Journal of Food Composition and Analysis 104 (2021) 104168

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original Research Article

Effects of maceration length after prefermentative cold soak: Detailed chromatic, phenolic and sensory composition of cabernet sauvignon, malbec and merlot wines

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ARTICLE INFO

Keywords:
Cold soak
Maceration
Phenolic compounds
Wine color
Sensory analysis

ABSTRACT

Cabernet Sauvignon, Malbec and Merlot grapes were processed with prefermentative cold soak (CS) for 5 days followed by a short maceration time of 5 days (CS + 5d), or a long maceration time of 10 days (CS + 10d). CS did not affect the basic chemistry of the wines, nor improved anthocyanins, polymeric pigments and total phenolics relative to Control wines (10 days maceration). Wine tannins were lowered in CS + 5d wines by 71 % (Cabernet Sauvignon) and by 29 % (Merlot). CIELab coordinates showed a negative impact on L*, C*, and copigmentation in CS wines indicating that these wines were lighter in color than their Control counterparts and these differences could be distinguished by the human eye. Astringency and bitterness were lower in CS + 5d wines, whereas CS + 10d wines showed enhanced fresh fruit aroma, body, bitterness, and astringency.

1. Introduction

Once red wine grapes ripeness and quality are deemed fit for a particular wine style, the most characteristic sensory features of red wines are defined during the maceration process (Casassa and Harbertson, 2014). Maceration entails the period in which grape solids (skins, seeds and, when present, stems), are in contact with the juice. From a physical viewpoint, phenolic compounds of sensory relevance such as skin anthocyanins (responsible for color), and seed- and skin-derived tannins (responsible for mouthfeel properties such as astringency), are extracted *via* a diffusive mass transfer process (Setford et al., 2017). Extraction from skins includes an initial leakage from the edges of broken skin cells that occurs at crushing, and a slower concentration-driven diffusion across the solid layers that occurs throughout the maceration period (Setford et al., 2017). Seed-derived tannins are located in the inner and outer integuments of the seeds and are lower in molecular weight but higher in concentration on a per berry basis than skin-derived tannins (Hanlin et al., 2011). They are also extracted into wine *via* a diffusion process, but their diffusion is contingent upon the concentration of tannin already present in the wine as well as a dissolution process that is independent of concentration (Setford et al., 2017). Maceration and the production of ethanol also allow the solubilization of aromas and aroma precursors leading to subsequent changes in aroma volatility.

During the prefermentative phase, coupled enzymatic oxidations favored by dissolved oxygen may occur in parallel with the extraction of phenolics, affecting the extraction of the latter (Macheix et al., 1991), as well as the release of wine aromas (Nikolantonaki et al., 2012). Subsequently, during alcoholic fermentation, the most salient chemical changes include the drop of the redox potential, the early occurrence of covalent reactions between anthocyanins and tannins to form polymeric pigments (Singleton and Trousdale, 1992), and the production of ethanol with its effects on esterification and copigmentation reactions. There is also evidence of adsorption and desorption mechanisms mediated by non-covalent reactions involving phenolics during maceration. For example, seed tannin extraction into wine may at least partly depend on disruption of non-covalent interactions between previously extracted tannins and non-phenolic materials through a desorption mechanism (Casassa et al., 2019). Other non-covalent reactions during maceration such as copigmentation between the flavylium form and/or quinoidal/hemiacetalic anthocyanin forms may enhance color in the early stages of maceration but the disruption of such reactions by ethanol may

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https://doi.org/10.1016/j.jfca.2021.104168
Received 23 August 2021; Received in revised form 6 September 2021; Accepted 9 September 2021
Available online 14 September 2021

result in color losses at the end of maceration (Somers and Evans, 1979). Lastly, the increase in the redox potential due to increasing levels of dissolved oxygen and decreasing levels of CO_2 during the post-alcoholic fermentation period may lead to chemical oxidation and the production of acetaldehyde and other carbonyl compounds, which can affect color and mouthfeel characteristics such as astringency (Sheridan and Elias, 2015).

Maceration techniques in red winemaking include protocols or technologies that are applied to red musts prior to the onset of alcoholic fermentation, during alcoholic fermentation, or after alcoholic fermentation, so long as the juice remains in contact with grape solids. There is a bevvy of maceration techniques available to winemakers, which range from relatively low intervention ones, such as whole cluster fermentation (Casassa et al., 2021), to more disruptive ones such as microwave-assisted extraction (Carew et al., 2014), or pulsed electric field extraction (El Darra et al., 2013), among others. However technically complex some of these techniques may be, arguably the two most important factors in determining the phenolic and sensory profile of red wines during maceration are temperature (Sacchi et al., 2005), and time (Casassa and Harbertson, 2014). These two factors affect the chemical and physical processes previously outlined above, but, importantly, they are also relatively easily adjustable during winemaking. For example, the winemaking technique known as prefermentative cold soak (CS) consists of allowing the contact of fermentation solids (skins, seeds and occasionally stems), with the juice in a non-alcoholic environment prior to the onset of alcoholic fermentation (Casassa and Sari, 2014). The absence of ethanol is ensured by keeping the must at low temperatures - in the range of 5–10 °C - for a variable period of time - from 1 to 12 days (Panprivech et al., 2015; Gordillo et al., 2010). As both anthocyanins and tannins are water-soluble, CS should favor the extraction of both phenolic classes (Casassa and Sari, 2014), if the increased solubility outweighs the decreased cellular permeability observed at lower temperatures (Sacchi et al., 2005), and/or the potentially detrimental effects of coupled enzymatic oxidations mediated by polyphenol-oxidases (Macheix et al., 1991).

Maceration time determines extraction peaks and retention of both anthocyanins and tannins. Anthocyanin extraction during maceration peaks after 5–7 days post-crushing (Romero-Cascales et al., 2005), whereas skin-derived tannins are also extracted early during maceration, following a sigmoid-type kinetic that fit a Boltzmann model (Cerpa-Calderón and Kennedy, 2008). However, seed-derived tannins are preferentially extracted during the post-fermentative maceration period, *i.e.*, from day 20 post-crushing onwards (Casassa et al., 2013). Whether enhanced tannin extraction during extended maceration is the result of true extraction of (primarily) seed-derived tannins or desorption of previously extracted tannins, or a combination of both mechanisms, the net result is that extended contact with fermentation solids lead to enhanced tannin extraction, and, concomitantly, enhanced perceived astringency (Casassa et al., 2013).

Whereas previous research has reported on the effect of the length of CS on phenolic characteristics (Panprivech et al., 2015), to our knowledge, no studies have focused on the impact of maceration time after completion of CS on phenolic and sensory characteristics. Winemakers have also empirically reported that CS seems to quickly and selectively extract anthocyanins and glycosylated bound aroma compounds, which will allow them to shorten the maceration time during alcoholic fermentation, thereby limiting tannin extraction. Therefore, the aim of the present experiment was to study the feasibility of such an approach in Cabernet Sauvignon, Malbec and Merlot grapes processed with CS followed by a short maceration time (5 days post-CS) and contrast its effect with a treatment based on CS followed by a long maceration time (10 days post-CS). The choice of these three cultivars was based on their distinctive volatile and, especially phenolic composition, with Malbec wines being generally richer in anthocyanins and fruitier in aromas, Merlot wines being distinctively higher in tannins and displaying a mix of vegetal and fruit aromas, and Cabernet Sauvignon wines being predominantly higher in vegetal aromas.

2. Materials and methods

2.1. Grapes

Own-rooted *Vitis vinifera* L. cvs. Cabernet Sauvignon, Malbec and Merlot grapes were selected from a vineyard trellised using vertical shoot positioning (VSP) system, located in Luján de Cuyo, Mendoza, Argentina (33°000 S, 68°510 W). Clusters (~ 1200 kg for each variety) were manually harvested into 18 kg plastic boxes on April 30th, April 10th, and March 25th, 2014, respectively (Table 1) and crushed on the same day. Visual inspection of the grapes revealed no symptoms of pest or mold damage. Four independent samples, each of 30 berries, were taken at harvest for each cv. and analyzed for berry weight and volume, seeds/berry, Brix (Atago, Tokyo, Japan), pH (Orion model 701-A, Thermo Scientific, Waltham, MA, USA) and titratable acidity.

2.2. Winemaking

Upon reception, grapes were crushed and destemmed (Metal Liniers model MTL 12, Mendoza, Argentina), and the musts were pumped into 100-L stainless steel tanks, which each tank receiving 75 ± 1 kg of must. The tanks were filled at 25 % increments within the replicates of each treatment to ensure consistency. The experimental design consisted of three maceration treatments for each of the three cultivars, replicated three times (n = 3, 27 separate fermentations).

Control wines were produced with a standard SO_2 addition of 50 mg/L followed by a maceration length of 10 days at 25.5 ± 0.5 °C. The cap management regime consisted of two daily full-volume pump-overs followed by two daily punch-downs (morning and afternoon, 1 min each). In addition to Control wines, a 5-day cold soak (CS) with a standard SO_2 addition of 50 mg/L was followed by two contrasting maceration lengths after completion of CS, namely 5 days and 10 days as follows. Cold soak with solid CO_2 pellets (dry ice) consisted of five days at 7 ± 2 °C achieved by an initial addition of 10 kg of CO_2 (Praxair SA, Mendoza, Argentina) during crushing. The temperature was maintained by keeping the tanks in a refrigerated room at 6 ± 2 °C during the duration of CS. Cap management during CS consisted of a 1-min punch down per day to keep the cap moist. Following completion of the 5-day CS period, the musts were subjected to either a 5-day fermentation/maceration period post-CS (CS + 5d), or a 10-day fermentation/maceration period post-CS (CS + 10d), under the same temperature and cap management conditions as Control wines. The total maceration length was thus 10 days for Control and CS + 5d wines and 15 days for CS + 10d wines. All tanks were inoculated 5 h after crush with a commercial yeast (EC-1118; Lallemand Inc., Copenhagen, Denmark) at a rate of 0.3 g/L, following a hydration protocol previously detailed (Casassa and Sari, 2014). At this moment, a 1.5 g/L tartaric acid addition was performed in all the tanks. Malolactic bacteria (Lalvin VP41,

Table 1
Harvest date and basic composition at harvest of Cabernet Sauvignon, Malbec, and Merlot grapes used for the winemaking treatments. Values represent the mean (± SEM) of four independent sample replicates taken at harvest (n = 30 berries).

Cultivar	Harvest date	Brix	pH	Titratable acidity (g/L tartaric acid)
Cabernet Sauvignon	4/30/2014	26.40 ± 0.13 b	3.64 ± 0.04 a	4.28 ± 0.15 a
Malbec	4/10/2014	24.80 ± 0.07 a	3.77 ± 0.02 b	5.75 ± 0.22 c
Merlot	3/25/2014	26.60 ± 0.09 b	3.76 ± 0.01 b	5.46 ± 0.19 b

[a]Different letters within a column indicate significant differences for Fisher LSD Test and $p < 0.05$.

Lallemand, Copenhagen, Denmark) were added 48 h after the onset of alcoholic fermentation for each treatment, at 10 mg/L. After completion of malolactic fermentation (malic acid < 0.20 g/L), confirmed by enzymatic analysis (Vintessential Laboratories, Victoria, Australia), the wines were racked, adjusted to 30 mg/L of free SO_2, and stored at 1 °C for 45 days. After this period, the wines were racked off the bitartrate crystals and brought to room temperature for 48 h. Prior to bottling, free SO_2 was adjusted to ensure 0.5 mg/L of molecular SO_2. The bottles were stored horizontally in a cellar and maintained at 12 ± 1 °C until needed.

2.3. Wine basic analysis

Ethanol content, titratable acidity (TA), volatile acidity (VA), and pH, were obtained using a FOSS Wine-Scan (FT- 120) rapid-scanning infrared Fourier-transform spectrometer (FOSS, Hillerod, Denmark). Reducing sugars were determined following a reference method (INV (Instituto Nacional de Vitivinicultura), 2013). Malic acid was determined enzymatically (Vintessential Laboratories, Victoria, Australia).

2.4. Spectrophotometric analysis

Spectrophotometric measurements to evaluate wine phenolics and chromatic characteristics were performed at key stages during the winemaking process, including day 10 (pressing for Control and CS + 5d wines, and day 5 of fermentation/maceration for CS + 10d wines), after completion of malolactic fermentation and after 3 months of bottle aging in the case of Merlot wines. For Cabernet Sauvignon and Malbec wines, these analyses were performed after 3 months of bottle aging, as this is a crucial time for gauging phenolic and chromatic characteristics (Panprivech et al., 2015). Prior to each of these measurements, the samples were centrifuged 30 min to 1600 g (Gelectronic G-49, Buenos Aires, Argentina), and then filtered through a 0.45-μm membrane (Sartorius, Goettingen, Germany).

Total phenolics (expressed as mg/L mg/L (+)-catechin equivalents, CE), were measured by reaction with ferric chloride, which targets phenolics containing vicinal dihydroxyls (Harbertson and Spayd, 2006), following a previously published method (Harbertson et al., 2003). Anthocyanins (expressed as mg/L malvidin-3-glucoside) were determined as detailed in Heredia et al. (2006). Briefly, wine samples (100 μL), were diluted in 400 μL of a model wine buffer (5 g/L potassium bitartrate, 12 % ethanol and adjusted to pH 3.3) and subsequently mixed in a 1.5 mL volume cuvette with 1 mL of a pH 1.8 buffer, incubated for 10 min at room temperature and the absorbance at 520 nm was then recorded. Protein precipitable tannins (mg/L CE), were determined by precipitation with bovine serum albumin (BSA, Fraction V, 1 g/L solution), after which they were resuspended with a buffer solution containing 5% triethanolamine (v/v) and 10 % sodium dodecyl sulfate (w/v) and subsequently reacted by ferric chloride as further detailed elsewhere (Harbertson et al., 2003). Large polymeric pigments (LPP), and small polymeric pigments (SPP) were measured as previously described (Harbertson et al., 2003). Briefly, wine samples (200 μL), were diluted in 300 μL of a model wine buffer (5 g/L potassium bitartrate, 12 % ethanol and adjusted to pH 3.3) and added with 1 mL of a pH 4.9 buffer (9.86 g/L of NaCl, 12 mL of glacial acetic acid, adjusted to pH 4.9 with NaOH), in a 1.5 mL cuvette, with the absorbance at 520 nm being subsequently recorded. Subsequently, 80 μL of 0.36 M potassium metabisulfite was added. After mixing and 10 min incubation the absorbance at 520 nm was determined. This absorbance represents the sum of large and small polymeric pigment in the original wine. In a 1.5 mL microfuge tube, wine samples (200 μL), were diluted with 300 μL of a model wine buffer and added with 1 mL of a BSA solution as detailed above. The mixture was allowed to stand at room temperature for 15 min and then centrifuged for 5 min at 13,500 *g* to pellet the tannin-protein precipitate. One mL of the supernatant was transferred to a cuvette, then 80 μL of 0.36 M potassium metabisulfite was added, and after 10 min the absorbance was determined at 520 nm. This absorbance represents the amount of polymeric pigment that did not precipitate with protein (small polymeric pigment, SPP), and the amount of large polymeric pigment (LPP) was calculated by subtracting the SPP value from the sum described above. Therefore, total polymeric pigments (TPP) were calculated as LPP + SPP. Characterization of wine color was undertaken using the CIELab system and by the determination of wine color intensity. The CIELab coordinates L* (lightness), C* (saturation or chroma), H* (hue angle), a* (green/red component), and b* (blue/-yellow component) were calculated as previously described (Pérez-Caballero et al., 2003), using MSCV™ software (Grupo de Color de La Rioja, Logroño, Spain). Lightness, a*, and b* were further considered to calculate the CIELab color difference (ΔE*) between each pair of wines for each of the three cultivars after 3 months of bottle aging. The ΔE* values were calculated as the Euclidean distance between two points, r and s, in the three-dimensional CIELab space as follows:

$$\Delta E^*_{r,s} = [(\Delta L^*_{r,s})^2 + (\Delta a^*_{r,s})^2 + (\Delta b^*_{r,s})^2]^{1/2}$$

Where $\Delta L^*_{r,s} = (L^*_r - L^*_s)$; $\Delta a^*_{r,s}$ and $\Delta b^*_{r,s}$ are defined in the same fashion.

Wine color intensity was determined by placing an aliquot of undiluted wine samples in 1 mm path-length quartz cuvettes, and the absorbances at 420, 520 and 620 nm were recorded. Wine color intensity was calculated as the sum of absorbances at 420, 520 and 620 nm, as previously detailed (Glories, 1984).

The copigmentation effectiveness index was calculated according to previous specifications (Gombau et al., 2019), considering the CIELab coordinates of a pure white colored solution as L* = 100.00, a* = 0.00, and b* = 0.00. All spectrophotometric measures were performed in a Perkin-Elmer Lambda 3B spectrophotometer (Norwalk, CT, USA).

2.5. Sensory descriptive analysis

Coincident with the last sampling point for phenolic and chromatic analyses at 3 months of bottle aging, the Malbec and Merlot wines and their replicates of the three treatments were subjected to sensory descriptive analysis. Two trained panels, each of 12 individuals, with ages ranging from 25 to 65 years, all of whom had extensive experience of wine sensory analysis were convened. Two formal evaluation sessions were held throughout the experiment, one for each cultivar, with two additional introductory sessions devoted to terminology development, attribute definition and exposure to standards. Terminology agreement, definition and consensus were established as previously described (Casassa and Sari, 2014). Briefly, panelists defined by consensus one color attribute (color saturation), five aroma attributes (overall aroma, fresh fruit, red fruit, dried fruit, cooked aroma, spicy), three taste attributes (sweetness, acidity and bitterness), six flavor (retronasal) attributes (fresh fruit, berry, dried fruit, herbal, spicy, hot), and four mouthfeel attributes (astringency, dryness, body and length) for each of which a definition and a standard, if applicable, were provided (Supplemental Table 1). During the training and evaluation sessions, the intensity of each attribute was assessed using a non-structured 10-cm line scale containing two reference points located at 1 cm of each end of the line. Wines and their replicates were presented in aliquots of 25 mL placed in ISO wine glasses covered with plastic lids to trap volatiles, following a balanced, complete block design. To minimize sensory carry-over, panelists were asked to rinse their mouth with mineral water and eat a cracker between samples following a sip and spit protocol.

2.6. Data analysis

Basic fruit chemical composition was analyzed by a one-way analysis of variance (ANOVA). The basic, phenolic, and chromatic composition of the wines of each cultivar were analyzed after 3 months of bottle aging by a series of fixed-effect one-way ANOVAs. In addition, the full

data set was reevaluated by a series of fixed effect two-way ANOVAs with interactions, including as main effects the grape cultivar and the three maceration techniques (Control, CS + 5d and CS + 10d), as well as the cultivar × maceration technique interaction (Supplemental Tables 2–4). In all cases, Fisher's LSD test was used as a post-hoc comparison of means with a 5% level for rejection of the null hypothesis. Data analysis was performed using XLSTAT v. 2019 (Addinsoft, Paris, France).

The data generated by the sensory descriptive analysis panel was first analyzed by multivariate analysis of variance (MANOVA) with all the dependent variables (*i.e.*, sensory descriptors), evaluated simultaneously, using the Wilks' Lambda test (Lawless and Heymann, 2010). Because MANOVA was significant for both sets of wines, the data were subsequently analyzed by a fixed-effect two-way ANOVA with interactions, including as main effects the panelists, the replicates, the wines, and the panelist × wines interaction, using Fisher's LSD as a post-hoc comparison of means with a 5% level for rejection of the null hypothesis. The sensory data set for each cultivar was also analyzed by principal component analysis, coupled with boot-strap confidence ellipses at 95 % confidence intervals using the sensory packet (product characterization) of XLSTAT v. 2019 (Addinsoft, Paris, France).

3. Results and discussion

3.1. Grapes and wine basic analysis

In this experiment, ripe Cabernet Sauvignon, Malbec, and Merlot grapes from Mendoza (Argentina) were processed in triplicate to assess the effect of the duration of maceration length after completion of the cold soak period (CS), on wine phenolic, chromatic, and sensory characteristics. Whereas a previous report explored the effect of different durations of the CS period on Cabernet Sauvignon wines (Panprivech et al., 2015), the present work focused instead on a fixed 5-day CS period and varied the duration of maceration length to 5 (CS + 5d) and 10 days (CS + 10d) post-CS, also including two other cultivars in which this approach has not been explored, namely Malbec and Merlot. Because tannins, and specifically seed-derived tannins, have been reported to be extracted after 20 days of maceration time in Merlot (Casassa et al., 2013), or, similarly, be progressively released as contact with fermentation solids is maintained in Cabernet Sauvignon (Casassa and Harbertson, 2014), it was expected that the CS + 5d treatment, with a total maceration length of 10 days, will allow the extraction of color components while avoiding excessive tannin extraction and preserving sensory characteristics especially in the case of Cabernet Sauvignon and Merlot wines.

In general, Merlot and Cabernet Sauvignon grapes were riper than Malbec grapes (Table 1), which was also reflected in the comparatively higher ethanol content of their respective resulting wines (Table 2). In Cabernet Sauvignon, the basic chemistry of the wines was unaffected by any of the maceration techniques, in agreement with previous reports (Casassa et al., 2016). In Malbec wines, the ethanol content was slightly lower in CS + 10d wines (about 0.56 % lower), whereas titratable acidity was slightly lower in Control wines, but these differences are unlikely to be a consequence of the maceration techniques and also unlikely to be of any sensory relevance. Indeed, previous research has shown that for wines with comparable levels with those of the present study, ethanol difference thresholds (orthonasally and retronasally, respectively) are 1.14 and 1.31 (Yu and Pickering, 2008), which are well above the 0.56 % ethanol variation observed in Malbec wines. In Merlot wines, titratable acidity in CS + 10d wines was about 0.5 g/L lower and pH 0.14 units higher than Control wine. Wines produced with extended maceration tend to result in comparatively higher pH wines (Casassa, Huff, et al., 2019). Because CS + 10d wines spent a total of 15 days in contact with fermentation solids, a comparatively higher pH may be expected in these wines. However, this trend was not observed for Cabernet Sauvignon and Malbec wines, suggesting a matrix-conditioned response to pH increases upon variable maceration lengths.

A two-way ANOVA confirmed the lack of effect of these maceration techniques in the basic composition of the wines (Supplemental Table 2), except for ethanol, which showed rather small and circumstantial variations and the mentioned differences in pH and titratable acidity in Merlot wines. These differences, however, are unlikely to be of sensory relevance.

3.2. Phenolic composition of the wines

Wines of the three cultivars were analyzed for their detailed phenolic (Table 3) and chromatic composition (Table 4), after 3 months of bottle aging. Complementarily, Merlot wines produced by the three maceration techniques were followed throughout winemaking for selected phenolic classes and chromatic features (Figs. 1 and 2, respectively). Supplemental Tables 3 and 4 show two-way ANOVA analyses separating the effect of cultivar, maceration technique, and their interaction on the phenolic classes and chromatic features.

In Cabernet Sauvignon after 3 months of bottle aging, the CS + 5d wines showed the lowest anthocyanin concentrations, whereas for Malbec wines, conversely, this was true for Control wines. This suggests that Malbec anthocyanins were more readily extractable than Cabernet Sauvignon anthocyanins during the CS period, and/or showed enhanced solubility and retention during CS, as previously suggested (Casassa and Sari, 2014).

Tracking of anthocyanins in Merlo twines started at day 10 post-

Table 2
One-way ANOVA of the basic chemical composition of Cabernet Sauvignon, Malbec, and Merlot wines produced applying a control treatment (10 days of maceration), cold soak (CS) followed by a short maceration (5 days) and CS followed by a long maceration (10 days). Values represent the mean (± SEM) of three tank replicates. Analyses were performed after 3 months of bottle aging.

Cultivar (cv.)	Maceration technique	Ethanol (% v/v)	Residual sugars (g/L)	Titratable acidity (g/L tartaric acid)	pH	Volatile acidity (g/L acetic acid)
Cabernet Sauvignon	Control	14.93 ± 0.12 b[a]	2.01 ± 0.11 a	5.23 ± 0.05 a	3.66 ± 0.01 ab	0.61 ± 0.02 a
	CS + 5d	15.36 ± 0.03 a	1.80 ± 0.00 a	5.50 ± 0.16 a	3.61 ± 0.01 b	0.39 ± 0.03 b
	CS + 10d	15.13 ± 0.03 ab	1.80 ± 0.00 a	5.13 ± 0.23 a	3.68 ± 0.02 a	0.51 ± 0.03 ab
p-value		0.051[b]	0.097	0.251	0.092	0.507
Malbec	Control	15.06 ± 0.05 a	1.83 ± 0.01 a	3.92 ± 0.07 b	4.08 ± 0.02 b	0.51 ± 0.02 a
	CS + 5d	15.20 ± 0.05 a	1.83 ± 0.00 a	4.12 ± 0.11 ab	3.99 ± 0.01 ab	0.53 ± 0.01 a
	CS + 10d	14.63 ± 0.04 b	1.93 ± 0.01 a	4.37 ± 0.08 a	3.93 ± 0.01 b	0.56 ± 0.02 a
p-value		**0.008**	0.267	**0.049**	0.111	0.583
Merlot	Control	15.27 ± 0.02 a	2.28 ± 0.02 a	5.47 ± 0.13 a	3.68 ± 0.01 b	0.50 ± 0.14 a
	CS + 5d	15.46 ± 0.03 a	3.27 ± 0.05 a	5.03 ± 0.09 b	3.73 ± 0.01 b	0.55 ± 0.12 a
	CS + 10d	15.31 ± 0.04 a	3.38 ± 0.08 a	4.95 ± 0.08 b	3.82 ± 0.01 a	0.62 ± 0.23 a
p-value		0.329	0.651	**0.049**	**0.019**	0.281

[a] Different letters within a column indicate significant differences for Fisher LSD Test and $p < 0.05$.
[b] Significant *p*-values ($p < 0.05$) are shown in bold.

Table 3
One-way ANOVA of the basic chemical composition of Cabernet Sauvignon, Malbec, and Merlot wines produced applying a control treatment, cold soak (CS) followed by a short maceration (5 days) and CS followed by a long maceration (10 days). Values represent the mean (± SEM) of three tank replicates. Analyses were performed after 3 months of bottle aging.

Cultivar (cv.)	Maceration technique	Anthocyanins (mg/L)	Tannins (mg/L)	Small Polymeric Pigments (AU)	Large Polymeric Pigments (AU)	Total Polymeric Pigments (AU)	Total phenolics (mg/L)
Cabernet Sauvignon	Control	210 ± 5 a[a]	213 ± 42 a	2.03 ± 0.03 a	0.99 ± 0.12 a	3.03 ± 0.12 a	839 ± 26 a
	CS + 5d	165 ± 6 b	61 ± 7 b	1.84 ± 0.12 a	0.42 ± 0.01 b	2.32 ± 0.11 b	515 ± 85 b
	CS + 10d	255 ± 9 a	190 ± 16 a	1.97 ± 0.01 a	0.95 ± 0.03 a	2.92 ± 0.03 a	673 ± 30 ab
	p-value	**0.003**[b]	**0.015**	0.233	**0.005**	**0.005**	**0.016**
Malbec	Control	390 ± 11 c	375 ± 19 a	1.92 ± 0.05 a	0.83 ± 0.15 a	2.70 ± 0.13 a	1198 ± 148 a
	CS + 5d	512 ± 5 b	300 ± 21 a	1.69 ± 0.11 a	0.57 ± 0.08 a	2.26 ± 0.07 b	1264 ± 137 a
	CS + 10d	562 ± 19 a	314 ± 30 a	1.35 ± 0.12 b	0.63 ± 0.05 a	1.98 ± 0.12 b	1280 ± 50 a
	p-value	**0.001**	0.146	**0.017**	0.271	**0.008**	0.881
Merlot	Control	288 ± 4 a	799 ± 8 a	1.92 ± 0.07 ab	1.63 ± 0.12 a	3.55 ± 0.14 a	2113 ± 72.55 a
	CS + 5d	328 ± 21 a	566 ± 49 b	2.25 ± 0.10 a	1.53 ± 0.11 a	3.78 ± 0.21 a	1730 ± 110 b
	CS + 10d	307 ± 13 a	824 ± 93 a	1.88 ± 0.11 b	1.66 ± 0.17 a	3.54 ± 0.26 a	2371 ± 130 a
	p-value	0.232	**0.046**	0.073	0.811	0.683	0.124

[a] Different letters within a column indicate significant differences for Fisher LSD Test and $p < 0.05$.
[b] Significant *p*-values ($p < 0.05$) are shown in bold.

Table 4
One-way ANOVA of the detailed chromatic composition of Cabernet Sauvignon, Malbec, and Merlot wines produced applying a control treatment, cold soak (CS) followed by a short maceration (5 days) and CS followed by a long maceration (10 days). Values represent the mean (± SEM) of three tank replicates. Analyses were performed after 3 months of bottle aging.

Cultivar (cv.)	Maceration technique	L* (CIELab units)	C* (CIELab units)	H* (CIELab units)	a* (CIELab units)	b* (CIELab units)	Wine color (AU 420 + 520 + 620)
Cabernet Sauvignon	Control	67.93 ± 0.27 c[a]	31.80 ± 0.38 a	8.64 ± 0.27 c	31.52 ± 0.07 a	4.93 ± 0.03 b	1.19 ± 0.06 a
	CS + 5d	76.60 ± 0.35 a	22.81 ± 0.13 c	12.16 ± 0.03 a	22.65 ± 0.23 c	4.85 ± 0.02 b	0.83 ± 0.04 c
	CS + 10d	72.17 ± 0.17 ab	28.94 ± 0.26 b	10.15 ± 0.11 b	28.99 ± 0.29 b	5.59 ± 0.18 a	1.02 ± 0.02 b
	p-value	**<0.0001**[b]	**< 0.0001**	**< 0.0002**	**< 0.0003**	**0.008**	**0.005**
Malbec	Control	48.60 ± 1.60 b	46.88 ± 0.97 a	1.21 ± 0.61 a	46.86 ± 0.97 a	0.99 ± 0.52 a	1.10 ± 0.05 a
	CS + 5d	53.63 ± 1.34 b	46.46 ± 2.99 a	0.41 ± 0.34 a	46.44 ± 2.99 a	0.36 ± 0.32 a	0.96 ± 0.04 a
	CS + 10d	62.20 ± 1.85 a	37.01 ± 1.99 b	0.97 ± 0.30 a	37.01 ± 1.97 b	0.41 ± 0.35 a	0.73 ± 0.04 b
	p-value	**0.003**	**0.031**	0.470	**0.031**	0.143	**0.004**
Merlot	Control	64.80 ± 0.49 b	35.78 ± 0.48 a	15.88 ± 0.30 b	34.41 ± 0.47 a	9.80 ± 0.22 a	1.37 ± 0.02 a
	CS + 5d	66.93 ± 0.14 ab	34.12 ± 0.21 a	18.93 ± 0.79 a	32.20 ± 0.35 b	11.26 ± 0.39 a	1.35 ± 0.06 a
	CS + 10d	68.56 ± 1.27 a	31.61 ± 0.91 b	18.51 ± 0.62 a	29.97 ± 0.75 c	10.05 ± 0.62 a	1.24 ± 0.05 a
	p-value	**0.041**	**0.008**	**0.025**	**0.004**	0.124	0.201

[a] Different letters within a column indicate significant differences for Fisher LSD Test and $p < 0.05$.
[b] Significant *p*-values ($p < 0.05$) are shown in bold.

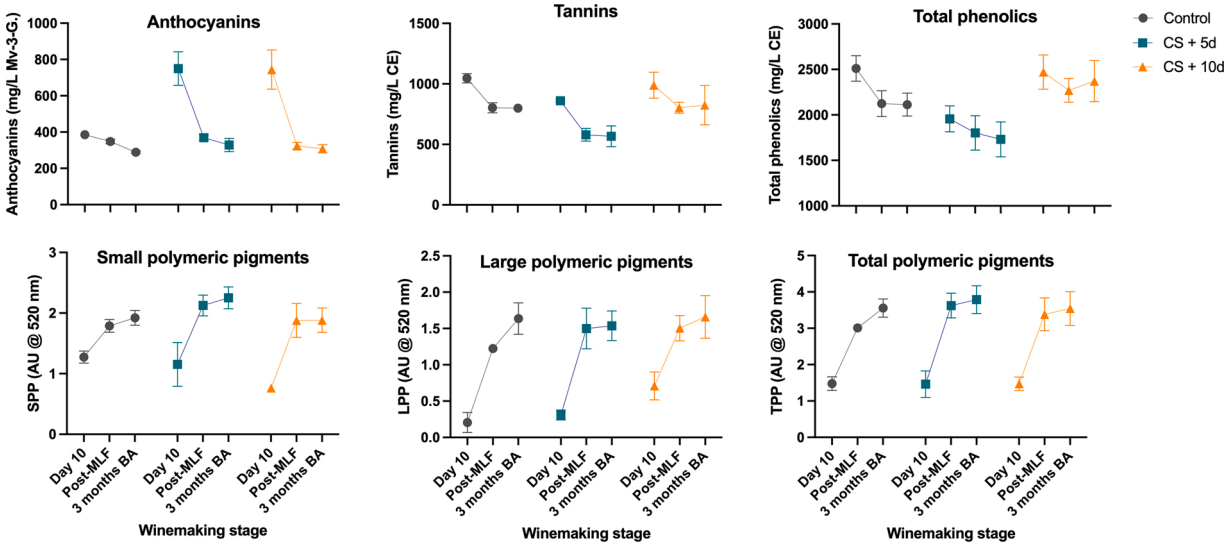

Fig. 1. Evolution during winemaking and bottle aging of selected phenolic classes and the tannin to anthocyanin ratio of Merlot wines produced applying a control treatment, cold soak (CS) followed by a short maceration (5 days) and CS followed by a long maceration (10 days). MLF: malolactic fermentation; BA: bottle aging; Mv-3-G: malvidin-3-glucoside; CE: catechin equivalent; SPP: Small polymeric pigments; LPP: Large polymeric pigments; TPP: Total polymeric pigments; AU: Absorbance units. Each value represents the average of three tank replicates and error bars represent the standard error of the mean.

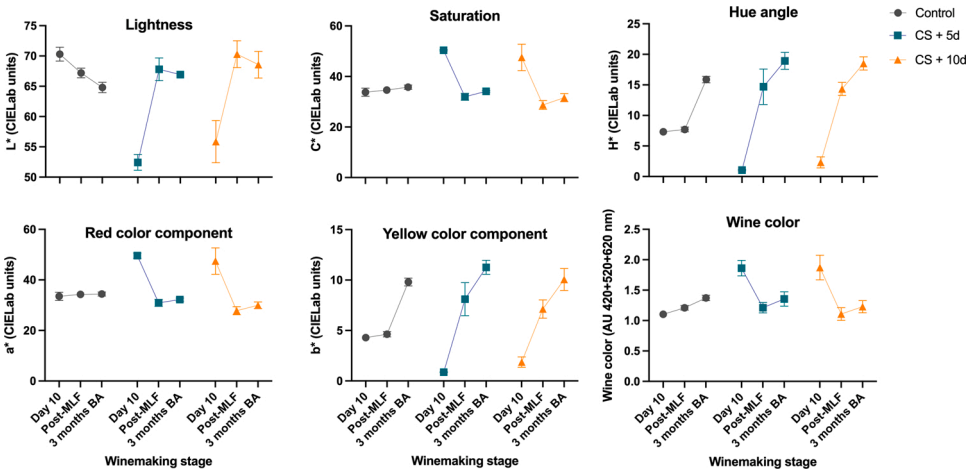

Fig. 2. Evolution during winemaking and bottle aging of the detailed chromatic composition of Merlot wines produced applying a control treatment, cold soak (CS) followed by a short maceration (5 days) and CS followed by a long maceration (10 days). MLF: malolactic fermentation; BA: bottle aging; AU: Absorbance units. Each value represents the average of three tank replicates and error bars represent the standard error of the mean.

crushing, and at that point anthocyanins were at their peak relative to their subsequent progression in the case of the CS + 5d and CS + 10d wines. Anthocyanin extraction typically peaks during the early stages of fermentation (Romero-Cascales et al., 2005). Therefore, CS + 5d wines were pressed off the fermentation solids at the peak of anthocyanin extraction (*i.e.,* day 10) whereas Control wines had comparatively much lower anthocyanin concentration at day 10. However, important losses of anthocyanins occurred in CS + 5d and CS + 10d wines after MLF, resulting in wines with equivalent anthocyanin levels than Control wines after 3 months of bottle aging (Table 3). Despite their solubility in an aqueous medium such as the one present during CS, previous research has reported a general nil effect of CS on anthocyanin extraction in Tannat (González-Neves et al., 2010), and Malbec wines (Casassa and Sari, 2014). Based on previous results and the results herein presented, extraction and retention of anthocyanins after CS may reflect not only actual extraction but also potential anthocyanin losses due for instance, coupled enzymatic oxidations with diphenols such as catechol and caftaric acid (Yokotsuka & Singleton, 1997). Because the enzymatic oxidation of musts occurs mainly prior to the addition of sufficient SO_2 to inactivate polyphenol-oxidase activity (Yokotsuka et al., 1997), increasing SO_2 additions prior to CS may preserve and enhance anthocyanin content and preserve wine color after CS on the resulting wines, as previously shown (Casassa et al., 2016).

Previous research on the effect of CS on tannin extraction reported no effect of this technique on Malbec and Barbera wines from Mendoza (Argentina) (Casassa et al., 2016), but, contrastingly positive effects on Sangiovese from Tuscany (Italy) (Parenti et al., 2004). Based on the latter, a cultivar-dependent response to tannin extraction during CS and winemaking was expected.

In the present work, tannins were unaffected by any of the winemaking techniques in Malbec, but, consistent with expectations, were significantly lower in CS + 5d wines in the case of Cabernet Sauvignon (71 % lower) and Merlot (29 % lower). In this regard, it is likely that for Cabernet Sauvignon and Merlot grapes, which are inherently high in extractable seed-derived tannins, a combination of initial low temperature and limited fermentation time, such as was the case of the CS + 5d treatment, limited seed-tannin extraction, and retention, thus resulting in overall lower tannin content in their respective wines.

Total phenolics, better referred to as "iron-reactive 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 colored ligands with, and thus quantify, monohydroxylated phenols and anthocyanins. Total phenolics were lower in CS wines in the case of Cabernet Sauvignon wines, and significantly lower in the case of CS + 5d wines in the case of Merlot wines (Table 3, Fig. 1). However, this trend for lower total phenolics in CS wines was not observed for Malbec wines. In the absence of cold soak, phenolic losses up to 20 % due to coupled enzymatic oxidations had been reported in Carignan, which presumably occurred during a short prefermentative phase or after the cessation of yeast activity (Cheynier et al., 1997). This is consistent with the overall lower phenolic content observed in Cabernet Sauvignon wines produced with CS and Merlot wines produced with CS + 5d.

Polymeric pigments are winemaking artifacts resulting from covalent reactions between anthocyanins and carbonyl compounds such as acetaldehyde and pyruvic acid (Adams et al., 2004), and as such, they can be quantified as small polymeric pigments, or SPP (Table 3 and Fig. 1). However, polymeric pigments can also be formed through covalent reactions between anthocyanins and tannins of variable molecular weights, constituting large polymeric pigments, or LPP (Adams et al., 2004). In addition to providing stable color, LPP can also precipitate salivary proteins and therefore elicit astringency (Casassa and Harbertson, 2014). Total polymeric pigments (TPP) simply represent the sum of SPP and LPP. Polymeric pigments were quantified in absorbance units (AU) due to the absence of a standard for polymeric pigment quantification. For the sake of brevity, SPP, LPP and total polymeric pigments are discussed together.

In Cabernet Sauvignon, SPP were not affected by any of the winemaking techniques. However, more than a half less LPP were produced in CS + 5d wines which saw shorter total maceration time, suggesting that extended maceration time may have been necessary for enhanced tannin extraction, which contribute to LPP formation (Casassa and Harbertson, 2014). In Malbec wines, CS + 10d resulted in lower levels of SPP and, overall, both CS techniques applied to Malbec and Cabernet Sauvignon resulted in lower amounts of TPP relative to Control wines, generally confirming a negative effect of CS on polymeric pigment formation (Supplemental Table 3). Previous research on CS applied to Cabernet Sauvignon has also confirmed a lack of effect of CS on polymeric pigment formation (Panprivech et al., 2015). As alluded to above, this could be tentatively attributed to a relatively lower extraction and molar amounts of tannins, if these are considered the limiting factor for polymeric pigment formation in CS wines.

In Merlot, an early formation of SPP over LPP was observed at day 10, with an overall improvement of SPP of 52 %, 95 % and 147 % from the initial reading to 3 months of bottle aging, in Control, CS + 5d and CS + 10d wines, respectively. However, by the time of the last sampling point at 3 months of bottle aging, SPP showed a significant improvement

only in CS + 5d wines (Table 3, Fig. 1). LPP formed more slowly but primarily increased after MLF. However, no treatment effects were observed for LPP. TPP were also unaffected by any of the winemaking techniques in Merlot wines. Our results therefore have conclusively shown a general lack of effect, or occasional negative effects, of cold soak on polymeric pigment formation.

3.3. Chromatic characteristics of the wines

The detailed chromatic features of the wines are presented in Table 4 whereas Fig. 3 shows the actual color representations and CIELab color differences (ΔE*) between pairs of Cabernet Sauvignon, Malbec, and Merlot wines. The visual color representations show the color of the wines as they will be seen through a 1 mm pathlength quartz cuvette, which explains why these may look lighter than they will be perceived in a regular ISO wine glass. Fig. 2 shows the evolution of the same chromatic features in Merlot wines throughout winemaking.

Irrespective of the cultivar, both CS alternatives had a negative impact on L*, generally indicating these wines were lighter in color than their Control counterparts. Similarly, changes in chroma (C*), which indicate a bias towards a dominant color component (a* or b*), also suggested the lowest color saturation in CS + 10d, all wines considered. Red hue values (a*) were consequently higher in Control wines relative to both CS alternatives. Taken as a whole, these results suggest an overall negative effect of CS on wine color characteristics. A previous report in which CS was applied to 6 different cultivars, including Merlot, Malbec, and Cabernet Sauvignon, showed that both C* and the red component of color (a*) were slightly higher in CS wines (Casassa et al., 2015). However, in the previous work, CS was limited to only 3 days. Interestingly, a longer duration of CS in the absence of CO_2 use, for 7 days (Casassa and Sari, 2014) or more (Panprivech et al., 2015), had been associated with nil or detrimental effects in wine color. This suggests that extending the CS period beyond 3 days may result in either ineffectual (but at the expense of tank usage and energy cost), or

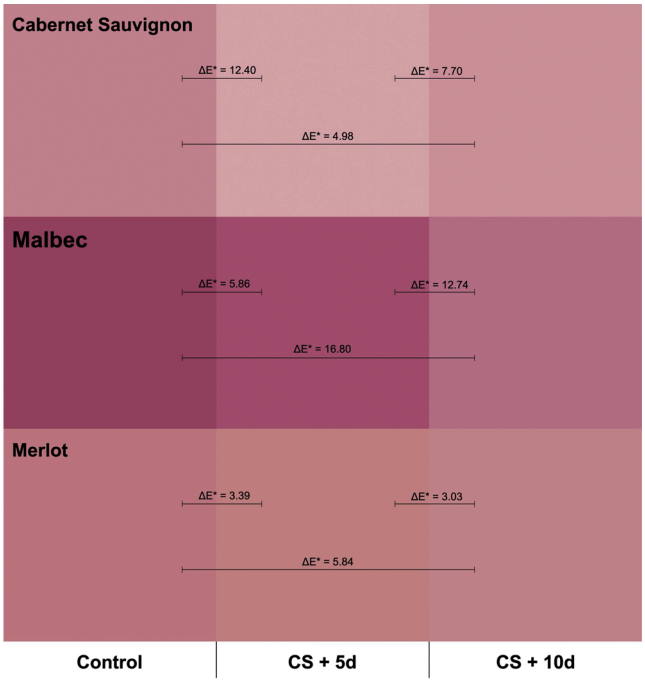

Fig. 3. CIELab color representations and CIELab color difference (ΔE*) between pairs of Cabernet Sauvignon, Malbec, and Merlot wines produced applying a control treatment, cold soak (CS) followed by a short maceration (5 days) and CS followed by a long maceration (10 days) after 3 months of bottle aging. Color panels depict wine samples analyzed through a quartz cuvette of 1 mm pathlength.

negative effects on wine color. This outcome is likely the result of coupled enzymatic oxidation reactions resulting in anthocyanin losses during cold soak, favored by increasing amounts of dissolved oxygen in the fermentation tanks undergoing CS (Casassa and Sari, 2014).

Hue angle (H*) is expressed in degrees; 0° indicates + a* (red), 90° indicates + b* (yellow), 180° indicates -a* (green), and 270° indicates -b* (blue). As shown in Table 3, Malbec wines had a more prominent red hue (*i.e.*, a*), than Cabernet Sauvignon and Malbec wines, which is consistent with these wines showing vastly lower H* values than the Cabernet Sauvignon and Merlot wines (Supplemental Table 4), as further confirmed in the CIELab color panels shown in Fig. 3. With regard to the winemaking techniques, H* was generally higher (*i.e.*, less red hue and more yellow hue) in CS wines in the case of Cabernet Sauvignon and Merlot wines, but no treatment effect was observed in the case of Malbec wines for H*. Similarly, very few treatment effects were observed for b*. In Port wines (tawnies and rubies), comparatively higher values of H* had been associated with browning resulting from oxidative aging under conditions of low aldehyde presence (Bakker et al., 1986). This further suggests that oxidative reactions did occur during the CS period in CS wines. Alternatively, it could also be offered that during the CS period certain phenolic precursors were extracted that further become oxidized over time, thus decreasing color saturation, and concomitantly increasing H*.

Wine color as measured by AU 420 + 520 + 620 nm has been the subject of much criticism as it only involves discrete absorbance values, as opposed to considering the whole spectrum of visible absorbance in the 380−770 nm range. Wine color was generally higher in Control wines in the case of Cabernet Sauvignon and Malbec wines, but this parameter showed no differences in the case of Merlot wines. However, Fig. 3 shows that even in the case of Merlot wines, Control wines were more saturated in color than CS+10d wines. More generally, Control wines were consistently more saturated in color than CS wines for all the wines considered. Indeed, a threshold ΔE* value of 3 CIELab units is considered enough for wines poured in a wine glass to be distinguishable by the human eye under practical conditions (Martínez et al., 2001). Fig. 3 shows that Control wines were not only more saturated but also could be distinguished from CS wines in all instances, with extremes as in the case of the comparison between Control and CS + 10d in Malbec wines, in which a ΔE* value of 16.80 was obtained.

Copigmentation is a transient phenomenon in young red wines, mediated by π-π non-covalent stackings between planar molecules and the flavylium cation, *Z*-chalcone or quinoidal anthocyanin forms, resulting in a hyperchromic shift (color enhancement) and a bathochromic shift (color shift towards more bluish hues). Enhanced copigmentation may lead to the progressive formation of polymeric pigments (Trouillas et al., 2016), thereby resulting in stable color and, potentially, desirable mouthfeel characteristics. In the present work, the copigmentation index relative to Control wines showed negative values for all CS wines, ranging from -24 % in Malbec CS + 10d wines to -5% in CS + 5d wines. Lesser effects in magnitude were observed for CS Merlot wines, but the copigmentation index reminded negative, nonetheless. Because the copigmentation index has been proposed as a measurement to evaluate the effectiveness of copigments, added or extracted into wine (Gombau et al., 2019), it can be concluded that CS, regardless of the maceration length that followed it, CS was unable to extract more copigments. A previous report, also in Malbec wines subjected to CS, confirmed these results whereby no effect of CS was observed on copigmentation relative to Control wines over a 790-day period (Casassa and Sari, 2014).

It is finally worth pointing out that wine color measured as AU 420 + 520 + 620 nm showed relatively comparable values between Cabernet Sauvignon and Malbec wines (and to a certain extent, also Merlot wines). For example, Control wines of both Cabernet Sauvignon and Malbec wines showed a wine color difference of only 0.09 AU between them (Table 4). However, Malbec wines showed vastly different values of L*, C*, H*, a* and b* than Cabernet Sauvignon wines. Indeed, the

wines of these two cultivars were accordingly vastly different in perceived color as seen by the human eye (Fig. 3). Because at the pH of most common young red table wines anthocyanins positively deviate from Beers law, perceived color increases more than proportionally with increasing concentrations of anthocyanins (Boulton, 2001), which was the case of the Malbec wines of the present study (Table 3). Thus, the observed differences in perceived wine color between Malbec and Cabernet Sauvignon wines as shown in Fig. 3 may be an indication of copigmentation, and more specifically, self-association stackings of flavylium forms (González-Manzano et al., 2008), as suggested elsewhere (Bakker et al., 1986). Further evidence of enhanced copigmentation in these Malbec wines relative to Cabernet Sauvignon and Merlot wines can be established based on comparatively higher C* values in these wines, which has been correlated with self-association of monomeric anthocyanins (González-Manzano et al., 2008), despite similar or even lower values of wine color (AU 420 + 520 + 620 nm).

3.4. Sensory descriptive analysis of Malbec and Merlot wines

Malbec and Merlot wines were analyzed by sensory descriptive analysis after 3 months of bottle aging, coincident with the last sampling point considered for phenolics, chromatic characteristics and copigmentation. Because a total of 20 sensory descriptors were assessed by the panelists for each experiment, it was considered that relying on ANOVA, which perform multiple tests for each descriptor, could artificially inflate the possibility to incur a type-I error (Lawless and Heymann, 2010). Because of the latter and the multicollinearity of sensory attributes, a MANOVA was initially performed to evaluate the significance of overall sensory differences among wine samples. MANOVA indicated that when all the sensory attributes were considered, winemaking treatments produced a significant sensory effect in Malbec (Wilks' Lambda, $p = 0.028$), and Merlot wines (Wilks' Lambda, $p = 0.002$), further justifying performing ANOVA.

Tables 5 and 6 present a three-way ANOVA of sensory descriptors for Malbec and Merlot wines, respectively, considering the effects of the winemaking treatments, the panelists, the replicate, and the panelist × wine interaction. Fig. 5 A and B show a more concise visualization of the most salient sensory aspects of Malbec and Merlot wines, respectively, by way of a PCA. The PCA analysis retained only discriminant descriptors and because the analysis was performed with the raw data including all the replicates, confidence ellipses were constructed with 95 % certainty, which provides significance testing (Lawless and Heymann, 2010). The ellipses represent empirical descriptions of the variability of the sensory evaluations, and if the ellipses do not superimpose, then the wines are significantly different from a sensory standpoint. In both sensory panels, the good performance of the panelists was evidenced by the general lack of panelist × wine interaction for most descriptors (Lawless and Heymann, 2010). Likewise, even though the three replicates of each winemaking treatment were included and analyzed, there was generally no significant effect of the replicate on the sensory descriptors of each treatment.

The 20 sensory descriptors pertaining color, aroma, taste, retronasal aroma (*i.e.*, flavor), and mouthfeel were evaluated along a 10-cm unstructured scale. In Malbec wines, color saturation, fresh fruit aromatic character, sweetness, bitterness, astringency, and body were affected by the winemaking treatments (Table 5). Control wines were highest in color saturation. This result agrees with the chromatic characteristics shown in Fig. 3, and with the comparatively higher copigmentation index of these wines relative to their CS counterparts (Fig. 4). Control wines were also higher in sweetness and bitterness. CS + 10d wines showed enhanced fresh fruit aroma and body, but also comparatively higher bitterness and astringency than CS + 5d wines (Fig. 5 A). CS + 5d wines did not show any distinctive sensory feature and their sensory profile gravitated in between that of Control and CS + 10d wines (Fig. 5A). One of the main purposes of the CS + 5d treatment was to allow for color and flavor extraction (primarily during the cold soak

Table 5
Three-way ANOVA of sensory descriptive terms of Malbec wines produced applying a control treatment, cold soak (CS) followed by a short maceration (5 days) and CS followed by a long maceration (10 days). Values represent the mean of 12 evaluations (n = 12). Evaluations were made along a 10-cm unstructured line scale. A: aroma; F: Flavor.

ANOVA effect	Color saturation	Overall aroma	Fresh fruit (A)	Red fruit (A)	Dried fruit (A)	Cooked (A)	Spicy (A)	Sweetness	Acidity	Bitterness	Fresh fruit (F)	Berry (F)	Dried fruit (F)	Herbal (F)	Spicy (F)	Hot (F)	Astringency	Dryness	Body	Length
Wine																				
Control	6.27 a[a]	6.99 a	2.42 b	2.08 a	0.75 a	1.04 a	0.94 a	5.74 a	5.78 a	6.36 a	2.06 a	1.99 a	0.79 a	0.71 a	1.21 a	2.65 a	6.04 ab	6.00 a	6.01 b	3.67 a
CS + 5d	6.15 ab	6.81 a	1.81 b	1.84 a	0.90 a	1.04 a	1.00 a	5.30 b	5.78 a	5.27 c	1.79 a	1.41 a	0.85 a	0.89 a	0.78 a	2.67 a	5.71 b	5.87 a	5.87 b	3.42 a
CS + 10d	5.86 b	7.14 a	3.46 a	1.67 a	0.99 a	0.91 a	0.83 a	5.26 b	5.98 a	5.82 b	1.93 a	1.37 a	1.11 a	0.52 a	1.36 a	3.38 a	6.13 a	6.00 a	6.45 a	3.51 a
p-value	**0.045**[b]	0.276	**0.002**	0.665	0.817	0.929	0.883	**0.049**	0.359	**<0.0001**	0.724	0.326	0.734	0.496	0.274	0.448	0.102	0.792	**0.001**	0.252
Panelist																				
p-value	0.126	**0.003**	**0.001**	**<0.0001**	**<0.0001**	**0.003**	**0.001**	**0.006**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.022**	**<0.0001**	**<0.0001**	**<0.0001**	**0.004**	**<0.0001**	**<0.0001**
Replicate																				
p-value	0.357	0.090	0.367	0.159	0.131	0.667	0.960	0.267	0.624	0.088	**0.033**	0.803	0.353	0.423	**0.015**	0.535	0.942	0.326	0.092	0.120
Panelist × Wine																				
p-value	0.972	0.972	0.927	0.512	0.729	0.900	0.392	0.907	0.425	0.284	0.823	0.473	0.923	0.648	0.593	0.979	0.563	0.558	0.208	0.100

[a] Different letters within a column indicate significant differences for Fisher LSD Test and $p < 0.05$.
[b] Significant *p*-values ($p < 0.05$) are shown in bold.

Table 6
Three-way ANOVA of sensory descriptive terms of Merlot wines produced applying a control treatment, cold soak (CS) followed by a short maceration (5 days) and CS followed by a long maceration (10 days). Values represent the mean of 12 evaluations (n = 12). Evaluations were made along a 10-cm unstructured line scale. A: aroma; F: Flavor.

ANOVA effect	Color saturation	Overall aroma	Fresh fruit (A)	Red fruit (A)	Dried fruit (A)	Cooked (A)	Spicy (A)	Sweetness	Acidity	Bitterness	Fresh fruit (F)	Berry (F)	Dried fruit (F)	Herbal (F)	Spicy (F)	Hot (F)	Astringency	Dryness	Body	Length
Wine																				
Control	6.55 a[a]	7.37 b	1.59 a	1.40 b	0.98 b	1.48 a	1.36 a	5.34 b	5.97 a	5.58 b	2.79 a	2.80 a	1.05 a	1.50 a	0.53 b	3.62 a	7.34 a	7.25 a	6.19 a	3.92 a
CS + 5d	6.53 a	7.29 b	1.59 a	1.78 ab	2.07 a	1.32 a	0.88 a	5.80 ab	5.66 a	5.98 ab	2.22 a	1.93 a	1.28 a	1.13 a	1.45 a	4.06 a	6.38 b	6.73 b	6.23 a	3.52 b
CS + 10d	6.75 a	8.04 a	0.96 b	1.86 a	1.82 ab	1.19 a	1.00 a	6.28 a	5.68 a	6.08 a	2.22 a	1.86 a	1.45 a	0.92 a	1.03 ab	4.10 a	7.46 a	7.65 a	6.42 a	3.46 b
p-value	0.309	**0.001**	**0.031**	0.077	**0.032**	0.685	0.532	**0.004**	0.217	0.088	0.450	0.165	0.552	0.424	0.132	0.709	**<0.0001**	**<0.0001**	0.498	**0.025**
Panelist																				
p-value	**0.010**[b]	0.061	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.103	0.074	**<0.0001**	**0.001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.007**	**0.020**	**<0.0001**	0.243	**0.000**	**0.002**	**<0.0001**
Replicate																				
p-value	0.154	0.089	**0.032**	**0.028**	0.066	0.154	0.125	0.247	0.861	0.386	0.632	0.196	0.295	0.503	0.700	**0.001**	0.295	0.270	0.416	0.570
Panelist × Wine																				
p-value	0.994	0.819	**0.002**	**0.001**	0.823	0.290	0.920	0.742	0.848	0.604	0.966	0.593	0.699	0.636	0.676	0.939	0.295	0.297	0.790	0.701

[a] Different letters within a column indicate significant differences for Fisher LSD Test and $p < 0.05$.
[b] Significant *p*-values ($p < 0.05$) are shown in bold.

Fig. 4. Copigmentation index of Cabernet Sauvignon, Malbec, and Merlot wines produced applying a Control treatment, cold soak (CS) followed by a short maceration (5 days) and CS followed by a long maceration (10 days) after 3 months of bottle aging. The Control wines were used as a baseline for calculation of the copigmentation index between pairs of wines. All the replicates were considered.

period), but also to limit tannin extraction, and specifically seed-derived tannins, during the later stages of the alcoholic fermentation process. Although Table 3 shows that tannin extraction measured by protein precipitation was not affected by any of the winemaking techniques in Malbec wines, the CS +5d wines were sensorially perceived as the less astringent wines (Fig. 5A). Overall, and except for a moderate increase in fresh fruit aroma of CS + 10d wines, the application of CS did not have any outstanding positive effects on Malbec wines. This result agrees with previous research also conducted on Malbec wines. For example, a study compared the sensory effects of CS for 7 days with and without CO_2 using a sensory panel (Casassa and Sari, 2014). Relative to control wines without CS, it was found that CS without CO_2 resulted in wines with lower colour and a noticeable acetaldehyde character, whereas CS with CO_2 produced wines with less fruity character than control wines (Casassa and Sari, 2014). Another study in which CS was shortened to 3 days and conducted under CO_2 reported that CS did not affect color components, aroma, astringency, and body relative to control wines (Casassa, Bolcato, et al., 2015). Therefore, the overarching conclusion of the present and the previous studies is that the application of CS appears to be of little or no merit in Malbec winemaking.

In Merlot wines, Control wines showed lower overall aroma intensity and dried fruit character, but higher astringency, dryness (relative to CS + 5d wines) and retronasal length. As also observed in their counterpart Malbec wines, CS + 5d wines were again sensorially placed in between Control and CS + 10d wines and showed an enhanced fresh fruit aroma (relative to CS + 10d wines), dried fruit aroma (relative to Control wines), and overall lower astringency and dryness (Table 6). Therefore, consistent with what was observed for Malbec wines, astringency, and dryness in CS + 5d wines were reduced relative to Control wines. Lastly, CS + 10d wines were characterized by higher overall aroma intensity (Table 6, Fig. 5B), and were perceived as more astringent and drier than CS + 5d wines, but not than Control wines. The current results in Merlot generally support the notion of a moderate impact of both alternatives of CS on the wines of this cultivar. In a previous report also on Merlot, CS with dry ice for 3 days had a slight positive effect on chromatic characteristics as measured by UV–vis spectroscopy, but no effects were found on visual color intensity, aroma, astringency, and body relative to Control wines (Casassa, Bolcato, et al., 2015). Another report in Merlot

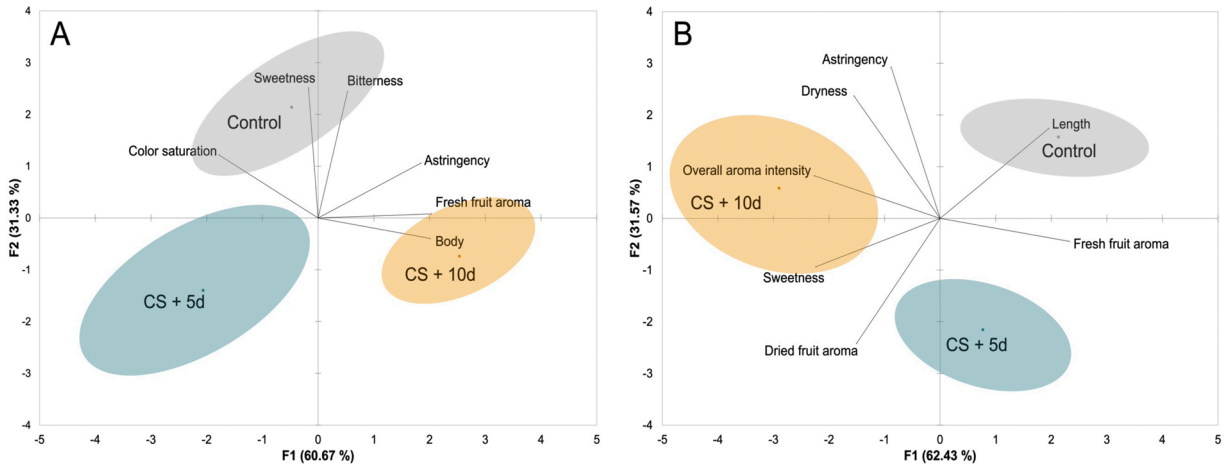

Fig. 5. Principal component analysis of descriptive sensory data of A) Malbec, and B) Merlot wines produced applying a control treatment, cold soak (CS) followed by a short maceration (5 days) and CS followed by a long maceration (10 days). Wines were evaluated by a trained sensory panel (n = 12), and only significant and discriminant descriptors were retained by the analysis.

from Umbria (Central Italy), in which CS was applied for 4 days at 8 °C found that CS wines had levels of ethyl hexanoate, ethyl octanoate, ethyl decanoate, diethyl succinate, and ethyl laurate which were 20 % higher in CS relative to Control wines produced without CS (De Santis and Frangipane, 2010). In Mencia wines, prefermentative CS was suggested to improve volatile composition by increasing the extraction of aroma precursors, but no effect on ethyl esters, nor odor activity values and recognition thresholds were reported (Mihnea et al., 2015). Contrastingly, CS applied to Aglianico, Primitivo and Nero di Troia red wines resulted in wines with increased ester concentrations, generally above their detection thresholds (Gambacorta et al., 2019). Lastly, the application of CS for 7 days at 8−10 °C in Cabernet Sauvignon enhanced the fruity, caramel, and floral aroma series, including esters and ß-damascenone within a total of 89 volatiles quantified (Cai et al., 2014). Esters are compounds known to provide aromatic lift and increase overall wine aroma, and ß-damascenone has been associated with enhanced fruitiness in red wines (Pineau et al., 2007). Although the studies of Cai et al. (2014); De Santis and Frangipane (2010) and Gambacorta et al. (2019), did not report sensory data to confirm if CS wines were indeed more aromatic, it could add support to the higher perceived fresh fruit aroma and aromatic intensity in CS + 10d wines of the present study (Fig. 5).

4. Conclusions

Cabernet Sauvignon, Malbec, and Merlot wines were produced to compare the effect of maceration length (5 and 10 days), after completion of a cold soak (CS) period of 5 days. One of the main premises of the present work hinged upon the possibility to enhance color and flavor extraction during CS while minimizing tannin extraction by reducing the length of maceration after completion of CS, with the expectation of cultivar-specific results. These maceration techniques did not produce any significant impact on the basic chemistry of the wines. There was also a nil effect on anthocyanin extraction upon application of CS in Cabernet Sauvignon and Merlot. Results indicated an overarching negative impact of CS on the chromatic composition of the wines, including wines of lower saturation and red color component and higher hue. These chromatic differences in favor of Control wines and to the detriment of CS wines were corroborated by both the CIELab color difference (ΔE*), and by sensory descriptive analysis. We hypothesize that color losses may have occurred during the CS period due to coupled enzymatic oxidation, or that, alternatively, certain easily oxidizable phenolic precursors were extracted during this period.

CS followed by a short maceration time (CS + 5d) effectively reduced astringency and bitterness perception in the resulting wines, but was ineffective at enhancing aroma and flavor components. It can therefore be an advisable protocol for highly tannic cultivars in which the stylistic goal is to curb tannin extraction into wine. Conversely, CS + 10d led to wines of higher body, astringency, fresh fruit aroma and enhanced aroma intensity. Enhanced aroma intensity and aromatic lift could be the result of ester formation and extraction of aroma precursors during CS, which were likely released after sufficient maceration time in the case of CS + 10d. However, the magnitude of these increments was modest and may likely escape consumer perception.

Present and past evidence of the effects of CS seem to point out that a shorter duration of CS (*e.g.*, 3 days), and diligent use of CO_2 should be instituted to minimize coupled enzymatic oxidation during CS. However, the winemaker should factor in the energy input and logistic constraints that this technique entails and contrast those with the modest and rather inconsistent positive effects of its application on phenolic chemistry, chromatic characteristics, and sensory features.

Funding sources

This project was partially funded by the Instituto Nacional de Tecnología Agropecuaria (INTA) Mendoza (Mendoza, Argentina).

CRediT authorship contribution statement

L. Federico Casassa is the corresponding author of the article, conceived and designed the experiment, helped in the execution of the experiment and compiled, analyzed the data and wrote the manuscript. **Esteban Bolcato** helped in the execution of the experiment. **Santiago Sari** contributed with data compilation, execution of the various analysis and data analysis. **Nora Barda** conducted the sensory analysis of the wines, contributed with data compilation, execution of the various analysis and data analysis from the sensory panel.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

Panel members of the INTI-INTA Villa Regina Wine Sensory Panel are thanked for their commitment to this study. Vanesa García is acknowledged and thanked for expert assistance on the analysis of grapes and wine samples.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jfca.2021.104168.

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