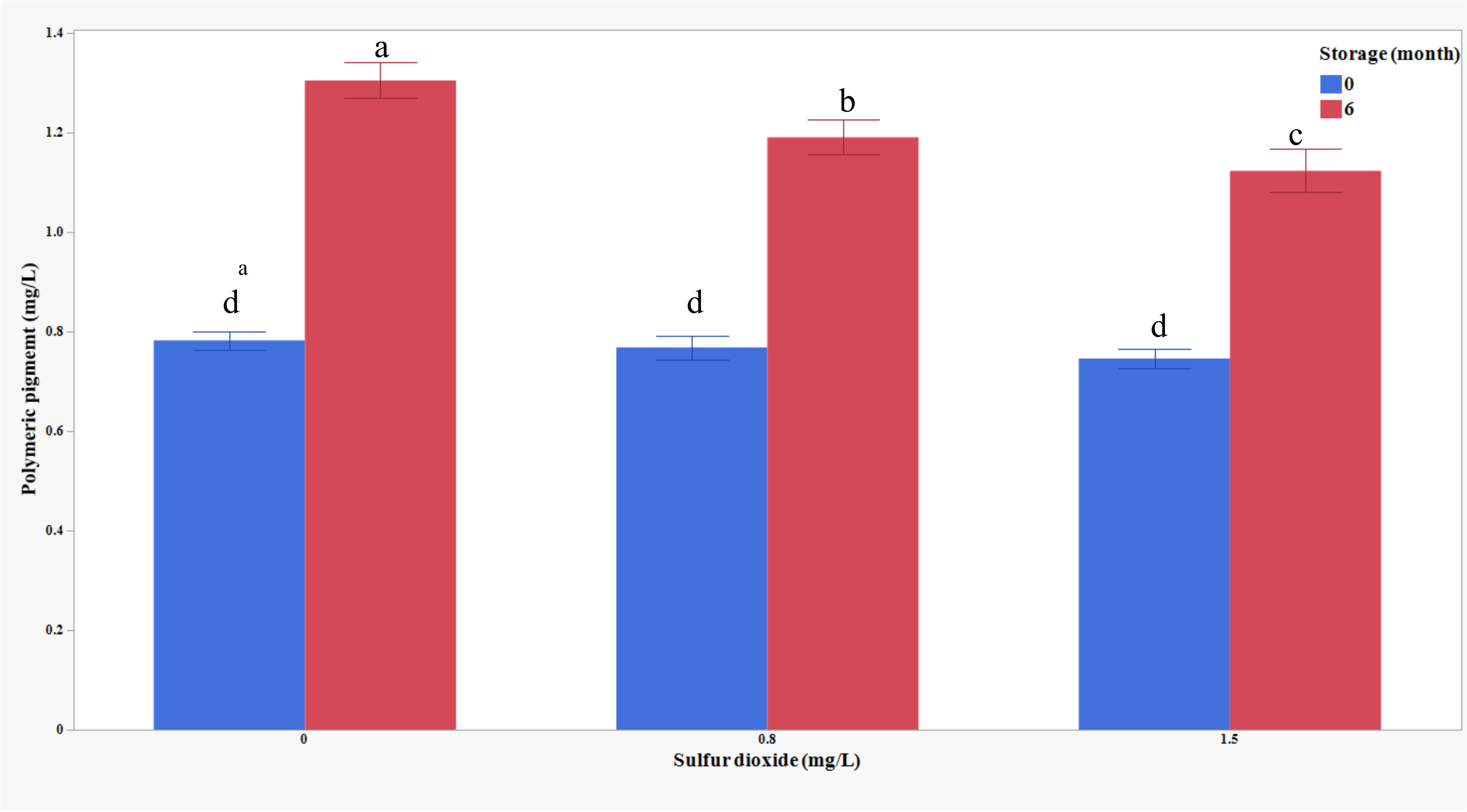


Figure 10. Effect of molecular sulfur dioxide and storage (0-and 6-months at 15°C) on polymeric pigment for wines in 2024.
[a]Means with different letters are significantly different ($p < 0.05$) according to Tukey's Honest Significance Difference (HSD) test.

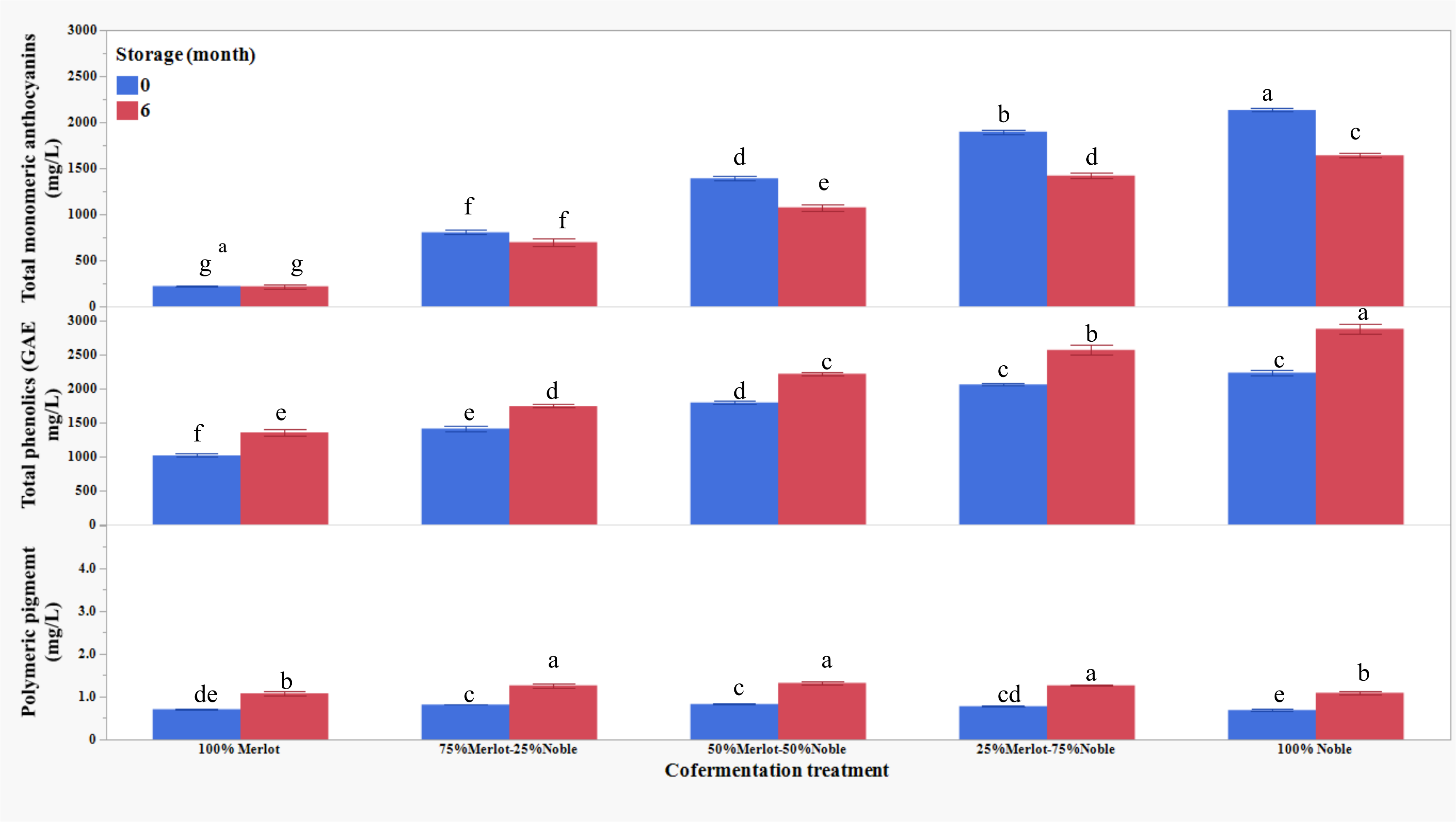


Figure 11. Effect of co-fermenation treatment and storage (0-and 6-months at 15°C) on polymeric pigment, total phenolics, and total monomeric anthocyanins for wines in 2024.

[a]Means with different letters are significantly different ($p < 0.05$) according to Tukey's Honest Significance Difference (HSD) tes

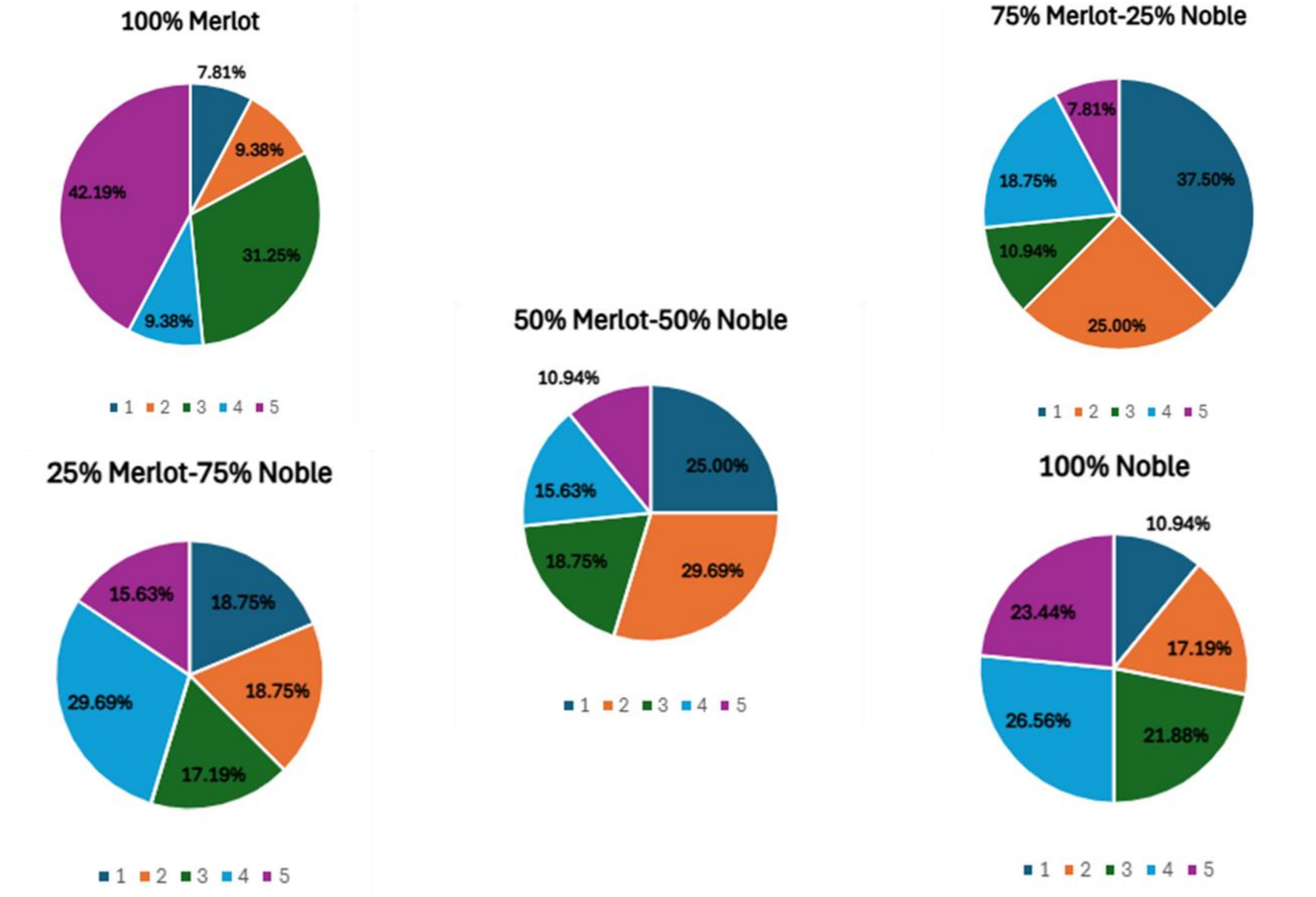
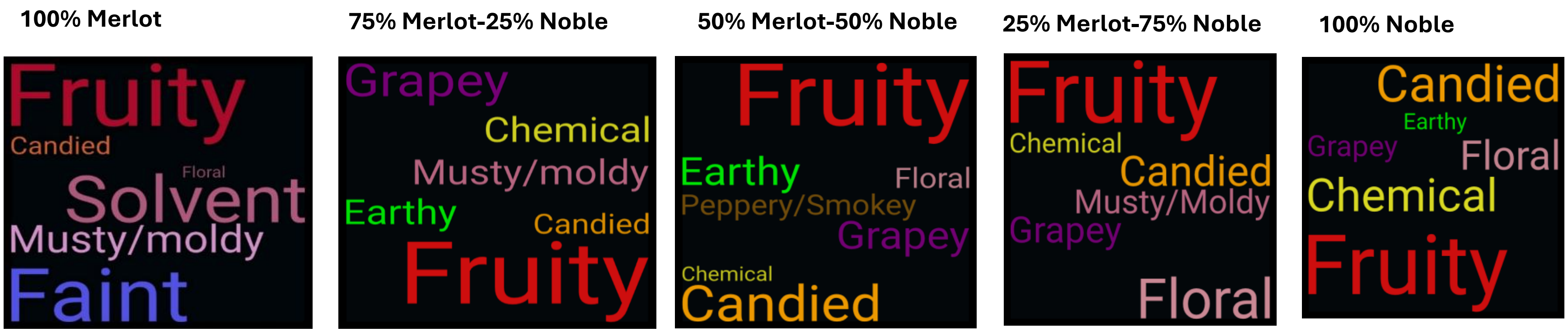


Figure 12. Consumer (n=64) ranked the wines produced from co-fermentation of Noble and Merlot grapes at 0.8 mg/L molecular sulfur dioxide (SO_2) in 2024. Wines were evaluated at 2-mo storage at 15°C. Ranking: 1 = most preferred to 5 = least preferred.



100% Merlot

75% Merlot-25% Noble

50% Merlot-50% Noble

25% Merlot-75% Noble

100% Noble

Figure 13. Word cloud for the top three descriptors given by consumers (n = 64) to describe the aroma of wines produced from co-fermentation of Noble and Merlot grapes at 0.8 mg/L molecular sulfur dioxide (SO_2) in 2024. The wines were evaluated at 2-mo storage at 15°C.