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Research Article

Chemical and Sensory Effects of Cofermentation and Blending of Malbec and Merlot Wines from the Central Coast of California

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Background and Aims. Cofermentation and blending are traditional winemaking practices. This study reports their comparative chemical and sensory outcomes. *Methods and Results*. Malbec and Merlot were made into monovarietal wines, cofermented (COF), blended postalcoholic (PAF), and postmalolactic fermentation (PMLF), at a 50/50 proportion. Wines were followed up to 3 years of bottle aging. Tannins were 50% higher in monovarietal Merlot wines, which improved production of large polymeric pigments in Merlot-based cofermented and blended wines. Addition of Malbec favored production of small polymeric pigments. After 3 years of bottle aging, polymeric pigments were higher in cofermented and blended wines. PMLF blended wines showed 15% improved copigmentation and 25% increase in wine colour. The perception of wine length was positively correlated with red fruit aroma ($R^2 = 0.94$ and $p = 0.006$) and negatively correlated with earthy aroma ($R^2 = 0.91$ and $p = 0.012$). *Conclusions*. Blending postalcoholic fermentation highlighted more of the individual character of each monovarietal wine in the final blend. Cofermentation and postmalolactic fermentation blending tended to equalize the sensory profile of the resulting wines but also showed higher complexity of aromas than monovarietal wines. *Significance of the Study*. Cofermentation and blending are both suitable winemaking practices for increasing the polymeric pigment content and the sensory complexity of the resulting wines.

1. Introduction

Vitis vinifera L. grapes, related species, and their corresponding wines show a wide array of unique chemical and sensory characteristics that are the result of their genetic makeup, which, in addition to environmental influences, is primarily informed by the grape cultivars (cvs) [1]. Wines made from single grape cultivars may be referred as varietal wines and there is currently documentation of wine being produced from 1,368 vine cvs, with further estimates that in Italy alone, 380 grapes cvs produce wines in commercial circulation [2]. These varietal wines contrast from wines produced by blending monovarietal wines prior or after completion of malolactic fermentation, or, less commonly, by cofermentation of different grape cvs. Cofermentation is a winemaking technique that may have been refined from a traditional viticultural practice known as "field blends," whereby different grape cvs are interplanted in the same block, harvested and vinified at the same time [3]. During cofermentation, two or more different grape cvs are harvested separately but crushed and fermented together, allowing the simultaneous extraction of phenolic

compounds, aroma precursors, and free aromas during alcoholic fermentation. This can be achieved by cofermenting red and white cvs, or by cofermenting two or more red cvs [4]. For example, in France, the Côte Rôtie appellation of the northern Rhône Valley permits the cofermentation of Syrah with up to 20% addition of Viognier, and in Italy, additions of Trebbiano or Malvasia (white) to Sangiovese (red) were practiced in the past. The practice of cofermenting white and red cvs allegedly favors copigmentation and colour stabilization whereby colourless "cofactors" mainly represented by flavonols, form stable and coloured planar stackings with anthocyanins, resulting in an hyperchromic shift (colour enhancement) and a bathochromic shift (colour shift towards more bluish hues) [5]. Certain white and red cvs may be reportedly richer in these skin cofactors, justifying their addition to red ferments during cofermentation. However, this latter assumption has never been scientifically validated. For example, a study in which Syrah was cofermented with different white cvs, including Viognier, Marsanne, Roussanne, Picpoul, and Grenache Blanc reported that cofermented wines were generally lower anthocyanin, although cofermentation with Viognier increased the aromatic complexity (i.e., more diversity of aromas) of the cofermented wines [6]. In another study focusing on the wines' volatile composition in which Monastrel (Mourvèdre) was cofermented with either Merlot or Cabernet Sauvignon, a 60 : 40 proportion of Monastrel : Merlot resulted in wines with higher aromatic complexity, and with more odorants in the fruity and sweet aroma series [7]. A study in which Cencibel (Tempranillo) was cofermented with Bobal and Moravia Agria reported all cofermented wines showed higher concentrations of bound terpenes and C13-norisoprenoids, implying a more complex chemical profile in cofermented wines [8]. Overall, the aggregate of current research seems to suggest cofermentation of red-red and red-white cvs has little merit in increasing copigmentation (and thus colour), but rather that the value of this practice resides in increasing the sensorial and chemical complexity of the resulting wines.

It is empirically known that different varietal wines have complementary chemical and sensory profiles. Thus, blending monovarietal wines postalcoholic or postmalolactic fermentation, a practice also referred to as coupage, has been and still remains a staple of modern winemaking. Although distinct sensory attributes of the components of a wine blend may combine in a nonlinear manner [9], blending is more technically reliable and logistically easier than cofermentation. This is because monovarietal wines with defined characteristics can be individually assessed through bench trials prior to blending, and many possible blends of two or more monovarietal wines can be assayed in small scale. In a study focusing on the chemistry of blending, Tempranillo based wines were blended with 25% and 10% (v/v) of either Graciano or Cabernet Sauvignon [10]. This study reported a higher concentration of peonidins in the Tempranillo-Graciano blends, of acetyl-glucoside anthocyanins and anthocyanin–pyruvic acid adducts in Tempranillo-Cabernet Sauvignon blends, and of flavanols in the blends from both grape varieties, which resulted in wines of better quality attributes than the base wine [11]. In another study, Cabernet Sauvignon, Merlot, and Cabernet franc monovarietal wines were used to produce 11 two-wine blends and four three-wine blends. It was found that the presence of an intense attribute in a given monovarietal wine was generally masked if the other monovarietal wines in the blend were rated high in some contrary attribute [12]. Although the latter study did not report on the malolactic fermentation status of the wines prior to blending, higher sensory scores and increased complexity in the blended wines were beyond simple averaging effects and several suppressing and amplifying effects were recorded.

Whereas the aggregate of research suggests that cofermentation and blending of monovarietal wines does indeed increase the chemical and sensory complexity of the blended wines, the effects of the timing of blending (e.g., immediately after alcoholic fermentation versus after malolactic fermentation), deserves further exploration. Indeed, the occurrence of alcoholic fermentation and subsequently that of malolactic fermentation affects the reactivity of wine phenolics [13], which in turn have sensory consequences in the blended wines. In the present work, two grape cvs of distinct chemical profile such as Malbec and Merlot were made into monovarietal wines, cofermented at a 50/50 proportion and a portion of their respective monovarietal wines blended postalcoholic and postmalolactic fermentation. Because phenolic reactivity seems to be more pronounced earlier during winemaking and because empirical observations suggest that there may be synergistic and enhancing effects on aroma due to cofermentation, it was hypothesized that cofermentation will result in positive improvements on the chemical and sensory composition of the resulting wines, over postalcoholic and postmalolactic fermentation blending.

2. Materials and Methods

2.1. Grapes and Vineyard Site. Malbec and Merlot grapes, grafted onto 1103P rootstock and trained into a vertical shoot positioning system (spur-pruned), were sourced from the Sunnybrook Ranch, in the Paso Robles American Viticultural Area (AVA) of San Luis Obispo County (Paso Robles, CA). Grapes (1.05 tons for each cvs) were manually harvested in 0.5 tons bins on October 10th, 2018 and transported to the research winery of the Wine and Viticulture Department (California Polytechnic State University San Luis Obispo, California, USA). Forty clusters ($n = 40$) were randomly taken from each grape cvs prior to crushing, as previously described in [14]. Clusters were sorted into three field replicates of 10 clusters each, manually destemmed, crushed manually in Ziplock bags and left to macerate for 4 h prior of determination of Brix, pH, titratable acidity, yeast assimilable nitrogen, and potassium (Supplemental Table 1). Brix was measured with a handheld refractometer (Vee Gee Scientific, Kirkland, WA, USA). Titratable acidity was measured by titrating a known quantity of juice (5 mL) in a deionized water solution against 0.067N NaOH (Fisher Scientific, Waltham, MA, USA) to

a pH endpoint of 8.2 [14]. Yeast assimilable nitrogen (YAN) and L-malic acid were measured enzymatically from juice utilizing an analyzer (Y15 Automatic Analyzer, Admeo, Angwin, CA, USA), and commercially available kits (Biosystems, Barcelona, Spain) [14].

2.2. Winemaking and Experimental Design. The experimental design included monovarietal wines of Malbec and Merlot, as well as treatments established prior to alcoholic fermentation, hereafter referred as cofermentation treatments (COF), and treatments in which the finished wines from these two varietal wines were blended postalcoholic fermentation (hereafter referred as PAF blending), and postmalolactic fermentation (hereafter referred as PMLF blending) (Supplemental Figure 1).

2.2.1. Monovarietal Wines. Upon arrival to the research winery, Malbec and Merlot grapes were independently processed using a destemmer-crusher (Bucher Vaslin, Niederweningen, Switzerland), with the rollers of the crusher disengaged [14]. The musts were placed separately in individual 60-L plastic fermentors (Speidel, Swabia, Germany), with each fermentor receiving 50 kg (±0.1 kg) of must. Fermentors were filled sequentially at 25% increments to ensure proper homogenization of the fruit and consistency on each fermentor. Immediately after crushing, 50 mg/L of SO_2 was added to each fermentor and incorporated with a 30 s punch-down. Diammonium phosphate (Fermaid K, Lallemand, Rexdale, ON, Canada) was added to raise the yeast assimilable nitrogen to 300 mg/L prior to alcoholic fermentation in all cases. Musts were inoculated with *Saccharomyces cerevisiae* (EC-1118, Lallemand, Rexdale, ON, Canada) at a rate of 30 g/hL 6 h after crushing. Six replicates of each of these monovarietal wines were produced, with three replicates ($n = 3$) kept as monovarietal wines and the other 3 replicates saved for further blending postalcoholic and postmalolactic fermentation (Supplemental Figure 1).

2.2.2. Cofermentation Wines (COF). Malbec and Merlot grapes were cofermented at 50% by weight for each varietal, with each fermentor receiving 25 kg (±0.1 kg) of must from each varietal after crushing in triplicate fermentations ($n = 3$). Must additions (by weight) to the fermentors were measured with a commercial scale (Adam Equipment, Oxford, NY, USA). Thereafter the same protocol applied to monovarietal wines was followed.

Alcoholic fermentation occurred at a temperature between 18.5 and 28°C (average: 24.2 ± 4°C), and the total maceration time was set to 14 days. Sugar consumption (determined with a temperature-compensated densimeter (Anton Paar, Graz, Austria)) and temperature curves during alcoholic fermentation showed good agreement among the different treatments and within tank replicates (data not shown). Cap management consisted of two 1-min punch-down per day (morning and afternoon) during the 14-day maceration period. Upon completion of alcoholic fermentation, free-run wines were transferred to 20-L glass carboys fitted with airlocks, with a fraction of these free-run wines, as previously explained, saved for immediate blending (postalcoholic fermentation blending) or for blending after completion of malolactic fermentation (postmalolactic fermentation blending). All wines showed no signs of onset of malolactic fermentation at the end of alcoholic fermentation as confirmed by no degradation of the original malic acid content (~2.75 g/L in Malbec wines and ~1.88 g/L in Merlot wines).

2.2.3. Postalcoholic Fermentation Blending Wines (PAF). Immediately after pressing, free-run Malbec and Merlot wines were blended at 50/50 proportion (by volume) in triplicate ($n = 3$), and subsequently placed in 20-L glass carboys fitted with airlocks. The wines were allowed to settle overnight and were all subsequently inoculated with malolactic bacteria *Oenococcus oenii* (VP41, Scott Laboratories, Petaluma, CA, USA) at a rate of 20 g/hL to undergo malolactic fermentation, including the allocated volume of monovarietal wines saved for postmalolactic fermentation blending (Supplemental Figure 1). Malolactic fermentation was monitored via enzymatic analysis of L-malic acid and L-lactic acid with an enzymatic analyzer (Admeo Y15, Angwin, CA, USA) using enzymatic analysis kits (Biosystems, Barcelona, Spain).

2.2.4. Postmalolactic Fermentation Blending Wines (PMLF). Once malolactic fermentation was completed (≤0.1 g/L-malic acid), the wines were racked, and the portion of malolactic fermentation-completed Malbec and Merlot wines saved for blending were blended at 50/50 proportion (by volume) in triplicate ($n = 3$) and placed in 20-L glass carboys fitted with airlocks. The full experimental design afforded 15 wines (5 treatments × 3 replicates) (Supplemental Figure 1). All wines were adjusted to 0.3 mg/L molecular SO_2 in December 2018. The wines were racked off in February 2019, cold-stabilized at 8 to 10°C for 14 days, and subsequently racked again and filtered through 8 μm cellulose filter pads (Vitner's Vault, Paso Robles, CA, USA), readjusted to 30 mg/L SO_2, and bottled in 750 mL bottles on March 2019. Bottles were closed using DIAM 5 micro-agglomerated corks (G3 Enterprises, Modesto, CA, USA) and stored at controlled temperature in cellar-like conditions (~14°C), until analysis, as previously described [14].

2.3. Wine Basic Chemical Composition. Wine titratable acidity (TA) and pH were measured following the same method detailed above for determination of juice TA and pH. Ethanol (% v/v) was measured by near-infrared spectroscopy using a Alcolyzer Wine M/ME analysis system (Anton Paar, Graz, Austria). Acetic acid, glucose, fructose, malic acid, and lactic acid were determined enzymatically using commercial enzymatic analysis kits (Admeo, Biosystems Group, Hollister, CA, USA).

2.4. Analysis of Phenolic Compounds and CIELab Wine Colour Parameters. Spectrophotometric measurements included analysis of phenolic compounds and colour parameters and were performed to evaluate the effect of the cofermentation, postalcoholic fermentation and postmalolactic fermentation blending on the evolution of phenolic compounds and chromatic characteristics during selected winemaking and bottle aging stages and up to 1260 days postcrushing (equivalent to 3 years of bottle aging). Wine samples were centrifuged at 15000 g in a microfuge (model 5415D; Eppendorf, Hamburg, Germany), and the supernatant transferred into clean 1 mL Eppendorf tubes prior to analysis [14]. Anthocyanins and total polymeric pigments (TPP) (herein defined as the sum of small polymeric pigments (SPP) and large polymeric pigments (LPP)) were measured as previously reported in [15]. Tannins were analyzed by protein precipitation [16]. Wine colour parameters, full visible spectrum scans (300 to 750 nm), and CIELab colour coordinates were determined in 1 mm pathlength quartz cuvettes. CIELab coordinates including L^* (lightness), C^* (saturation), H^* (hue angle), a^* (green/red component), and b^* (blue/yellow component) were calculated using the Cary WinUV colour software (version 6.0, Startek Technology, Boronia, Vic., Australia) under a D65 illuminant. To explore overall chromatic differences between treatments, the CIELab colour difference (ΔE^*) between any given pair of wines was calculated as the Euclidean distance between two points (r and s), in the three-dimensional CIELab space as follows: $\Delta E^*r, s = [(\Delta L^*r, s)^2 + (\Delta a^*r, s)^2 + (\Delta b^*r, s)^2\ ]^{1/2}$ as previously described [17]. A ΔE^* value ≥ 2.0 indicates that two colours are distinguishably different from each other to the naked human eye. ΔE^* was calculated using the averaged CIELab parameters of three replicates for each treatment and taken at the last sampling point (3 years of bottle aging). The copigmentation effectiveness index was calculated according to previous specifications [18], considering the CIELab coordinates of a pure white coloured solution as $L^* = 100.00$, $a^* = 0.00$, and $b^* = 0.00$. Visual depiction of the wines after 3 years of bottle aging was accomplished using the MSCV™ software (Grupo de Color de La Rioja, Logroño, Spain) considering the standard CIELab conditions (10° standard observer and the illuminant D65) and recorded in a 1 mm-path length quartz cuvette. All spectrophotometric measurements were made with a Cary 60 UV-Vis spectrophotometer equipped with an 18-sample cell autosampler (Agilent Technologies, Santa Clara, CA, USA).

2.5. Analysis of Monomeric Anthocyanins, Anthocyanin-Derived Pigments, and Flavonols. The wines were analyzed by HPLC-diode array detector (DAD) with peak identity confirmed by MS at pressing (days 14) and after 70 (end of malolactic fermentation), 540 (~1 year of bottle aging), and 1260 days postcrushing (3 years of bottle aging), as previously described in [14]. Prior to analysis, the wines were centrifuged for 10 min at 15000 g (Eppendorf 5430 R, Hamburg, Germany) and filtered through a 0.45 μm membrane (Sartorius, Goettingen, Germany). The wines were analyzed in an Agilent 1100 series HPLC system coupled to a DAD (Agilent Technologies), as described in [19], with minor modifications in [14]. Separation occurred in a Zorbax SB-C18 column (4.6 mm × 150 mm, 3.5 μm particle size, Agilent Technologies) thermostated at 40°C and protected by a guard column of the same packing material. Peak identity was confirmed using a Waters Acquity I-Classultra-performance liquid chromatography system connected to an AB Sciex 4000 Q-Trap MS/MS (Waters, Milford, MA, USA). The column eluent, under the same operational conditions described above, was directed to the mass spectrometer on positive ionization mode, and compound detection was performed by multiple reaction monitoring (MRM), using the MS/MS transitions reported in Supplemental Table 2. Monomeric anthocyanins were quantified using malvidin-3-glucoside chloride as standard (Extrasynthèse, Lyon, France), and a standard calibration curve ($R^2 = 0.99$). Flavonols were quantified using quercetin-3-glucoside (Sigma-Aldrich, St Louis, MO, USA) as standard and a standard calibration curve ($R^2 = 0.99$).

2.6. Sensory Analysis. The wines were analyzed by descriptive analysis after 3 months of bottle aging as previously described [20]. Ten panelists (5 females, 5 males) aged 21 to 44 years of age, participated in this study. No information about the nature of the study was provided to reduce bias, and the Cal Poly Institutional Review Board (IRB protocol # 2019-058) for human subject participation approved the project. Panelists were trained biweekly for a total of nine 90-min sessions. During these sessions the panelists were made familiar with the use of the unstructured line scale, exposed to the experimental wines, deliberated, and agreed upon the sensory characteristics (descriptors) that the wines displayed. A total of 12 descriptors were selected by consensus including descriptors for colour: rubyand purple; for orthonasal aroma: red fruit, baked fruit, spice, vegetal, tobacco, herbal, and earthy; and lastly, for taste and mouthfeel: acidity and astringency, respectively. Standards of colour, aroma, and taste and mouthfeel characteristics (the latter presented at two levels: low and high) were presented to the panelists to calibrate and adjust accordingly and were always available during the training sessions (Supplemental Table 3*). Length was measured in seconds and defined to the panelists as "the overall length of sensation on the palate immediately after expectoration of the wine" (Supplemental Table 3). The experimental wines were formally assessed over four evaluation sessions during which each wine replicate was presented four times. The sessions were held in individual sensory booths. The three-wine replicates of each treatment were presented in a sequential monadic way according to random block design using the RedJade software (RedJade Sensory Solutions, Martinez, CA, USA). Wines were assessed in clear ISO wine glasses labelled with three-digit random code numbers covered with plastic lids (to trap volatiles). For aroma and taste/mouthfeel, panelists evaluated the wines under red lighting to prevent bias due to colour. Color was assessed separately from aroma and taste/mouthfeel to ensure independent ratings. During the evaluation sessions, panelists were given 30 mL of wine and their*

responses were registered on tablets (Apple, Cupertino, CA, USA) on a 10 cm unstructured line scale. Unsalted crackers (Nabisco, East Hanover, NJ, USA), deionised water, and spittoons were provided to panelists during sessions that evaluated aroma and taste/mouthfeel attributes. Panelists were instructed to wait 1 min and consume a cracker and water before moving on the next wine during the taste and mouthfeel assessment sessions.

2.7. Statistical Analysis. All wines were produced in triplicate ($n = 3$). The fruit data was analyzed by a Student-T test ($p < 0.05$). The basic chemical, phenolic, colour, and HPLC-DAD-MS data of the wines were analyzed by a one-way analysis of variance (ANOVA). Linear and nonlinear regressions were used to determine correlations between selected phenolic parameters and final polymeric pigment formation. The descriptive sensory data were analyzed by a three-way ANOVA considering the separate effects of wines, replicates, and panelists as well as the wine × panelist interaction. In all cases, Fisher's LSD test was used as a posthoc comparison of means with a 5% level for rejection of the null hypothesis using the XLSTAT v. 2019 software (Addinsoft, Paris, France). The individual performance of each panelist was evaluated by analyzing interaction plots generated by the Panel Check software (version 1.4.0, Nofima, Trømso, Norway). Principal Component Analysis (PCA) using the correlation matrix with no rotation was applied to sensory data set, including the replicates, using R software version 3.4.0 (R Development Core Team 2021, Vienna, Austria). Confidence ellipses indicating 95% confidence intervals were based on the multivariate distribution of Hotelling's test for $p < 0.05$ and were constructed using the SensoMineR panellipse function of R as described in [21]. Regression analyses between sensory descriptors were carried out using R software (R Development Core Team 2021, Vienna, Austria), with the packets ggplot2 and ggpmisc (R package version 0.4.6. https://CRAN.R-project.org/package=ggpmisc). All remaining graphical representations were prepared with GraphPad Prism software version 9.0 (GraphPad Soft-ware, San Diego, CA, USA).

3. Results and Discussion

This experiment was designed to assess and compare the chemical and sensory implications of cofermentation of two single red varietals (COF), and their respective blends assembled either after alcoholic fermentation (PAF) or after malolactic fermentation (PMLF) (Supplemental Figure 1). The choice of Malbec and Merlot hinged upon their distinctive and complementary phenolic profiles, with Malbec monovarietal wines being generally higher in anthocyanins with moderate to low tannin levels, and Merlot monovarietal wines being higher in tannins with moderate levels of anthocyanins [22].

3.1. Basic Chemical Composition of Grapes and Wines. Because of the need of cofermentation, which entails blending of each varietal immediately postcrushing, Malbec and Merlot grapes were harvested at the same time. Whereas both varietals were considered ripe from a winemaking perspective, Malbec grapes were riper than Merlot grapes, having about 1.4 Brix more than Merlot grapes (23.7 Brix and 22.3 Brix, respectively, Supplemental Table 1). Accordingly, acidity and pH also reflected relatively lower ripeness in Merlot fruit, whereby pH was lower and acidity was 0.8 g/L higher in Merlot fruit (Supplemental Table 1).

Table 1 shows the basic chemical composition of the finished wines. Monovarietal Malbec and Merlot wines showed the largest variation in alcohol content (ABV), being 1.2% v/v higher in Malbec wines. Being ethanol the volumetrically most important component of wines after water, a 1.2% ABV difference in favor of Malbec wines may enhance viscosity [23], which in turn, can have an effect on perceived mouthfeel and length, as well as in the volatility of wine aromas [24]. Cofermented and blended wines, on the other hand, showed relatively smaller differences in ethanol content within them and compared with monovarietal Malbec wines. Lactic acid content was highest in Malbec wines, and lowest in Merlot wines, with the remaining parameters of the basic chemistry of the wines showing no differences within treatments.

3.2. Evolution of the Phenolic Composition of the Wines during Winemaking and Bottle Aging. Selected phenolic classes known to have a direct sensory impact were followed during key stages of the winemaking process, including alcoholic fermentation (day 5), pressing, postalcoholic fermentation (day 14), end of malolactic fermentation (day 70), and after 1 (day 540) and 3 years (day 1260) of bottle aging. These phenolic classes included anthocyanins (Figure 1(a)), tannins (Figure 1(b)), total phenolics (Figure 1(c)), nontannin phenolics (Figure 1(d)), small (Figure 1(e)), large (Figure 1(f)), and total polymeric pigments (Figure 1(g)).

During alcoholic fermentation, total anthocyanins were the highest in Malbec wines, the lowest in Merlot wines, and intermediate in cofermented wines (Figure 1(a)). These trends were generally maintained at pressing with PAF blended wines displaying almost identical values relative to their cofermented counterparts. An expected decrease in anthocyanins occurred in all the wines after completion of malolactic fermentation, with monovarietal Malbec wines showing a 38% decrease in their anthocyanin content. At this point (after MLF), cofermented wines achieved an equivalent anthocyanin content than that of monovarietal Malbec wines, even though these were cofermented with Merlot, which had a comparatively lower anthocyanin content. Indeed, the opposite was found, suggesting Merlot may contribute to anthocyanin extraction, retention, and stabilization from Malbec musts and wines during cofermentation, possibly via the contribution with non-coloured phenolics. Protein precipitable tannins were 50% higher in Merlot wines relative to monovarietal Malbec wines at pressing (Figure 1(b)). Thus, a possible role of tannins into favoring anthocyanin solubility can also be posited to explain enhanced anthocyanin retention in cofermented Malbec and Merlot wines. Nonetheless, after 3 years of bottle

Table 1: One-way analysis of variance (ANOVA) of the basic chemical composition of monovarietal, cofermented, and blended Malbec and Merlot wines. Values represent the mean of three tank replicates followed by the standard error of the mean ($n = 3$).

Winemaking treatment	Ethanol (% v/v)	pH	Titratable acidity (g/L tartaric acid)	Glucose + Fructose (g/L)	Malic acid (g/L)	Lactic acid (g/L)	Acetic acid (g/L)	Acetaldehyde (mg/L)
Malbec	14.11 ± 0.08 a (*)	4.01 ± 0.02 ab	4.61 ± 0.06 a	0.31 ± 0.01 b	0.10 ± 0.00 a	1.37 ± 0.02 a	0.23 ± 0.00 b	7.33 ± 0.67 c
Merlot	12.91 ± 0.05 d	3.96 ± 0.01 c	4.63 ± 0.08 a	0.29 ± 0.01 d	0.09 ± 0.01 a	0.97 ± 0.01 e	0.29 ± 0.01 a	8.33 ± 0.88 bc
COF	13.92 ± 0.02 b	4.03 ± 0.01 a	4.39 ± 0.03 b	0.30 ± 0.00 bc	0.09 ± 0.01 a	1.28 ± 0.01 b	0.27 ± 0.01 a	11.67 ± 0.33 ab
PAF	13.66 ± 0.01 c	3.99 ± 0.01 bc	4.41 ± 0.01 b	0.34 ± 0.00 a	0.08 ± 0.01 a	1.22 ± 0.01 c	0.30 ± 0.01 a	13.67 ± 1.33 a
PMLF	13.56 ± 0.01 c	3.96 ± 0.01 c	4.49 ± 0.02 ab	0.24 ± 0.00 cd	0.09 ± 0.00 a	1.17 ± 0.01 d	0.28 ± 0.01 a	8.67 ± 1.86 bc
p value	**<0.0001**	**0.0060**	**0.0150**	**<0.0001**	0.6351	**<0.0001**	**0.0040**	**0.0150**

(*) Different letters within a column for each variety indicate significant differences for Fisher's LSD test and $p < 0.05$. Significant p values are shown in bold fonts. COF: cofermentation; PAF: postalcoholic fermentation blending; PMLF: postmalolactic fermentation blending.

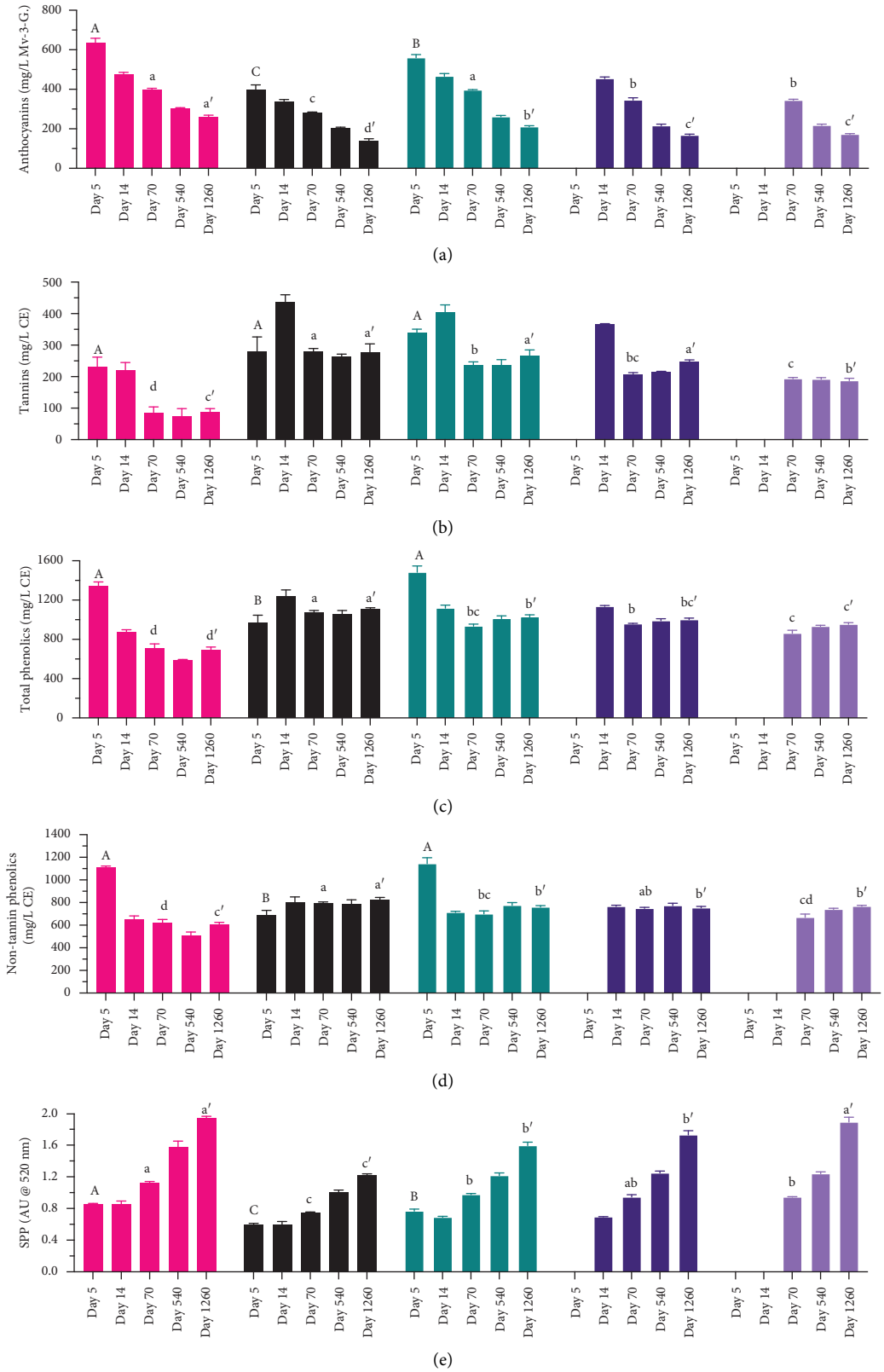

Figure 1: Continued.

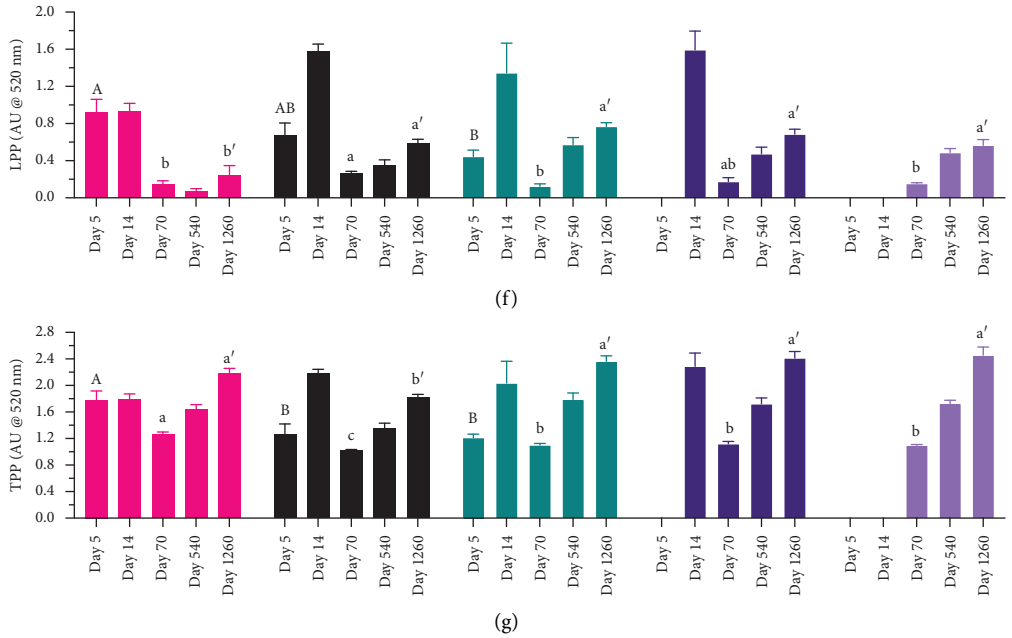

Figure 1: Evolution of (a) anthocyanins, (b) tannins, (c) total phenolics, (d) nontannin phenolics, (e) small polymeric pigments (SPP), (f) large polymeric pigments (LPP), and (g) total polymeric pigments (TPP) throughout winemaking and bottle aging of the monovarietal, cofermented, and blended Malbec and Merlot wines. Malbec (■), Merlot (■), COF (■), PAF (■), and PMLF (■). Different letters at day 5 (alcoholic fermentation, capital fonts), day 70 (completion of malolactic fermentation, lower fonts), and day 1260 (3 years of bottle aging, apostrophed fonts), indicate significant differences for Fisher's LSD test and $p < 0.05$. EQ: equivalents; CE: catechin equivalents; AU: absorbance units; COF: cofermentation; PAF: postalcoholic fermentation blending; and PMLF: post-malolactic fermentation blending.

aging, monovarietal Malbec wines retained the highest anthocyanin content, whereas all cofermented wines had lower anthocyanin content compared with monovarietal Malbec wines. At this point, MalMerPAF and MalMerPMLF had similar anthocyanin content. Overall, the anthocyanin content and profile of cofermented and blended wines was positively affected by cofermentation or blending with monovarietal Malbec wines. Lastly, blended wines showed lower anthocyanin values than those registered in Malbec wines (but higher relative to monovarietal Merlot wines) (Figure 1(a)).

Although modulated by factors such as ethanol, pH, and the total phenolic content [25], wine tannins are largely responsible for the tactile sensation of astringency, and thus their content herein measured by protein precipitation can be used as a surrogate of perceived astringency [26]. No differences between monovarietal and cofermented were observed during fermentation (Figure 1(b)). However, at pressing, the tannin content of Merlot wines was about 90% higher than that of Malbec wines, possibly due to extraction of seed-derived tannins, as previously shown in Cabernet Sauvignon wines [27]. A general decrease in tannins was observed postmalolactic fermentation. At the last sampling point after 3 years of bottle aging, monovarietal Merlot, cofermented, and PMLF blended wines showed comparable levels of tannins. This contrasts with the 67% decrease in tannin in Malbec wines from pressing to the end of the study. In cofermented and PAF blended wines the contribution of Merlot was to maintain tannin levels comparable to those of monovarietal Merlot wines.

Total phenolics include all phenolics containing vicinal dihydroxyls, including tannins, flavan-3-ols, and flavonols. However, monohydroxylated phenols and anthocyanins are not included in this measurement because the reagent used to measure total phenolics is ferric chloride, and iron is unable to form coloured ligands, and thus quantify monohydroxylated phenols and anthocyanins [28]. Nontannin phenolics are represented by monomeric flavan-3-ols, dimers, and up to pentameric tannins. As such, both total phenolics and nontannin phenolics provides insights in the full phenolic content of the wines excluding anthocyanins. During fermentation, total phenolics were higher in monovarietal Malbec and cofermented wines, and significantly lower in monovarietal Merlot wines (Figure 1(c)). After completion of malolactic fermentation, total phenolics were significantly higher in Merlot wines, on accounts of the enhanced tannin extraction during maceration recorded in these wines (Figure 1(b)). Contrastingly, a 44% decrease in total phenolics occurred in monovarietal Malbec wines. Reflecting the previous trend uncovered for tannins, cofermented and blended wines showed intermediate values of total phenolics and nontannin phenolics postmalolactic fermentation, and generally, throughout bottle aging (Figure 1(d)).

Polymeric pigments are winemaking artifacts formed through covalent reactions between anthocyanins and tannins and can be fractionated into small (SPP) and large (LPP) polymeric pigments. Small polymeric pigments include pyranoanthocyanins such as vitisins and low molecular weight tannin-anthocyanin adducts, some of them

mediated by acetaldehyde [29]. In the present work, a steady increase in SPP was observed in all the wines (Figure 1(e)). For example, SPP formation increased more than two-fold in both monovarietal Malbec and Merlot wines from fermentation to 3 years bottle aging, albeit this increase in SPP formation was especially noticeable in Malbec wines. After 3 years of bottle aging, monovarietal Malbec and PMLF blended wines showed the highest SPP content, whereas monovarietal Merlot wines showed the lowest. Formation of SPP in red wines is mainly regulated by the content of monomeric anthocyanins [30]. This could explain why SPP formation was favored by blending with Malbec wines post-malolactic fermentation. Cofermented and PAF blended wines, although higher in SPP content than that monovarietal Merlot wines, were lower in SPP relative to monovarietal Malbec wines.

Large polymeric pigments include pigmented tannins of high molecular weight. Because of their molecular weight and structure, these pigments can precipitate with proteins therefore participating in the tactile sensation of astringency [31]. LPP formation followed a more erratic behavior than that in SPP formation, peaking at pressing (in parallel with the peak observed in tannin extraction), then decreasing thereafter to resume their formation again after 3 years of bottle aging. Overall, addition of Merlot to blends favored LPP production (Figure 1(f)). However, when the total polymeric pigment content was accounted for, Merlot monovarietal wines showed significantly lower levels of polymeric pigments than the other treatments (Figure 1(g)), and this result was primarily driven by their comparatively lower SPP content, suggesting than anthocyanins were the limiting factor for further polymeric pigment development in Merlot wines. A previous study conducted in Cabernet Sauvignon and Syrah wines submitted to accelerated aging related final polymeric pigment formation with the initial concentration of anthocyanins, stating that other factors, such as the tannin to anthocyanin ratio or the initial tannin content were poor predictors of final polymeric pigment content [32]. Similarly, in the present study, the initial tannin content of the wines showed no linear relationship with their final polymeric pigment content ($p = 0.735$, $R^2 = 0.01$, data not shown). In agreement with [32], in the present study the initial wine anthocyanin content was a good linear predictor of polymeric pigment formation in aged wines (Figure 2(a)). However, the parameter that best modeled polymeric pigment formation over time was the initial anthocyanin to tannin ratio (A:T), which followed a quadratic response (Figure 2(b)). This trend suggested that an initial A:T ratio of 1.5 to 1.75 would maximize polymeric pigment formation during aging. Merlot wines, which showed the lowest initial A:T ratio (around 0.75), and Malbec wines, which showed the highest initial A:T ratio (above 2) resulted in comparatively lower final levels of polymeric pigments than that in cofermented and blended wines (Figure 2(b)). Previous research on Sangiovese has also related comparatively lower production of polymeric pigments with an initial low A:T ratio [33]. Cofermented and blended wines showed improved formation of polymeric pigments, in agreement with the quadratic relation that suggested an initial (i.e., at pressing) anthocyanin to tannin ratio of 1.5 to 1.75 would maximize such formation. However, it should be noted that the quadratic response to polymeric pigment formation was obtained by a regression against the A:T ratio, which is a relative term.

3.3. Detailed Anthocyanin and Flavonol Composition of the Wines. The detailed composition of monomeric anthocyanins and flavonols of the wines, determined by HPLC-DAD-MS, were assessed at the end of the maceration period (day 14), and reassessed at days 70 (end of malolactic fermentation), 540 (~1 year of bottle aging), and 1260 (3 years of bottle aging) (Figure 3). Additionally, Supplemental Figure 2 shows representative HPLC-DAD chromatograms of the wines of the monovarietal, cofermented, and blended Malbec and Merlot wines, highlighting the evolution of the six quantitatively most important monomeric anthocyanins throughout winemaking and bottle aging. However, a total of 18 anthocyanins were determined, quantified, and grouped as glycosylated and acylated forms, as well as anthocyanin-derived pigments, which include vitisins A and B. The most abundant anthocyanins were the glucosides of delphinidin, petunidin, and malvidin, the acetyl-glucosides of petunidin and malvidin, and the coumaroyl-glucoside of malvidin (Supplemental Figure 2A). Monovarietal Malbec wines showed higher concentration of all anthocyanin classes at pressing, whereas monovarietal Merlot wines showed the lowest. Malolactic fermentation (day 70) resulted in a decrease in monomeric anthocyanins across all the wines (Supplemental Figure 2B), with further decreases observed after 1 year of bottle aging (Supplemental Figure 2C). As expected, the most drastic decrease in anthocyanins was observed after 3 years of bottle aging, which accounted for a 71, 79, 77, and 84% anthocyanin loss in Malbec, Merlot, COF, and PAF wines, respectively (Figure 3(a)). PMLF wines saw an 80% decrease in anthocyanins from day 70 to 3 years of bottle aging. The HPLC chromatograms and peak identity also reflected this drastic decrease of monomeric anthocyanins after 3 years of bottle aging, with only residual levels of malvidin-3-glucoside left in the wines. At this time as well, significantly higher concentrations of malvidin-3-glucoside were present in monovarietal Malbec wines, followed by cofermented wines (Supplemental Figure 2D). Overall, there was a gradual decrease of monoglucosilated and acylated anthocyanins throughout aging, whereas the composition of anthocyanin-derived pigments remained relatively stable.

Figure 3(b) shows the detailed flavonol composition of the wines. The determination of the detailed flavonol composition of these wines was undertaken due to the role of flavonols as copigmentation factors [34], usually resulting in both hyperchromic and bathochromic shifts in the visible spectrum of the wines [4]. Flavonols may also play a role in mouthfeel properties, such as the perception of a velvety astringency subquality [35]. Flavonols, grouped as quercetin

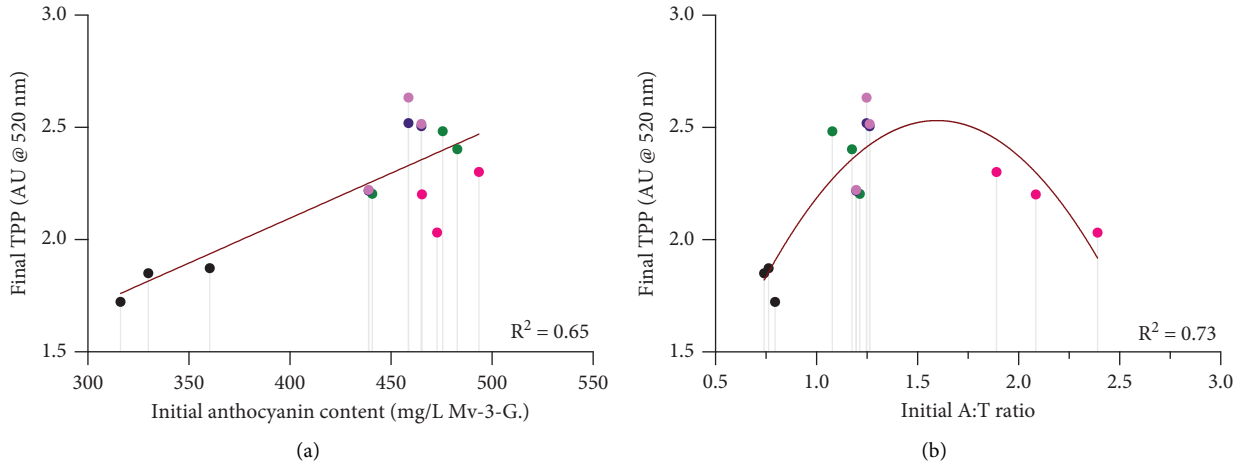

FIGURE 2: Scatter plots showing the relationship between final total polymeric pigment content (TPP) in the wines ($n = 15$) after 3 years of bottle aging versus, (a) initial anthocyanin content, and (b) initial anthocyanin to tannin (A:T) ratio. Malbec (●), Merlot (●), COF (●), PAF (●), and PMLF (●). COF: cofermentation; PAF: postalcoholic fermentation blending; and PMLF: postmalolactic fermentation blending.

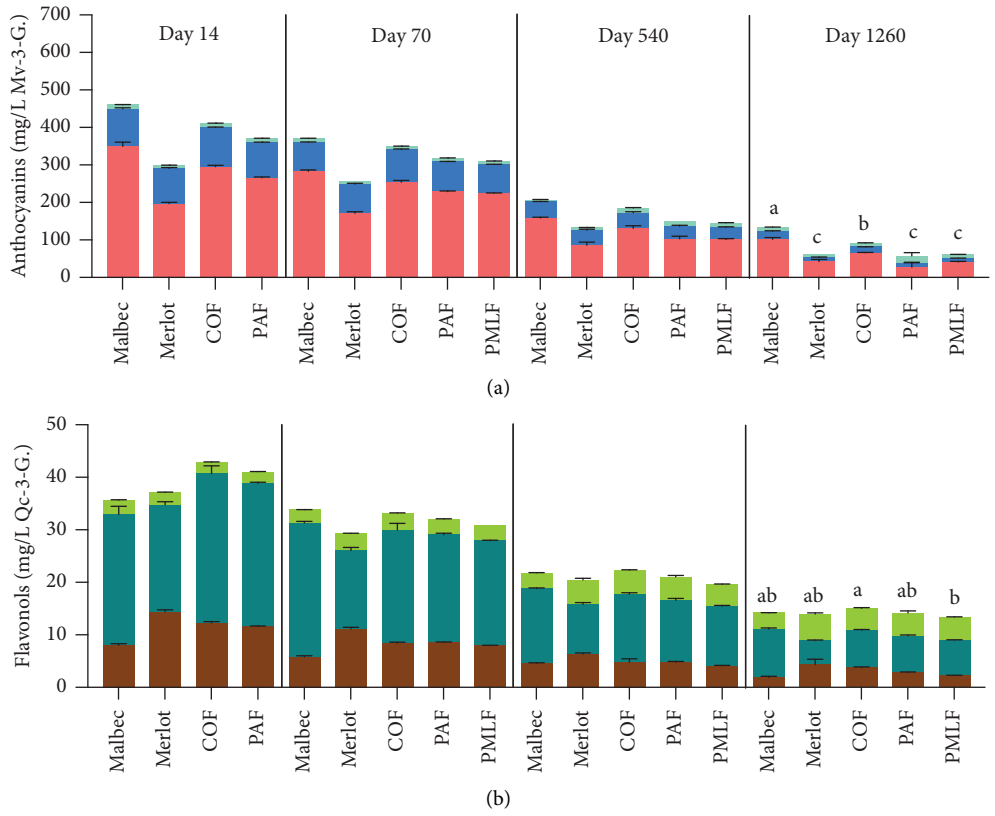

FIGURE 3: Evolution during winemaking (day 14: pressing; day 70: postmalolactic fermentation) and extended bottle aging (day 540 : 1 year of bottle aging; day 1260 : 3 years of bottle aging) of (a) monoglucosilated (●), acylated (●), and anthocyanin-derived pigments (●), and (b) Quercetin derivatives (●), other flavonols (●), and flavonol aglycones (●). Mv-3-G.: malvidin-3-glucoside equivalents; Qc-3-G.: quercetin-3-glucoside equivalents. COF: cofermentation; PAF: postalcoholic fermentation blending; and PMLF: postmalolactic fermentation blending.

derivatives (quercetin-3-glucoside and quercetin-3-glucuronide), other flavonols (including myricetin-3-glucoside, laricitrin-3-glucoside, kaempferol-3-glucoside, and isorhamnetin-3-glucoside), and flavonol aglycones were higher in cofermented wines at pressing. A study from Spain reported that cofermentation of Monastrell with Cabernet Sauvignon and Merlot wines resulted in enhanced copigmentation [4], and although in the latter study flavonols were not determined, and enhanced extraction and/or addition of the flavonol rutin to wine fermentations had been accordingly linked with enhanced copigmentation [36]. After 3 years of bottle aging, there was an expected, albeit

gradual, increase in the proportion of flavonol aglycones in all the wines. Indeed, flavonol aglycones are formed via acid hydrolysis from their respective glycosylated forms, and thus they progressively increase throughout aging [37]. COF wines maintained comparatively higher levels of total flavonols after 3 years of bottle aging, but only relative to PMLF wines.

3.4. Chromatic Composition and Copigmentation of the Wines during Winemaking and Aging. Figure 4 shows the full visible spectrum scans captured in the monovarietal and blended wines throughout winemaking and bottle aging. Figure 5(a) shows the copigmentation index in relation to the percentual increase or decrease in wine colour (AU 420 + 520+620 nm) in the blended wines relative to each of the two monovarietal wines (Figure 5(b)). Lastly, the actual visual aspect of the wines after 3 years of bottle aging, modeled using the CIELab parameters, is shown in Figure 6. During alcoholic fermentation and at pressing, Merlot wines consistently showed lower absorbance values in the 450 to 600 nm range (Figure 4). Addition of Merlot as coferment to Malbec in the case of the COF wines lowered both the copigmentation index and wine colour on these cofermented wines. Interestingly, as previously shown, at pressing, MarMerCOF wines showed higher levels of flavonols, including myricetin-3-glucoside, laricitrin-3-glucoside, kaempferol-3-glucoside, and isorhamnetin-3-glucoside, which were grouped as "other flavonols" in Figure 3(b). Flavonols act as copigmentation factors, and experiments in which rutin (a flavanol) was supplemented to Cabernet Sauvignon fermentations resulted in enhanced copigmentation [36]. However, under the present experimental conditions, this putative enhanced copigmentation effect related to a higher concentration of flavonols was not observed, suggesting that different flavonols may differ in their ability to copigment with monomeric anthocyanins. After completion of malolactic fermentation (day 70 post-crush), a substantial decrease in the colour of the wines was observed (Figure 4). This is likely due to the concomitant increase in pH typically observed after completion of malolactic fermentation, as well as due to the effect of the SO_2 added to signal the completion of malolactic fermentation. Overall, initial chromatic differences between treatments remained stable after MLF, although none of the treatments improved copigmentation relative to monovarietal Malbec wines at this time. However, after approximately 1 year bottle aging, PAF and PMLF blended wines showed both improved copigmentation and colour relative to either of their monovarietal counterparts (Figure 5). After 3 years of bottle aging, PMLF blended wines showed improved copigmentation index relative to monovarietal wines, which in turn resulted in a 15% improvement and a 25% improvement in wine colour relative to monovarietal Malbec and Merlot wines, respectively. A closer look at the absorbance spectrum of the wines in the 490 nm to 590 nm range (Figure 4, inset) showed an increase in absorbance at 520 nm in PAF and especially in PMLF wines in addition to a bathochromic shift relative to Merlot wines. A bathochromic shift typically results in bluer hues and it is a characteristic spectral feature of copigmentation [5, 38]. This result compares favorably with the observed increases in the copigmentation index in PAF and PMLF wines. Figure 6 also confirmed these results, whereby ΔE values as high as 4.52 CIELab units were observed when comparing PMLF with Merlot wines. Likewise, ΔE values of 3.17 CIELab units were recorded between PAF and Merlot wines, further confirming previously observed positive effects of PAF blending on copigmentation and wine colour. Interestingly, COF wines showed less colour improvement. Although these wines were still more visibly saturated than Merlot wines (ΔE = 3.38), they showed no improvement in colour relative to Malbec wines (ΔE = 1.43), despite having consistently higher levels of flavonols (Figure 3(b)). Differential colorimetry studies evaluating anthocyanin-flavonol interactions in model wines have conclusively shown that certain combinations of flavonols do not always result in enhancement of wine colour [38], as observed in the MarMerCOF wines of the present study.

Overall, PMLF wines showed the highest colour, and thus the best improvement in chromatic characteristics relative to cofermentation and monovarietal Merlot wines. Chromatic differences of lesser magnitude, but nonetheless measurable, were observed between cofermented and blended treatments.

3.5. Sensory Descriptive Analysis. The wines were evaluated using descriptive analysis [20] after 3 months of bottle aging. The sensory data set was analyzed by a 3-way ANOVA (Supplemental Table 4) as well as by principal component analysis with confidence ellipses (Figure 7). The sensory data set was further reassessed by linear regression analysis (Figure 8), whereby wine length (in seconds, post-expectoration) was regressed against descriptive sensory attributes. Figure 7 shows a principal component analysis (PCA), which explained approximately 80% of the variability of the data set with two components. Furthermore, the confidence ellipses were constructed with 95% certainty according to the Hotelling's test, which provides significant testing. Therefore, if the ellipses do not superimpose, it can be assumed that the wines are significantly different from a sensory viewpoint. Principal component 1 (PC1), which explained 65% of the variability, clearly separated monovarietal Malbec (negative dimension of PC1) from Merlot wines (positive dimension of PC1), with cofermented and blended wines located towards the center of the PCA. PC1 was mostly weighted on colour attributes and astringency. Merlot wines were characterized by ruby colour, astringency, spice, tobacco, vegetal, and earthy notes. Enhanced astringency in Merlot wines may be explained by their comparatively higher levels of protein precipitable tannins (Figure 1(b)). Vegetal and earthy notes can be attributed to methoxy-pyrazines, which are volatiles bearing vegetal notes commonly found in Cabernet Sauvignon and Merlot grapes and wines [39]. These vegetal notes in Merlot may also be explained by the fact Merlot wines were made from comparatively less ripe fruit (22.3 Brix) than Malbec wines (23.7

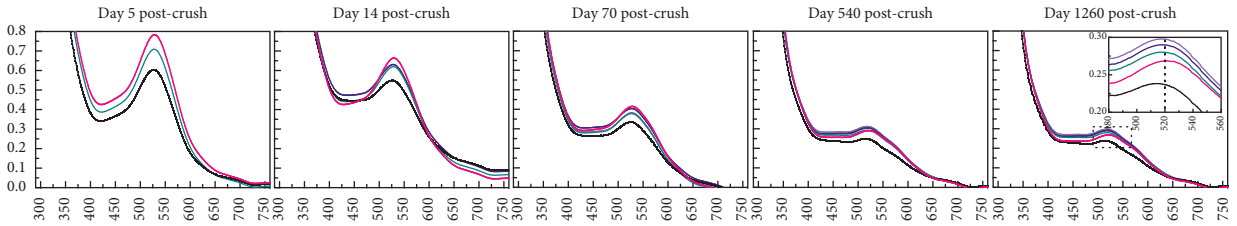

FIGURE 4: Full visible absorption spectrum scans recorded throughout winemaking and up to 3 years of bottle aging (day 1260 postcrush) of monovarietal, cofermented, and blended Malbec and Merlot wines. Malbec (—), Merlot (—), COF (—), PAF (—), and PMLF (—). Lines represent the average of all treatment replicates ($n = 3$). COF: cofermentation; PAF: postalcoholic fermentation blending; and PMLF: postmalolactic fermentation blending.

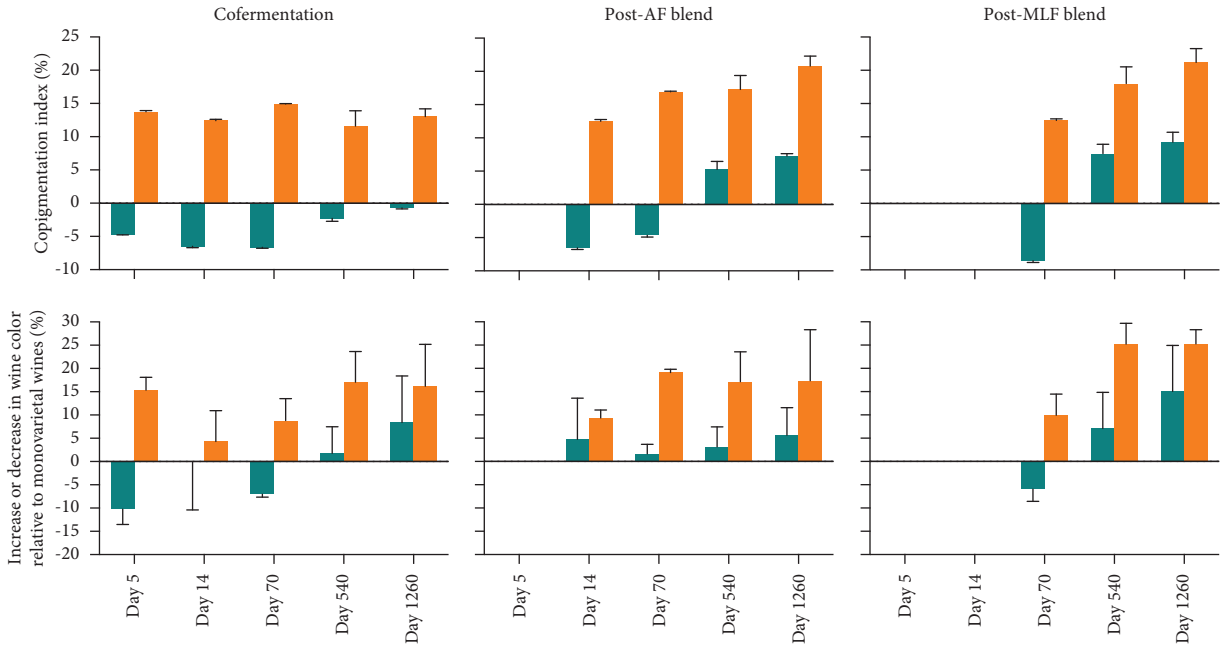

FIGURE 5: (a) Copigmentation index and (b) percentual increase or decrease of wine colour (AU 420 + 520+620 nm) of cofermentation, postalcoholic fermentation blending (post-AF blend) and postmalolactic fermentation blending (post-MLF blend) wines throughout winemaking and bottle aging. Monovarietal wines of Malbec and Merlot were used as a baseline for calculation of the copigmentation index between pairs of wines: wines compared against Malbec wines (■) and wines compared against Merlot wines (■). All the replicates were considered.

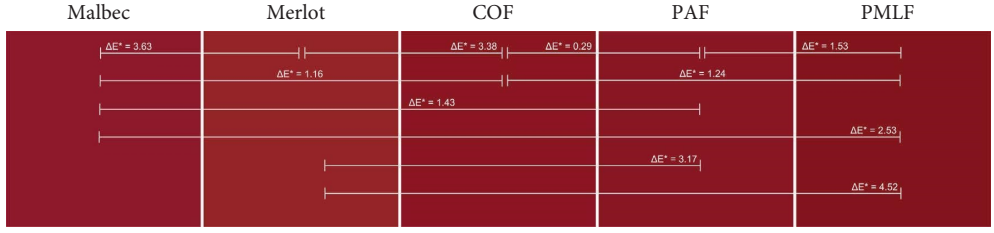

FIGURE 6: Visual depiction of the actual colour of the wines obtained via CIELab values (through a 1 mm pathlength quartz cuvette), of the monovarietal, cofermented, and blended Malbec and Merlot wines ($n = 3$) after 3 years of bottle aging. ΔE^* values are shown between any given pair of treatments. COF: cofermentation; PAF: postalcoholic fermentation blending; and PMLF: postmalolactic fermentation blending.

Brix) (Supplemental Table 1). Monovarietal Malbec wines, located in the negative dimension of PC1, were characterized by red fruit and baked fruit aromas, and purple colour. In the present study, the standard for purple colour was assembled by producing a wine-like solution that was higher in saturation and blue hue than the ruby colour standard, which in turn was predominantly red, less blue in hue but also higher in yellow/brown hues than purple colour (Supplemental Table 3). Herein, perceived purple colour in monovarietal Malbec wines may be linked with enhanced

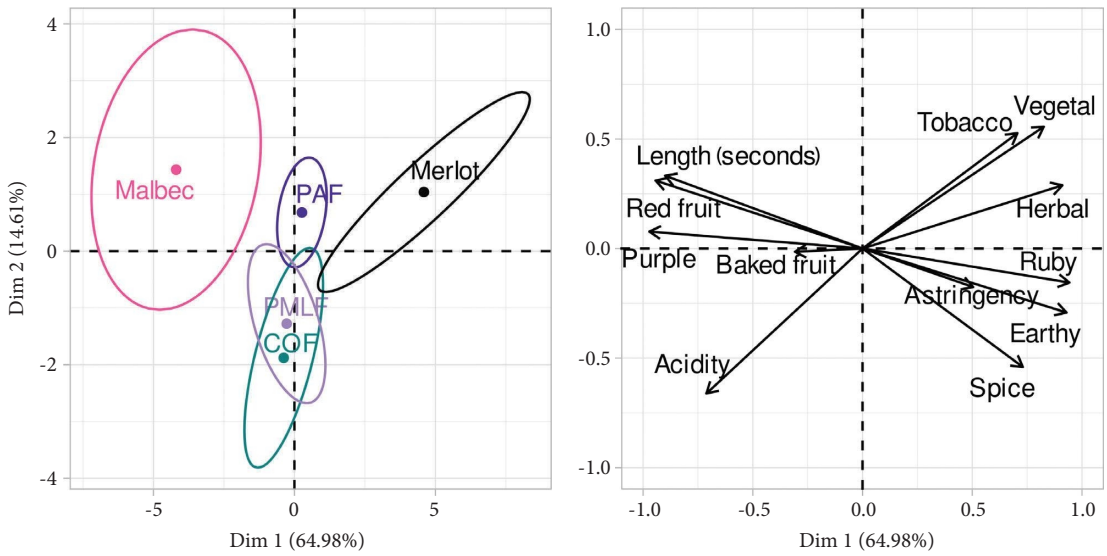

Figure 7: Principal component analysis of descriptive sensory data of monovarietal, cofermented, and blended Malbec and Merlot wines evaluated by a trained sensory panel ($n = 10$), showing wine scores (a) and sensory loadings (b). Malbec (——), Merlot (——), COF (——), PAF (——), and PMLF (——). Confidence ellipses indicate 95% confidence intervals. COF: cofermentation; PAF: postalcoholic fermentation blending; and PMLF: postmalolactic fermentation blending.

copigmentation, which typically results in a bathochromic shift thereby enhancing blue hues, as well as an hyperchromic shift [40]. This enhanced colour was observed analytically in the monovarietal Malbec wines of the present study (Figure 5). Moreover, monovarietal Malbec wines showed the lowest astringency, which correlated with the lowest level of protein precipitable tannins (Figure 1(b)), the lowest perception of earthy and vegetal character but the longest length of flavor postexpectoration (Figure 7 and Supplemental Table 4).

Cofermented and blended wines placed in the center of the PCA, with the corresponding confidence ellipses of COF and PMLF fully superimposed, whereas PAF wines were more different than COF and PMLF blended wines and closer to the sensory profile of each monovarietal wine. This suggest that COF and PMLF blending produced sensorially similar wines, and that early blending postalcoholic fermentation preserved and highlighted more of the individual character of each monovarietal wine in the final blend. As an example of the latter, postalcoholic fermentation blending with Merlot in PAF wines enhanced the vegetal character of these wines, as vegetal was a salient attribute of Merlot wines (Supplemental Table 4). Conversely, both cofermentation and postmalolactic fermentation blending tended to equalize the sensory profile of the resulting wines along with the enhancement of acidity and perceived spice aroma in these wines. No enhancement of the vegetal component or any attribute was observed in PMLF wines, suggesting fewer dominant aromas but a wider array of them without any salient aromatic descriptors, a sensory feature often associated with complexity. Overall, whereas monovarietal Malbec and Merlot wines were separated along PC1, indeed denoting the largest sensory difference between these wines, cofermented and blended wines were separated along PC2, which was weighted mostly on acidity and aroma attributes such as spice, tobacco, and vegetal.

If indeed, complexity is defined as a wide array of aromas and flavors without any of these dominating the sensory profile of the wines, the results herein presented are well-aligned with previous findings. For example, a study in which Syrah was either cofermented or blended (post-MLF) with Viognier, Marsanne, Roussanne, Picpoul, and Grenache Blanc reported that Viognier used either during cofermentation or blend after MLF increased the aromatic complexity of the resulting wines [6]. In the previous study, cofermented Syrah-Viognier wines were perceived as higher in citrus and cherry aroma than that in SY wines, whereas Syrah-Viognier wines blended post-MLF blended wines were higher in black fruit and cherry aromas than that in unblended Syrah wines. A study in California in which blends of Cabernet Sauvignon, Merlot, and Zinfandel were produced (reportedly after alcoholic fermentation and with no indications of malolactic fermentation status at the time of blending) showed that the individual sensory intensities of each monovarietal wine were lessened by blending, which in turn resulted in improved consumer acceptance [41]. Overall, the present results argue in favor of a more complex sensory profile, albeit less varietally marked in cofermented and PMLF blended wines relative to monovarietal wines.

To tease out the sensory basis that underpin wine length, linear regressions were established between each of the sensory attributes and wine length (Figure 8). Length was defined as overall duration of sensation on the palate immediately after expectoration. To gain further insights into a potential effect of cofermentation and blending in these correlations, the data was also segregated as a function of each winemaking treatment as shown in Figure 8. Consequently, each point in Figure 8 corresponds with 108 sensory

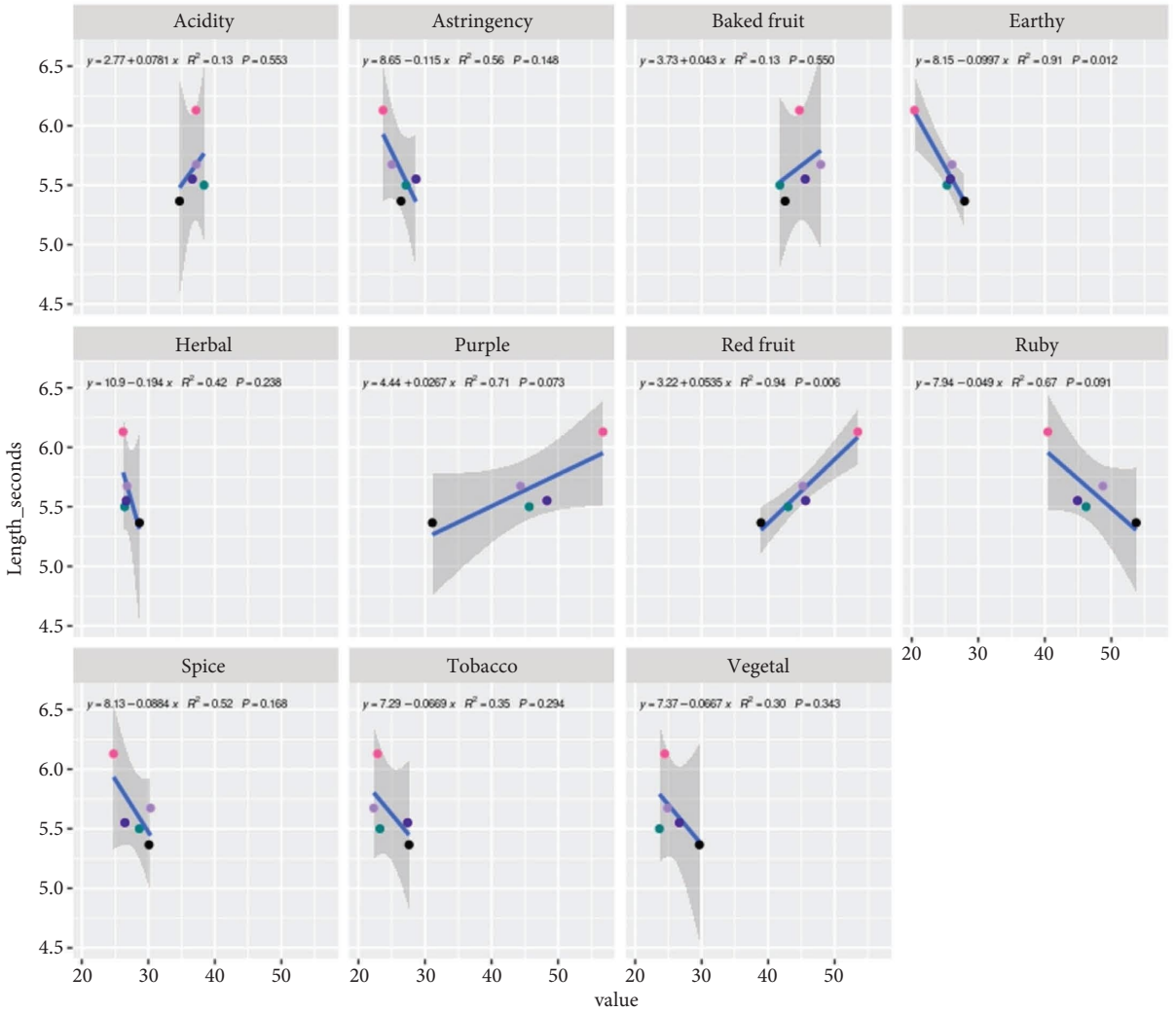

FIGURE 8: Linear regression analysis and confidence intervals between length (in seconds, postexpectoration) and descriptive sensory attributes of monovarietal, cofermented, and blended Malbec and Merlot wines. Malbec (●), Merlot (●), COF (●), PAF (●), and PMLF (●). COF: cofermentation; PAF: postalcoholic fermentation blending; and PMLF: postmalolactic fermentation blending. Each data point represents the average value ($n = 108$) for a given descriptor on each winemaking treatment.

evaluations. Out of 11 sensory attributes, only two showed significant correlations with length. The perception of red fruit aroma was positively and significantly ($p = 0.006$) related with wine length, with Malbec wines being higher in red fruit aroma perception as well as length. Moreover, the red fruit aroma attribute was the highest ranked aroma attribute across all the wines but especially in Malbec wines (Supplemental Table 4). Conversely, a negative correlation between wine length and earthy aroma was found ($p = 0.012$), with Merlot wines having the lowest perceived length. Interestingly, astringency was not correlated with length, but the red fruit character was, suggesting the panelists associated length with the duration of fruit flavors after expectoration [42], assuming orthonasal perception of red fruit aroma was replicated retronasally. Although not statistically significant, acidity and baked fruit perception also correlated positively with wine length, whereas astringency, herbal, vegetal, spice, and tobacco notes were negatively correlated with wine length. Wine length and finish are often part of regular wine terminology, though they are usually ill-defined, due to their inherent complexity and multisensory origin. For example, wine finish is defined as *"the aromatic and sapid sensations that linger following swallowing or expectoration"* [43]. A study of wine finish by time-intensity descriptive analysis applied to model white wines concluded that fruity flavors finished earlier than the other flavors, including coconut, floral, and mushroom flavors. The wines of the present study generally recorded shorter a length (5.72 seconds on average) and the prevalence of fruity aromas (and potentially fruity flavors) as being determinants of wine length. This could be due to the fact that the panelists were instructed to fully expectorate the samples instead of swallowing them, which could have further shortened perceived wine length relative to what a consumer would perceive when effectively swallowing the wine. More chemical and sensory evidence will be needed to unequivocally establish a causal relationship between fruity aromas (herein perceived and

recorded orthonasally) and wine length (in which these aromas are perceived retronasally). Further consideration should be given to the possibility that wine length may not be solely due to retronasal flavor, but that taste and tactile sensations may influence perceived flavor [3], and thus wine length. Nonetheless, present results argue in favor of a trend of fruity aromas related with wine length and earthy and vegetal aromas related with decreased wine length perception postexpectoration

4. Conclusions

The present study reported for the first time the separate effect of cofermentation of Malbec and Merlot, and their respective blends which were assembled either after completion of alcoholic fermentation and/or after malolactic fermentation. In general, blended wines seemed to have anthocyanin contents that reflected more the anthocyanin content of monovarietal Malbec wines, whereas their tannin content reflected more closely that of monovarietal Merlot wines. This suggests that chemically salient aspects of the phenolic profile of a given wine varietal may carry on to define the chemical profile of their respective cofermentation or blended wines. Contrary to previous assumptions, cofermentation did not favor copigmentation, despite this practice resulting in significant increases in the flavonol content of the wines. However, blending postmalolactic fermentation did, which in turn resulted in moderate improvements on the colour of these wines. This suggests that not all flavonols enhance copigmentation and that potential copigments may not have to be necessarily present early during fermentation. Should these specific flavonols be retained in the wines after alcoholic fermentation, as seems to be herein the case, they may contribute to copigmentation and improve colour. Long-term formation of total polymeric pigments was favored in cofermented and blended wines, which showed an initial anthocyanin to tannin ratio of about 1.25, and allowed us to suggest that an initial (i.e., at pressing) anthocyanin to tannin ratio of 1.5 to 1.75 would maximize such formation

Accepting that correlation does not implies causation, this study showed that wine length was positively associated with the orthonasal perception of red fruit aroma, and conversely, negatively associated with the orthonasal perception of earthy aromas. This suggests that panelists may associate wine length with fruit flavors perceived orthonasally (if these are replicated retronasally), rather than with tactile sensations such as astringency. Our results also suggest that blending soon after alcoholic fermentation will preserve the intrinsic salient sensory features of the blended monovarietal wines more, as opposed to cofermentation or postmalolactic fermentation blending, which tend to equalize and bring down any salient sensory feature of the monovarietal wines. Overall, cofermented and blended wines generally showed higher complexity than monovarietal wines.

Some specific practical insight for winemakers could be derived from these findings. For example, it is possible to infer that if blending post-MLF is instituted over cofermentation, even more favorable results can be obtained. Indeed, in the present study, Merlot fruit was harvested while still not fully ripe. However, improved phenolic maturity and a later harvest date in this Merlot fruit could have resulted in, for example, enhanced anthocyanin extractability. Perhaps, timing of harvest and finely matching the phenolic composition of two or more wine varietals post-MLF might be more impactful than forcing harvest dates to allow them to marry earlier during alcoholic fermentation and maceration.

Lastly, because of the traditional dimension attached to cofermentation, this practice will probably carry more allure to both winemakers and wine aficionados alike. Although logistically simpler than cofermentation, blending only remains a traditional and appellation-enforced practice mostly limited to certain iconic wine regions such as Bordeaux or Champagne, in France. Our results argue in favor of both practices as suitable for increasing the polymeric pigment content and the sensory complexity of the resulting wines.

Data Availability

All data is kept on institutional computers under the supervision of university officials.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Supplemental Table 1. Grape chemistry at harvest of Malbec and Merlot grapes. Averages followed by the standard error of the mean (SEM) ($n = 3$). *Supplemental Table 2*. MRM transition of pigments identified in monovarietal, cofermented, and blended Malbec and Merlot wines. *Supplemental Table 3*. Attributes and detailed composition of the standards used during the training and formal evaluation sessions. *Supplemental Table 4*. Three-way analysis of variance (ANOVA) with interaction showing mean separation and p values of descriptive sensory attributes of monovarietal, cofermented, and blended Malbec and Merlot wines. Results presented in mm (100 mm unstructured line scale). The main effects and interactions between selected ANOVA factors are also shown. *Supplemental Figure 1*. Diagram of the experimental design for production of the monovarietal, cofermented, and blended Malbec and Merlot wines. COF: cofermentation; PAF: postalcoholic fermentation blending; and PMLF: postmalolactic fermentation blending. *Supplemental Figure 2*. Representative chromatograms of monovarietal, cofermented, and blended Malbec and Merlot wines. COF: cofermentation; PAF:

postalcoholic fermentation blending; and PMLF: post-malolactic fermentation blending throughout winemaking and bottle aging. (A) day 14 (pressing), (B) day 70 (post-malolactic fermentation), (C) day 540 (~1 year of bottle aging), and (D) day 1260 (3 years of bottle aging). Peak identity: (1) delphinidin-3-glucoside; (2) petunidin-3-glucoside; (3) malvidin-3-glucoside; (4) petunidin-acetyl-glucoside; (5) malvidin-acetyl-glucoside; and (6) malvidin-coumaroyl-glucoside. (*Supplementary Materials*)

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