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Impact of closure type and storage temperature on chemical and sensory composition of Malbec wines (Mendoza, Argentina) during aging in bottle

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Abstract

Malbec is the flagship variety of Argentina mainly due to its high oenological value and plasticity to obtain different wine styles. During bottled aging, the chemical and organoleptic composition of wines is subject to changes depending on the aging conditions (closure, oxygen level, temperature, time). However, the combined effect of these factors on chemical composition and organoleptic characteristics of Malbec wines has not been studied yet. Wines were bottled with screw cap and natural cork and were kept in chambers at 15ºC and 25ºC for 2 years. Sampling was performed at 2, 4, 6, 9, 12, 15, 18, 21 and 24 months. Concentrations of free sulfur dioxide, dissolved oxygen, anthocyanins, tannins, esters, volatile phenols, organic acids, and color saturation decreased during the storage process. While, the formation of polymeric pigments, the color attributes (lightness and hue) and the levels of alcohols, norisoprenoids, furanoids and terpenoids increased. At 24 months, Malbec wines were organoleptically different. Wines kept at 15ºC were associated with high sensory perceptions in color intensity and violet tint, those presented a positive correlation with free sulfur dioxide, tannins, and anthocyanins levels. On the contrary, wines aged at 25ºC were linked with high sensory perceptions of dried vegetative and dried fruit aromas. These descriptors were positively correlated with norisoprenoids, furanoids, and terpenoids. In general, the chemical composition and organoleptic attributes of bottled Malbec wines (Mendoza, Argentina) were stable respect closure type employed, but highly sensitive to the combined effect of time and storage temperature. This finding is key to making decisions about the wine style searched, and costs (e.g. refrigeration) involved in the conservation period until consumption.

Keywords

Aging, storage temperature, cork, screw cap, phenolics, aromas, Malbec wines

1. Introduction

The optimal agro-ecological conditions for Malbec (*Vitis vinifera* L.) grapegrowing have turned their wine into an emblem of Argentina. Mendoza province accounts for more than 80% of the cultivated area for this variety in the country, and their wines sold in bottles constitute the most exported product of this industry.

In addition, due to its phenolic composition, characterized by high levels of anthocyanins and tannins, it has a great aging potential (Fanzone, Peña-Neira, Jofre, Assof, & Zamora, 2010).

Chemical composition and sensory attributes of young wines can change during the aging process. These modifications will depend on their initial composition (e.g. variety, climate, soil, viticultural and oenological management) and storage conditions (temperature, closure type and time, among other~~s~~). The absence of control in these parameters may decrease the useful life and wine quality (Avizcuri et al., 2013; Casassa, Bolcato, Sari, Fanzone, & Jofré, 2016; Garde-Cerdán, Marsellés-Fontanet, Arias-Gil, Ancín-Azpilicueta, & Martín-Belloso, 2008; Robinson et al., 2010; Waterhouse, Sacks, & Jeffery, 2016c).

During this stage, both the wine color and aromas are modified. The monomeric anthocyanins polymerize or react with other compounds generating more stable pigments, typical of aged wines (Fulcrand, Dueñas, Salas, & Cheynier, 2006; Waterhouse et al., 2016c). On the other hand, the floral-fruit profile of the young wines (fermentative aromas) are chemically changed (e.g. by intermolecular interactions, oxidations, reductions, hydrolysis) towards more complex aromas (furanoids generation and precursors release) (Jackson, 2008; Linsenmeier, Rauhut, & Sponholz, 2010).

The closure type employed in bottling acts as the only permeable membrane between the wine and the environment. This membrane regulates the ingress of oxygen from the outside (Lopes, Saucier, Teissedre, & Glories, 2007) and the migration of some volatile compounds from wine by absorption phenomena (Capone, Sefton, Pretorius, & Høy, 2003; Pickering, Blake, & Kotseridis, 2009; Silva, Julien, Jourdes, & Teissedre, 2011). Thus, closures with higher oxygen permeability would favor color stabilization and would promote oxidation reactions of phenolic and aroma compounds. Caillé et al. (2010) found that after 10 months of conservation, Grenache red wines sealed with natural cork and synthetic stoppers presented greater intensity of color, hue, caramel and red fruit attributes; and lower vegetative and animal (reductive) aromas than those closed with screw caps.

The storage temperature of bottled wines exerts a significant effect on the kinetics of the reactions occurred during aging (Scrimgeour, Nordestgaard, Lloyd, & Wilkes, 2015). Regarding this, Butzke, Vogt, and

Chacón-Rodríguez (2012) monitored the exposure of a model wine to the environment temperature during three weeks of commercial shipments and found an aging trend like 18 months, compared to a wine stored in the cellar (13ºC). The accumulated heat exposure of the wines was estimated using ethyl carbamate formation. Likewise, other authors Puech, Vidal, Pegaz, Riou, and Vuchot (2006) observed that red wines preserved at low and stable temperatures (around 14ºC), for 36 months, maintained higher anthocyanins levels and showed lower yellow hue, oxidized and fruity aromas, than those preserved at variable and higher temperatures (26ºC).

As mentioned above, the changes produced during wine storage will also depend on the grape variety and origin. Cassino, Tsolakis, Bonello, Osella, and Osella (2019) observed greater variability in volatile and non-volatile composition of red wines (Barbera, Nebbiolo, Ruchè and Grignolino) preserved in optimal storage conditions (12ºC, 4 years), due to the wine type and geographical origin than by the age of the wine. Instead, the behavior of Malbec wines from different zones of Mendoza and California during 5 years of aging in bottles showed a significant decrease in the anthocyanin content compared to the init ial samples, associated in a 40% to the time factor and in 20% to the region (Agazzi et al., 2018).

To the best of our knowledge, there has been no report so far the combined effect of different aging factors on the chemical composition of Malbec wines. Therefore, the first aim of the present work was to study the effect of temperature, closure type and time of conservation on phenolic and volatile composition of Malbec wines (Mendoza, Argentina) during 24 months of aging. Taking into account their influence on organoleptic characteristics, the second aim was to correlate the analytical variables with the sensory attributes by means of regression techniques (PLS).

2. Materials and methods

2.1. Wine samples

Malbec grapes (San Pablo, Tupungato, Mendoza) of 2015 season were harvested at 24°Brix and vinified following standardized oenological protocols for this variety by Fincas Patagónicas S.A winery. The alcoholic fermentation was conducted with commercial yeasts (EC1118; Lallemand, Montreal, Canada) in a stainless steel tank of 500 hL. After maceration was completed (21 days), free run wines were collected in another tank where malolactic fermentation was performed with a commercial *Oenococcus oeni* culture (VP-41, Lallemand). Then, the wine was racked off the lees, adjusted to 40 mg L^{-1} of free SO_2, and stabilized at 0-1ºC for 30 days. Before bottling, free SO_2 was adjusted to 0.70 mg L^{-1} of SO_2, and the wine was industrially fractioned (without filtration) in 750 mL transparent glass bottles. Bottles were closed with screw caps (30 x 60 mm) with Saran/Tin seal (SC; Astro, Arpex Internacional, Mendoza, Argentina) and natural corks of 24 x 45 mm (C; Portocork, CA, USA). All wine manipulations were performed with high purity nitrogen gaseous. This Included: the purged with the nitrogen of empty bottles to avoid oxygen pick-up and scavengers of headspace before bottles plugged. A total of 220 bottles were filled. By both closures types employed (c), the headspace was 5.09 mL. Bottled Malbec wines were kept into termostatizated chambers at two storage temperature (T): 15 ± 2.4ºC (15ºC) and 25 ± 1.9ºC (25ºC) during 2 years. The temperature was recorded with sensors (LogTag Recorder, Auckland, New Zealand) located in each chamber, every half hour. Sampling (bottles in triplicate) was performed at 2, 4, 6, 9, 12, 15, 18, 21 and 24 months. Chemical and microbiological analyses were carried out at each sampling. Chemical and microbiological analyses were carried out at each sampling point in triplicate. The microbiological stability of the wines was observed by detection analyses of total yeast, *Brettanomyces/Dekkera*, lactic and acetic bacterias, according to the method proposed by the OIV (OIV, 2010).

2.2. Dissolved oxygen concentration

Dissolved oxygen concentration (DOC), was measured by luminescence non-destructive technology Fibox 3 Trace fiber-optic oxygen-meter (PreSens, GmbH, Regensburg, Germany). Dissolved oxygen measurements were using Planar Oxygen-Sensitive Spot-PSt3 sensor (PreSens, Germany) glued in the middle of the glass bottle prior to filling with wine. Sixteen bottles were prepared for this purpose. Eight for bottles with cork closure (4 for 15ºC and 4 for 25ºC) and eight for screw cap (4 for 15ºC and 4 for 25ºC). The bottles were equally distributed in the bottling line during bottling. Manufacturer calibration was used for all the sensors during oxygen measurements. To the beginning of assay and at each sampling times were taken oxygen measurement without shaking bottles before measurement.

2.3. Standard oenological parameters

Standard parameters (ethanol, pH, total acidity, volatile acidity, malic, tartaric, lactic and citric acids) in Malbec wines, were measured using a platinum diamond ATR single reflection sampling module cell mounted in a Bruker Alpha instrument (Bruker Optics GmbH, Ettlingen, Germany). The MIR spectra of samples were recorded on OPUS software (Bruker Optics). Free sulfur dioxide measurements were carried out with FIAstar™ 5000 analyzer (Foss Analytical, Hillerød, Denmark).

2.4. Phenolic composition and color parameters

Large and small polymeric pigments, anthocyanins and tannins were evaluated by spectroscopy methods (Harbertson, Picciotto, & Adams, 2003). CIELAB parameters [L*(Lightness, 0 black and 100 white), C^*_{ab} (chroma, saturation), h_{ab} (tone; red, green, yellow) and the a*b*(red/green; yellow/blue) coordinates], were calculated from the absorption spectra by using the CromaLab® software. Color difference (ΔE^*_{ab}) was calculated as the Euclidean distance between two points (1 and 2) in three-dimensional (L*a* b*) space. ΔE^*_{ab} (L^*_1, a^*_1, b^*_1; L^*_2, a^*_2, b^*_2) = $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, where $\Delta L^* = L^*_1 - L^*_2$; $\Delta a^* = a^*_1 - a^*_2$ and $\Delta b^* = b^*_1 - b^*_2$.

2.5. Volatile compounds

The analysis of volatile compounds of Malbec wines was done by solid phase microextraction-gas chromatography/mass spectrometry methodology, according to Úbeda, del Barrio-Galán, Peña-Neira, Medel-Marabolí, and Durán-Guerrero (2017). 4-methyl-2-pentanol (0.75 mg L^{-1}) as an internal standard (Merck, Darmstadt, Germany) was used. Gas chromatography analysis was carried out on the 7890B Agilent GC system coupled to a quadrupole mass spectrometer Agilent 5977 inert (Agilent Technologies, Palo Alto, CA, USA). All data were recorded using MS ChemStation (Agilent Technologies, Palo Alto, CA, USA). Compounds identification was based on the comparison with authentic reference standards. However, some compounds tentative identification was carried out by comparison of mass spectra obtained from each compound with reference spectra on the NIST98 library and also comparing their retention index with ones found in the literature. Data were obtained from calibration curves with reference standards (relative area *vs*. concentration). The relative concentration was calculated by dividing the peak area of the target ion of each compound by the peak area of the target ion of the internal standard.

2.6. Descriptive sensory analysis

At 24 months of aging, Malbec wines were evaluated by sensory descriptive analysis with 15 trained judges, in the adequate tasting room. Previously, during two months, judges were trained into the identification of aromas, flavors, and wine color. Evaluated attributes in Malbec wines were agreed by panelists, and reference standards (low, medium and high levels) for every descriptor were prepared according to Noble et al. (1987). Reference standards are were prepared as indicated in Supplementary Table 1. Organoleptic characteristics analyzed were visual parameters (color intensity and violet tint), odorant descriptors [aromatic intensity, dried fruit (blueberry, strawberry and raspberry jam), caramelized and dried vegetative (tea and tobacco)], and mouthfeel sensations (astringency, acidity and bitter). Mouthfeel sensations and odorant descriptors were evaluated using black glass, while the visual parameters with white glass. Malbec wines were punctuated on an unstructured scale (in duplicate in two sessions) using an application of Soldesa software (Mendoza, Argentina).

2.7. Statistical analysis

Data were analyzed by multifactor analysis of variance using the Infostat/P 2017 software (Universidad Nacional de Córdoba, Argentina). Tuckey test was used to determinate significative differences with a confidence level of 95%. The sensory data for each attribute were analyzed using a suggested method by Varela and Ares (2014) of the mixed model to considerate the interaction panelist-sample, and R with SensoMineR package was used. Correlation and predictions of sensory and analytical variables were done by partial least squares regression (PLS), with R software.

3. Results and Discussion

3.1. Standard oenological parameters

At the beginning of the trial, the Malbec wine had 14 %v/v ethanol, 5.57 g L^{-1} total acidity, 0.46 g L^{-1} volatile acidity and pH 3.55. During the aging of wines, no significant differences were observed in the evolution of these oenological parameters by the effect of closure type and storage temperature, as shown in Supplementary Table 2. At the end of the process, for all treatments, the ethanol content of wines showed no significant differences in relation to the initial wine, whereas total acidity and pH decreased by 14% and 0.15 units, respectively, and volatile acidity was close to 0.77 g L^{-1}. These results are in agreement with similar studies carried out with other grape varieties (Garde-Cerdán et al., 2008; Hopfer, Buffon, Ebeler, & Heymann, 2013; Ortega-Heras, González-Sanjosé, & González-Huerta, 2007).

During Malbec wines aging, a decrease in tartaric, malic and citric acid concentrations was observed. Of these, tartaric acid (H^2T) showed the greatest concentration variation ($\Delta C_{\text{0-24 months}}$ = 0.48 g L^{-1}) probably do it would precipitate as potassium bitartrate (KHT). The KHT precipitation could generate a shift of thermodynamic equilibrium ($H2T/HT^-$) towards the bitartrate formation. When this equilibrium is modified, hydronium ions are released into the medium, which would contribute to the decrease of pH and total acidity of wines, as has been observed in this study (Boulton, Singleton, Bisson, & Kunkee, 1999; Waterhouse, Sacks, & Jeffery, 2016a). By other hand, Malbec wines showed no microbiological spoilage during aging (Supplementary Table 3) so the 65% increase on volatile acidity observed could be associated to oxidative chemical processes, as suggested by Clarke and Bakker (2004).

3.2. Oxygen and sulfur dioxide

In general, the dissolved oxygen concentration (DOC) decreased as the aging time (t) of Malbec wines increased (Fig. 1A). According to Dimkou et al. (2011), "the dissolved oxygen concentration would reflect the net balance between the dissolution of oxygen from the headspace into the wine and consumption by the wine itself". Thus, DOC would represent the residual oxygen content resulting from different physical and chemical phenomena that occur during bottle aging. Between 0 and 2 months of storage, a slight increase in DOC was observed which remained constant until month 4. This could be due to the fact that, during the early months of storage, the dissolved oxygen concentration in wine would increase by diffusive phenomena from the headspace (Dimkou et al., 2011) and, furthermore, because the dissolution rate of oxygen would be higher than the rate of oxygen consumption by chemical reactions (Laurie, Law, Joslin, & Waterhouse, 2008; Ugliano, 2013). From sixth to 24th months of aging a decrease in DOC was observed, probably due to the rate of dissolution of oxygen would be lower than the rate of consumption.

Figure 1

On the other hand, the evolution of the DOC over aging time was dependent on the interaction between temperature and closure (T-c, $p<0.0001$). At the beginning of the trial, bottles sealed with natural cork (C) and stored at 15ºC and 25ºC, showed a dissolved oxygen concentration 91±10 μg L^{-1}, and 87±3 μg L^{-1}, respectively; while those with screw cap (SC) presented 51±7 μg L^{-1} DOC at 15ºC, and 49±10 μg L^{-1} DOC at 25ºC. The difference observed in the initial DOC between C and SC could be due to the fact that the pressure applied to the cork at bottling would accelerate the dissolution of oxygen from headspace into wine in this period (Dimkou et al., 2011; Ugliano, 2013), which could have influenced the DOC until the fourth month of conservation, since wines were bottled with the same volume of headspace (5.09 mL). In the same way, throughout the whole process, DOC-SC was 1.32 times lower than DOC-C. This could be due to cork has a greater diffusion of oxygen (2-6 μL/day) than screw cap (<1 μL/day) (Silva et al., 2011). Also, DOC of Malbec wines kept at 15ºC (cork, 73.89±1.74 μg L^{-1}; screw cap, 53.65±1.34 μg L^{-1}) was higher than at 25ºC (cork, 66.86±1.51 μg L^{-1}; screw cap, 50.42±1.48 μg L^{-1}). The effect of temperature could be explained on one hand, since low aging temperatures favor the dissolution of oxygen ($\Delta H_{O2/H20\ (298\ K,\ 1\ atm)}$ 14.13 kJ.mol^{-1}) (Karbowiak et al., 2010); and, on the other hand, because elevated conservation temperatures would promote chemical reactions where oxygen is involved. Molecular oxygen is an ineffective oxidizing agent for reacting directly with wine components. To participate in oxidative reactions, in presence of Fe^{+2} and at wine pH, oxygen is transformed into hydroperoxyl radicals (HOO$_{\cdot}$) by an endothermic process (Karbowiak et al., 2010; Ugliano, 2013).

Fig. 1B shows the evolution of free sulfur dioxide concentration (fSO_2) during the aging of Malbec wines. The fSO_2 decreased progressively until 12 months of storage. This is consistent with other observations that correlate the high availability of oxygen with the rapid decrease in fSO_2 (Dimkou, Ugliano, Diéval, Vidal, & Jung, 2013; Lopes et al., 2009). The decrease of fSO_2 would be associated with reactions involving the formed hydroperoxyl radical in previous stages. $HOO^{\cdot}$ reacts with phenolic compounds generating hydrogen peroxide (H_2O_2), which in presence of Fe^{+2} o Cu^{+}, would unspecifically oxidizeall organic compounds of wine. But this process is controlled by fSO_2 which competes with Fe^{+2} and Cu^{+} by H_2O_2 (Danilewicz, 2011). In acidic medium, metabisulfite, and H_2O_2 react irreversibly producing sulphuric acid and water and, in this way, the organic fraction of wine would be protected (Elias & Waterhouse, 2010; Scrimgeour et al., 2015). By the other hand, differences observed during the evolution of fSO_2 were associated with changes in aging temperatures, but not with closure types. During the first year of aging, Malbec wines conserved at 25ºC presented 21% less fSO_2 than those at 15ºC. This could be due to the fact that high conservation temperatures would promote the formation of monosulfonated flavanols, products of the direct reaction between tannins and bisulfite, with the consequent decrease of fSO_2 (Arapitsas, Speri, Angeli, Perenzoni, & Mattivi, 2014). Also, the concentration of hydrogen peroxide (C_{H2O2}) of the medium could affect the concentration of fSO_2. The C_{H2O2} increases as the process temperature increases (Karbowiak et al., 2010), and consequently, the reaction velocity between this chemical specie with fSO_2 is increased, which would lead to the decrease of fSO_2. Finally, the storage temperature would affect the molecular SO_2/fSO_2 equilibrium, at 25ºC there would be a higher molecular $S0_2$ concentration and a lower concentration of fSO_2 than at 15ºC (Scrimgeour et al., 2015).

3.3. Phenolic composition

Figure 2 shows the evolution of different phenolic compounds during aging wines. Data obtained from the three-way ANOVA model, show that interaction between storage temperature and aging time had a significant effect ($p \leq 0.001$) on phenolic compounds of bottle-aged Malbec wines. But closure type had no effect on the evolution of phenolic composition ($p>0.05$).

Figure 2

As expected, during aging, anthocyanin concentration (AN) decreased in Malbec wines (Fig. 2A). Some authors have proposed AN reacts with bisulfite ions forming colorless and stable adducts at pH of wines (Berké, Chèze, Vercauteren, & Deffieux, 1998), but this would not explain the differences observed by the effect of storage temperature. Through kinetic studies on AN evolution, reaction orders and specific velocity constants (k_v) were determined by differential method (Bendito, Estela, & Maya, 2018; Pardue, 1989). The reaction orders for 15ºC and 25ºC were 3.66 and 2.21, respectively, showing that anthocyanin reactions occur through complex mechanisms (Supplementary Figure 1). At 15ºC k_v was 1.06 x 10^{-08}, while at 25ºC k_v was 1.40 x 10^{-04}. Because of the difference in k_vs values, it was found that at 25ºC AN fell 2.55 times more than at 15ºC, between 2 and 24 months of aging. This may be due to different reactions where these compounds are involved. AN could react by H_2O_2, generated during molecular oxygen activation (***section 3.2***), and this process is favored by the increased storage temperature (Lopes, Tristan, et al., 2007; Özkan, Yemenicioglu, Asefi, & Cemeroglu, 2002). In addition, due to high temperatures during aging, AN could be degraded to phenolic acids (yellow pigments) with the consequent formation of chalcones (Fulcrand et al., 2006; He et al., 2012). Villamor, Harbertson, and Ross (2009) showed that the difference in anthocyanin level of Cabernet Sauvignon and Merlot wines was a function of storage temperature (at high conservation temperatures, AN remaining in wines were lower than at low aging temperatures). Other authors (Lago-Vanzela et al., 2014) observed a greater loss in AN when Violet red wines were subjected to aging at 50ºC compared to control (15ºC). So far the loss of anthocyanins by degradation and bleaching processes has been discussed. Subsequently, other reactions that also participate will be analyzed.

As shown in Fig. 2B, tannin level (TN) decreased in Malbec wines during storage. It was observed that at 15ºC TN was higher than at 25ºC, during a completely aging process. It has been estimated that high temperatures and low pH (wine) accelerate the loss of high molecular weight tannins, probably due to the cleavage of interflavan C-C bonds with the consequent formation of low molecular weight species (Cheynier et al., 2006; Vidal, Cartalade, Souquet, Fulcrand, & Cheynier, 2002; Villamor et al., 2009).

In addition, during aging TN and AN may react either directly by forming flavanol anthocyanin (F -A^+) or anthocyanin-flavanol (A^+-F) adducts, or by generating molecular aggregates through the intervention of ethanal (Fulcrand et al., 2006). These formed compounds, called polymeric pigments (PP), are responsible for the color stability in wines over time (Blanco-Vega, Gómez-Alonso, & Hermosín-Gutiérrez, 2014; Karbowiak et al., 2010; Kilmister, Mazza, Baker, Faulkner, & Downey, 2014; Waterhouse, Sacks, & Jeffery, 2016b). In general, PP is classified into large polymeric pigments (LPP) and small polymeric pigments (SPP) which, respectively, precipitate and do not precipitate with proteins. SPP would contain anthocyanin-derived compounds originating by different mechanisms (direct reaction, cross-linking reaction, cycloaddition). LPP would be formed by anthocyanins that have reacted directly with polymeric flavan-3-ols (tannins) or polymeric pigments that have been formed by acetaldehyde cross-linking (Dueñas, Fulcrand, & Cheynier, 2006; Harbertson et al., 2003; Versari, Laurie, Ricci, Laghi, & Parpinello, 2014). PP increased along the Malbec wine aging process (Fig. 2C and 2D). SPP had a similar evolution between wines kept at 15ºC and 25ºC and, at the end of conservation; its content did not show significant differences between both temperatures. Although, in early stages of conservation it was observed that its formation at 15ºC was delayed with respect to 25ºC. On the other hand, like SPP, LPP showed similar evolutions during the aging time at 15ºC and 25ºC. However, during the process, the temperature had a significant effect on its contents. At 15ºC, Malbec wines had lower LPP contents than those at 25ºC. This could be due to the fact that, at wine pH and high conservation temperatures, LPP would have less contribution to F-A^+ adducts. Fulcrand et al. (2006) proposed, that under these conditions, the conversion of the chalcone into thermo-degradation products is faster than the reaction between hydrated anthocyanins and flavanol carbocations.

3.4. Volatile composition

Different authors have demonstrated how different conservation conditions affect the evolution of individual volatile compounds in white and red wines (Cassino et al., 2019; Guaita et al., 2013; Moreira, Lopes, Ferreira, Cabral, & Pinho, 2016; Pereira, Cacho, & Marques, 2014), but this study evaluated how these conditions affect the profileof different chemical families that comprise the aroma of Malbec wines.

The volatile composition of Malbec wines at bottling was mainly composed by alcohols (92%), esters (4%) and organic acids (3%); while the sum of norisoprenoids, terpenoids, furanoids, and volatile phenols was less than 1%. Finalized the aging period (24 months) it was observed that the initial total concentration (279.40 mg L^{-1}) increased 1.18 times in Malbec wines stored at 15ºC and 1.53 times in those kept to 25ºC. In addition, the percentual contribution of different chemical families to total volatile composition was modified. At both conservation temperatures, an increase in concentrations of alcohols, norisoprenoids, terpenoids, and furanoids, and a decrease of esters, organic acids, and volatile phenols were observed (Table 1). Supplementary Table 4 shows the chemical compounds that make up each family studied.

Table 1

Data obtained from the three-way ANOVA model, show that time had a significant effect (p=0.001) on the evolution of AO and volatile phenols, interaction T-t on alcohols, esters, terpenoids, and furanoids (p<0.01), and interactions c-T (p=0.0377) and T-t (p<0.0001) on norisoprenoids. In this study, it was observed that closures employed at bottling (C or SC) did not influence on the evolution of the majority of volatile chemical families during Malbec wines aging. This could be due to the fact that Malbec wines have a high polyphenolic composition, compared to other red varieties, which could generate greater resistance to degradative processes induced by the entry of oxygen through corks (Fanzone et al., 2012, 2010; Silva et al., 2011).

In young Malbec wines, alcohols (258.61 mg L^{-1}) were the most abundant compounds and, over the first year of aging, their concentrations increased. This behavior was also observed by Garde-Cerdán et al. (2008) in Parellada wines maintained at room temperature for 6 months. By the other hand, Malbec wines did not show significant differences in alcohols by storage temperature effect until 21 months of aging. At 24 months, wines kept at 15ºC showed 22% more than initial wines, while those conserved at 25ºC had 59% more quantity (Table 1). However, this would not lead to organoleptic defects, since alcohols concentrations were below their perception thresholds (Ayestarán et al., 2019; Clarke & Bakker, 2004).

Between 2 and 18 months of aging, Malbec wines showed differences on esters by conservation temperature. At bottling, the esters content was 12.26 mg L^{-1} and after 18 months its concentration decreased 39% to 15ºC and 56% to 25ºC (Table 1). These results would indicate that high storage temperatures increase acid hydrolysis of esters, with the consequent loss of fruity aromas characteristic of young wines (Cejudo-Bastante, Hermosín-Gutiérrez, & Pérez-Coello, 2013; Hopfer et al., 2013; Jackson, 2008; Pérez-Prieto, López-Roca, & Gómez-Plaza, 2003; Scrimgeour et al., 2015).

The total concentration of organic acids in initial Malbec wines was 7.37 mg L^{-1}, and decreased along with conservation without T showing significant effects on its evolution (Table 1). Organic acids decline during aging would be associated with esterification reactions of these compounds with alcohols, mainly, ethanol (Cassino et al., 2019; Pérez-Prieto et al., 2003).

Other volatile compounds (norisoprenoids, terpenoids, furanoids, and volatile phenols) were found in low concentrations (ppt to ppb) in aged Malbec wines, but they have great importance at the sensory level due to their low perception thresholds (Linsenmeier et al., 2010; Pereira et al., 2014; Piombino, Moio, & Genovese, 2019). In young Malbec wines, the norisoprenoids content was 362.67 μg L^{-1}, and its concentration was increased as storage time extended. This would be related to the release of norisopronoid aglycons from glycosides that are hydrolyzed at wine pH during aging (Black et al., 2012; Slaghenaufi & Ugliano, 2018; Waterhouse et al., 2016c)

Figure 3

Along the whole process, at both conservation temperatures, norisoprenoids presented a similar evolution pattern; however, at 15ºC, the norisoprenoids content was 2.35 times (on average) lower than at 25ºC (Table 1, Fig. 3). This could be associated with to the increased hydrolysis rate of glycosylated precursors favored by the higher storage temperature (Clarke & Bakker, 2004; Daniel, Capone, Sefton, & Elsey, 2009; Hopfer et al., 2013; Robinson et al., 2010). In addition, from 12 months of aging, Malbec wines stored at 25ºC showed significant differences in norisoprenoids contents between closures used at bottling (Fig. 3). Malbec wines sealed with SC had a superior norisoprenoids concentration than those with C and this could be related to the absorption of some norisoprenoids by cork closures as have proposed by different authors

(Capone et al., 2003; Skouroumounis et al., 2005).

Like norisoprenoids, free terpenoid compounds increased during the aging of Malbec wines as a consequence of hydrolytic reactions on β-glycosidic bonds of their precursors (Linsenmeier et al., 2010). After 2 months of storage, terpenoids began to differentiate by temperature. Wines at 25ºC had a higher terpenoid content compared to 15ºC (Table 1), which could indicate that the release of these aglycons from glycosides would be accelerated by the higher storage temperature (Hopfer et al., 2013; Robinson et al., 2010). Thus, at the end of aging, Malbec wines stored at 15ºC and 25ºC increased the terpenoids concentration of 25% and 47%, respectively, in relation to wines at bottling time.

The content of furanoid compounds , described as “honey” and “toasty caramel”, was enhanced during the conservation of Malbec wines (Table 1); and at 15ºC, furanoids was 2.13 times (on average) lower than at 25ºC. This would be due to that carbohydrate degradation, which is where furanoids is derived from (Maillard's reaction), increasing its velocity as storage temperature is higher (Clarke& Bakker, 2004; Pereira, Albuquerque, Ferreira, Cacho, & Marques, 2011; Waterhouse et al., 2016c). By another hand, the total concentration of volatile phenols in initial Malbec wines was 65.69 $\mu g\ L^{-1}$, and volatile phenols decreased along with conservation without T showing significant effects on its evolution. Fernández De Simón, Cadahía, Hernández, and Estrella (2006) observed the same behavior of volatile phenols during Tempranillo wines aging, and they proposed that this would be explained by the participation of these compounds in various degrading chemical reactions.

3.5. Wine color

Data obtained from the three-way ANOVA model, show that only interaction T-t had a significant effect ($p<0.05$) on lightness (L*), chroma (C^*_{ab}) and hue (h_{ab}) of Malbec wines during aging. Figure 4 shows wines' location on the color plane (a*b*) in the CIELAB space and L* evolution during aging.

Figure 4

Fig. 4A shows the evolution of C^*_{ab} and h_{ab} during Malbec wines aging. Chroma decreased over the whole process. After 4 months of conservation, significant differences in their values were found depending on storage temperatures. At the end of aging, C^*_{ab} fell 20% at 15ºC and 40% at 25ºC. Hue increased during storage and was also affected by aging temperature. Comparing Malbec wines at bottling with those aged for 24 months, an increase in h_{ab} of 4° and 18° to 15ºC and 25ºC, respectively, was observed. The decline on violet hues and the development of red-orangehues in wines during aging could be related to the decrease of anthocyanins (Fig. 2A) and pH values, and the increasing of SPP (Fig. 2C), related to orange tones (Avizcuri, Sáenz-Navajas, Echávarri, Ferreira, & Fernández-Zurbano, 2016). C^*_{ab} and lightness are related to variations in large polymer pigments during wine aging (Casassa & Sari, 2007; Sáenz-Navajas, Echavarri, Ferreira, & Fernández-Zurbano, 2011). In addition, C^*_{ab} presents a negative correlation with L* and, as expected, when the former increased, the latter decreased (Fig. 4B). After 6 months, L* showed significant differences between wines stored at 15ºC and 25ºC. At 24 months, L* presented a relative percentual increase (on average) of 8% at 15ºC and 16% at 25ºC in relation to initial wines.

This study also evaluated the color difference (ΔE^*_{ab}) between wines preserved at 15ºC and 25ºC; being ΔE^*_{ab} >3 the color difference appreciable by an average-observer (Martínez, Melgosa, Pérez, Hita, & Negueruela, 2001). In general, ΔE^*_{ab} increased from bottling to 15 months of aging and then remained constant until the end of the process. However, from the sixth month onwards differences in this parameter were clearly perceptible (ΔE^*_{ab} >3). At the end of aging, the relative contribution of chroma (% $\Delta^2 C^*_{ab}$) and hue (%$\Delta^2 H_{ab}$) were the biggest (>40%) over the observed color difference (11.3 CIELAB units).

The color difference between wines at each sampling point and initial wines at 15ºC and 25ºC was also evaluated. Malbec wines kept at 25ºC showed ΔE^*_{ab}>3 at 6 months, while those at 15ºC had the same difference at 15 months. This fact might indicate that oxidative and thermodegradation reactions would be accelerated by high storage temperatures during the aging period (Arapitsas et al., 2014; Gómez-Plaza, Gil-Muñoz, López-Roca, Martínez-Cutillas, & Fernández-Fernández, 2002; Gómez-Plaza, Gil-Muñoz, López-Roca, & Martínez, 2000; Hopfer et al., 2013). At 24 months, the wines stored at 25ºC presented 2-fold difference in color than those stored at 15ºC. At this moment, 25ºC wines were characterized by an increase in tonality

($\%\Delta^2H_{ab}=23$) and a decrease in saturation ($\%\Delta^2C^*_{ab}=57$), whereas 15ºC wines showed a decrease in saturation ($\%\Delta^2C^*_{ab}=70$).

3.6. Sensorial analysis

At the end of the aging period, Malbec wines were evaluated by quantitative descriptive analysis, and results were submitted to statistical analysis. Mixed linear models showed that color intensity (CI), violet tint (VT), caramelized (CD) and dried vegetative (VG) were attributes that differentiated wines according to conservation conditions ($p<0.05$). ANOVA models showed that wines kept at 15ºC had higher CI and VT than those stored at 25ºC. In agreement with these results, Espitia-López (2011) observed that Ruby Cabernet wines aged during 6 months at 18ºC presented lower CI than ones at 4ºC. By other hand, Malbec wines aged at 25ºC showed higher CD and VG than those conserved at 15ºC. High store temperatures would promote acid degradation of sugars (pentoses, hexoses) with the consequent formation of volatile compounds such as furans and pyrans, related to CD attributes (Waterhouse et al., 2016c). In addition, at 25ºC, Malbec wines sealed with SC had higher VG scores compared to those with C. The VG attribute, linked to tobacco aroma, could be associated to norisoprenoids compounds present in Malbec wines (Slaghenaufi & Ugliano, 2018); therefore, differences found for this descriptor could be related to differential absorption of norisoprenoids by stoppers (Skouroumounis et al., 2005).

3.7. Correlation between chemical and sensory variables

Figure 5 shows the correlation between analytical variables and organoleptic descriptors of Malbec wines aged for 24 months, analyzed by multivariate partial least squares regression (PLS). In this study, only analytical variables dependent on temperature and/or closure were considered. Analytical variables predicted 87% of the variability of organoleptic descriptors (Fig. 5A). Also, Malbec wines were differentiated by storage temperature effect, but not by the closure type used at bottling (Fig. 5B).

Figure 5

On the other hand, Malbec wines aged at 15ºC were associated with high CI and VT perceptions, while those kept at 25ºC had low values. Additionally, CI and VT showed a positive correlation ($r>0.90$) with fSO2, TN, AN and C^*_{ab}, and negative with LPP, L* and h_{ab}. These analytical variables explained more than 96% of the variability found for CI and VT descriptors (localized on the outer circle of the biplot, Fig. 5A). By another hand, aromatic intensity, mouthfeel attributes (astringency, acidity, and bitterness) and caramelized aroma (located on the inner circle, Figure 5A) were poorly predicted (<50%) by analytical variables. Also, these latter attributes only contributed 5% to the overall variability of Malbec wines (Component 2, Fig. 5B).

Malbec wines stored at 25ºC were associated with elevated VG and dried fruit (DF) aromatic intensities. These descriptors were positively correlated with terpenoids, furanoids, and norisoprenoids. Vegetative aroma, such as tea and tobacco notes, could be related to norisoprenoids, whereas dried fruit with furanoids compounds (Riberau-Gayon, Glories, A. Maujean, & Dubourdieu, 2006; Slaghenaufi & Ugliano, 2018).

PLS correlation between sensory and analytical variables of wines stored at different temperatures was also evaluated by different authors (Hopfer et al., 2013; Hopfer, Ebeler, & Heymann, 2012; Robinson et al., 2010). Hopfer et al. (2013) observed that Cabernet Sauvignon wines conserved at 10ºC had higher perceptions of color saturation, fresh fruit, and floral aromas than those conserved at 40ºC. They also showed a positive correlation between those attributes and concentrations of anthocyanins and sulfur compounds in these wines.

4. Conclusions

A comprehensive study of the aging process of Argentinean Malbec wines, providing an interesting insight, no reported so far, was conducted on the impact of different factors (closure type, storage temperature and time) on their chemical and sensory properties. Malbec wine profile changed during bottle conservation. In these changes, the factor more important was the interaction of time with the temperature conservation. At two years, wines were chemical (volatile and phenolic composition), physical (color) and sensory different by storage temperature. But, in Malbec wine, were not generated important differences in the chemical and sensory profile by used closure type. Additionally, the sensory description was in good agreement with the analytical results, with 87% of prediction ability (PLS). Malbec wines stored at 15ºC were characterized by greater color intensity and violet tint. While, the higher temperature developed more aromas of dried fruit (jam of red fruits) and dried vegetative (tobacco, tea). In conclusion, according to the Malbec wine style demanded by consumers, the oenologist could plane the maturation strategy, considering the joint effect of temperature and time as critical variables of aging. In this way, it would be possible to drive the process towards aromatic complexity and color stability or maintain the freshness and vivacity of the wine.

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Figure Captions

Fig. 1. Evolution of dissolved oxygen (DOC) and free sulfur dioxide(B, fSO_2) in Malbec wines during aging at two storage temperatures (15ºC and 25ºC).

Fig. 2. Evolution of phenolic compounds in Malbec wines during aging at two storage temperatures (15ºC and 25ºC). (A) Anthocyanins. (B) Tannins. (C) SPP, small polymeric pigments. (D) LPP, large polymeric pigments.

Fig. 3. Effect of closure, storage temperature and aging time on the evolution of norisoprenoids of Malbec wines. C, cork; SC, screw cap; 15, storage temperature 15ºC; 25, storage temperature 25ºC; ND, norisoprenoids concentration.

Fig. 4. CIELAB parameters during aging of bottled Malbec wines. (A) Plane (a*b*); chroma (C^*_{ab}) is a vector that links the dot (wine location) with the origin of coordinates; hue (h_{ab}) is the angle of this vector; t_0, Malbec wines at bottling; t_f, Malbec wines at 24 months of aging. (B) lightness (L*). Black and grey dots are Malbec wines stored at 15ºC and 25ºC, respectively, during aging.

Fig. 5. Partial least squares regression analysis of analytical and sensory variables of Malbec wines at 24 months of aging. (A) Correlation between analytical and sensory variables. fSO2, free sulfur dioxide; AN, anthocyanins; TN, tannins; LPP, large polymeric pigments; L*, lightness; C^*_{ab}, chroma; h_{ab}, hue; AL, alcohols;

ET, esters; ND, norisoprenoids; TD, terpenoids; FN, furanoids. (B) Wine distribution plot. C, cork; SC, screw cap; 15, storage temperature 15ºC; 25, storage temperature 25ºC; a, b, and c are replicates.

Table Captions

Table 1. Evolution of different volatile chemical families in Malbec wines during aging at two storage temperatures (15ºC and 25ºC).

Supplementary material

Figures

Supplementary Fig. 1. Kinetic studies on anthocyanin evolution at different temperatures: reaction orders (n) and specific velocity constants (kv).

Tables

Supplementary Table 1. Reference standards for aged Malbec wines.

Supplementary Table 2. Evolution of oenological parameters in Malbec wines during different conditions of aging.

Supplementary Table 3. Evolution of microbiological stability of Malbec wines during different conditions of aging.

Supplementary Table 4. Evolution of individual volatiles compounds in Malbec wines during different conditions of aging.

Table 1. Evolution of different volatile chemical families in Malbec wines during aging at two storage temperatures (15°C and 25°C).

[1] VCF, volatile chemical families. Represents the sum of individual compounds. Mean values (3 replicates), SDR% between brackets. Analyses are described in the Materials and Methods section.

		Aging time (months)				
VCF[1]	T[2]	2	6	12	15	18
Alcohols (mg L^{-1})	15°C	388.58 (13.35) cA	380.85 (6.28) bcA	330.27 (6.71) abcA	350.88 (12.32) abcA	381.45 (17.05) bcA
	25°C	341.83 (8) bcA	418.67 (9.7)dA	324.94 (4.27) abA	339.71 (4.87) bcA	396.72 (9.12) dA
Esters (mg L^{-1})	15°C	13.94 (3.15) dB	11.17 (9.1) dB	8.48 (6.35) cB	6.09 (15.03) abB	7.37 (15.59) bcB
	25°C	12.69 (7.59) cA	10.84 (11.88) bA	6.12 (6.44) a A	4.76 (12.21) a A	5.84 (14.3) a A
Organic acids (mg L^{-1})	15°C	7.04 (6.43) bA	7.5 (20.11) bA	5.96 (9.07) abA	6.21 (5.14) abA	6.23 (13.14) abA
	25°C	7.74 (8.55) cdA	8.44 (8.45) dA	6.13 (10.82) abA	5.58 (2.76) a A	6.58 (3.95) abcA
Norisoprenoids (µg L^{-1})	15°C	386.66 (6.68) a A	407.89 (4.49) a A	437.87 (3.55) a A	687.67 (1.68) bA	891.27 (9.98) cA
	25°C	518.9 (11.39) abB	762.12 (9.16) bB	1216.69 (7.27) cB	1572.36 (14.49) cB	2221.07 (12.63) dB
Terpenoids (µg L^{-1})	15°C	69.97 (4.98) bcA	70.41 (7.23) bcA	66.28 (4.97) abA	69.55 (4.03) bcA	78.23 (0.15) cA
	25°C	70.86 (4.95) bA	87.19 (5.84) dB	72.38 (4.09) abB	75.5 (3.06) bcB	84.45 (8.04) cdB
Furanoids (µg L^{-1})	15°C	268.36 (3.35) abA	317.75 (18.6) bA	353.06 (8.03) bA	456.05 (5.48) cA	522.21 (11.39) cdA
	25°C	348.88 (8.34) bB	642.07 (12.83) cB	794.32 (2.04) cdB	880.65 (5.46) dB	1064.02 (8.71) eB
Volatil phenols (µg L^{-1})	15°C	63.78 (28.32) cA	56.52 (29.28) bcA	38.31 (20.8) abA	38.07 (8.44) abB	36.42 (10.64) abA
	25°C	68.43 (17.28) bA	64.54 (15.49) bA	35.85 (33.38) a A	30.85 (3.25) a A	37.63 (6.51) a A

[2] T, storage temperatures.

Different lowercase letters (on row) indicate significant differences among ageing time ($p \leq 0.05$). Tukey´s (HSD) test ($\alpha = 0.05$).

Different capital letters indicate significant differences between temperatures for each VCF ($p \leq 0.05$). Tukey´s (HSD) test ($\alpha = 0.05$).

Highlights

- Malbec wines aged in bottles modify their color and aroma.
- Regardless of closures, the wine evolution was mainly influenced by the temperature.
- The wine color changed ($\Delta E^*_{ab}>3$) after 6 months of aging (25ºC) or 15 months (15ºC).
- The storage temperature impact significantly in the sensory profile of Malbec wines.

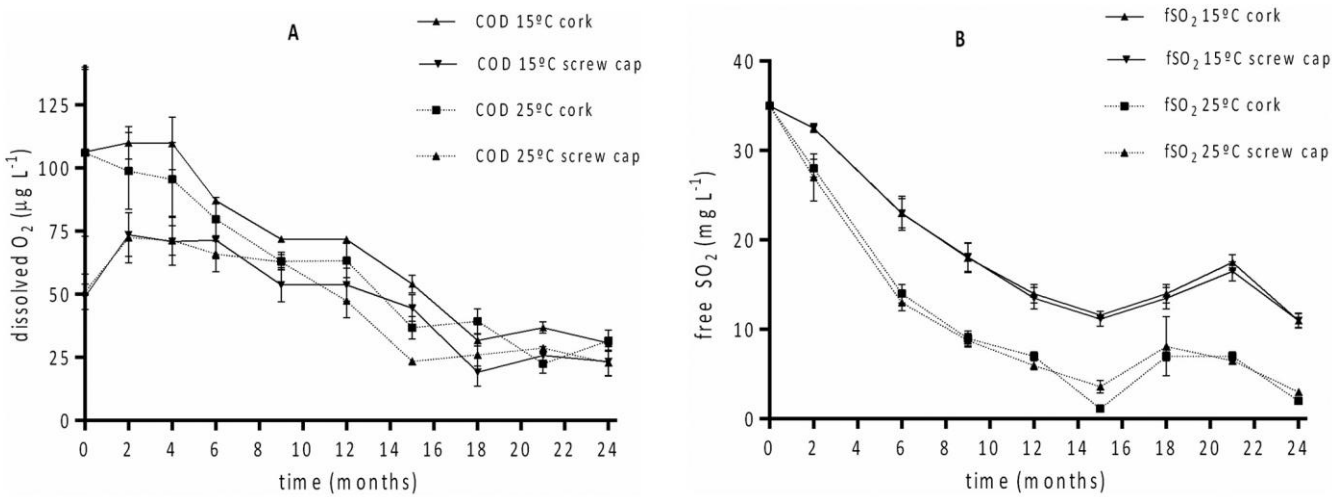
A
COD 15ºC cork
COD 15ºC screw cap
COD 25ºC cork
COD 25ºC screw cap
dissolved O_2 ($\mu g\ L^{-1}$)
0
25
50
75
100
125
0
2
4
6
8
10
12
14
16
18
20
22
24
time (months)
B
fSO_2 15ºC cork
fSO_2 15ºC screw cap
fSO_2 25ºC cork
fSO_2 25ºC screw cap
free SO_2 ($mg\ L^{-1}$)
0
10
20
30
40
0
2
4
6
8
10
12
14
16
18
20
22
24
time (months)

Figure 1

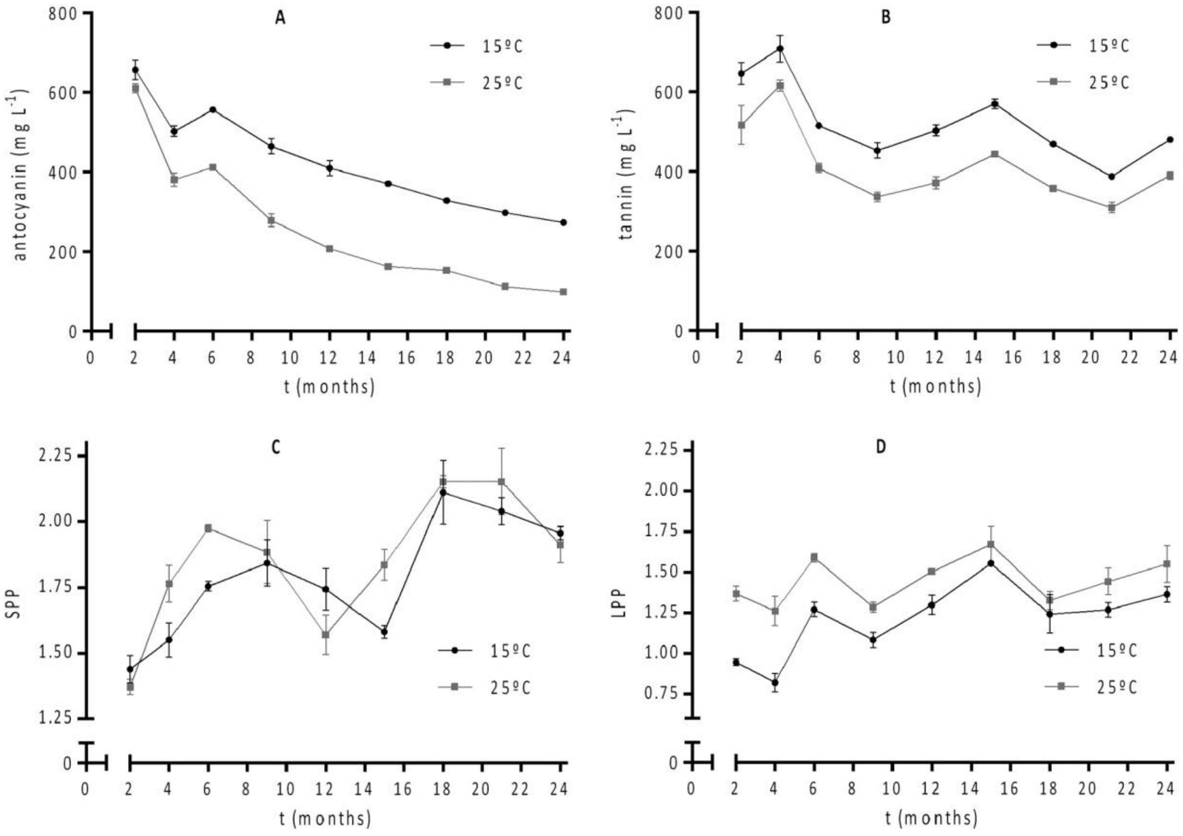A
B
C
D
antocyanin (mg L^{-1})
tannin (mg L^{-1})
SPP
LPP
t (months)
15ºC
25ºC
0
200
400
600
800
1.25
1.50
1.75
2.00
2.25
0.75
1.00
0 2 4 6 8 10 12 14 16 18 20 22 24

Figure 2

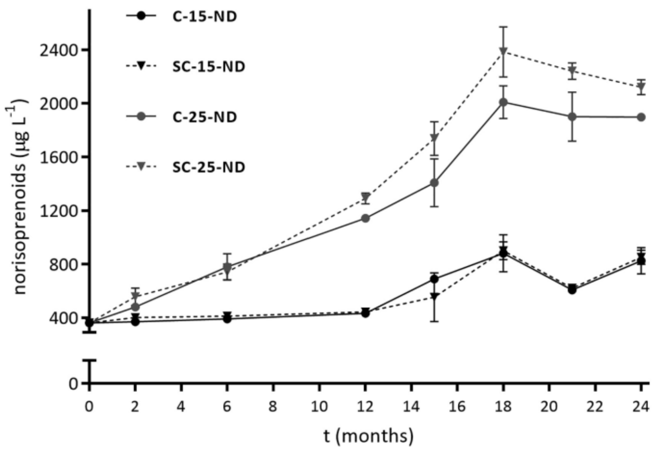
C-15-ND
SC-15-ND
C-25-ND
SC-25-ND
2400
2000
1600
1200
800
400
0
norisoprenoids (μg L^{-1})
0
2
4
6
8
10
12
14
16
18
20
22
24
t (months)

Figure 3

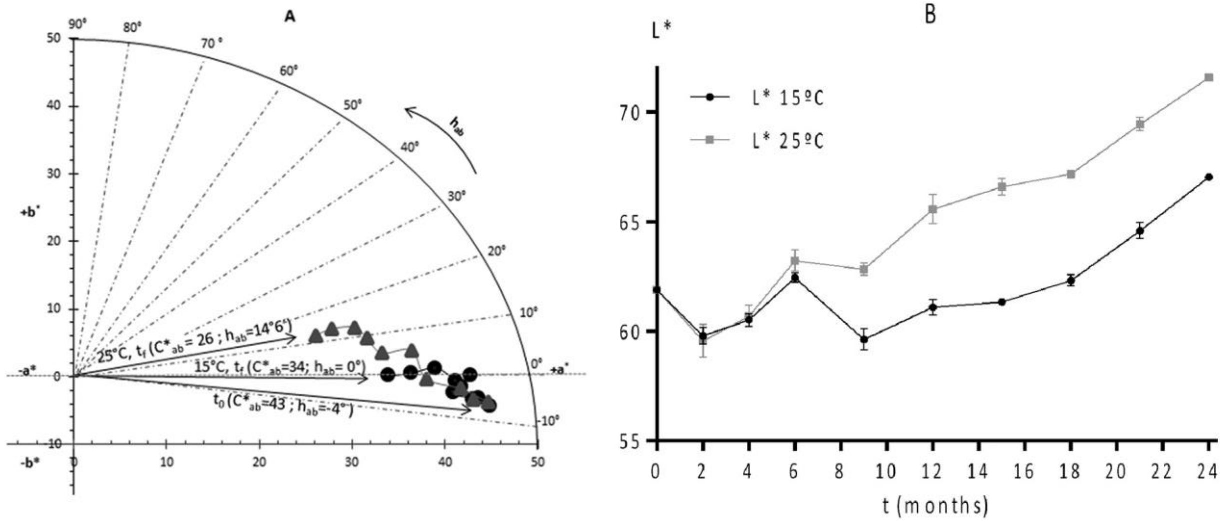
A
90°
80°
70°
60°
50°
40°
30°
20°
10°
0°
-10°
h_ab
+b*
-b*
-a*
+a*
50
40
30
20
10
0
10
0
10
20
30
40
50
25°C, t_f (C*_ab = 26 ; h_ab=14°6')
15°C, t_f (C*_ab=34; h_ab= 0°)
t_0 (C*_ab=43 ; h_ab=-4°)
B
L*
L* 15ºC
L* 25ºC
70
65
60
55
0
2
4
6
8
10
12
14
16
18
20
22
24
t (months)

Figure 4

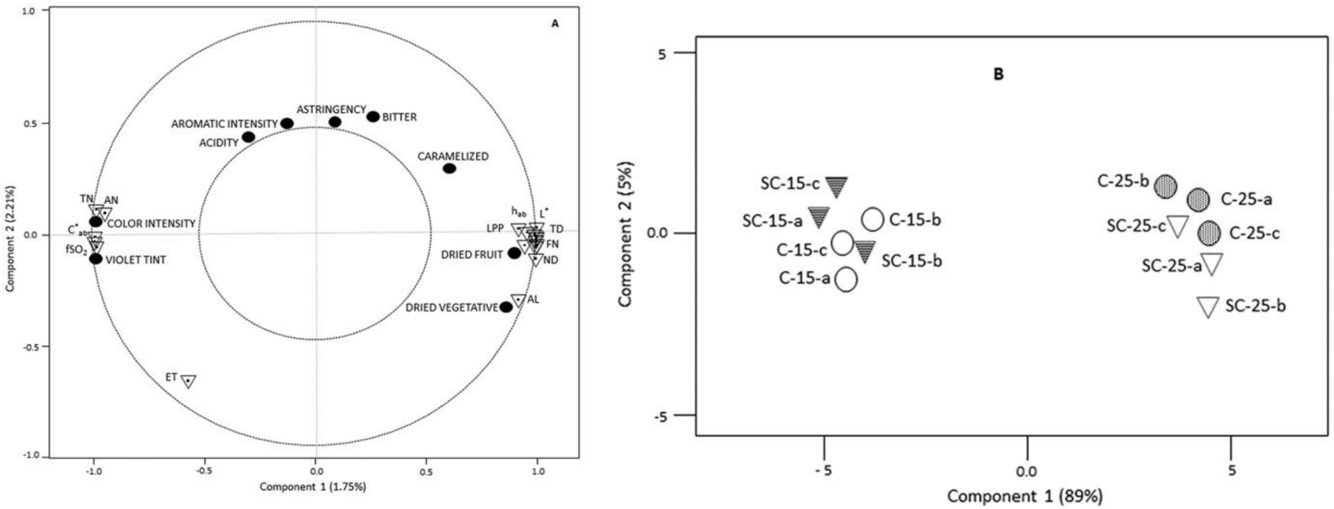
A
AROMATIC INTENSITY
ASTRINGENCY
BITTER
ACIDITY
CARAMELIZED
TN
AN
COLOR INTENSITY
C*ab
fSO2
VIOLET TINT
hab
L*
LPP
TD
FN
ND
DRIED FRUIT
DRIED VEGETATIVE
AL
ET
Component 2 (2.21%)
Component 1 (1.75%)
1.0
0.5
0.0
-0.5
-1.0
B
SC-15-c
SC-15-a
C-15-b
C-15-c
SC-15-b
C-15-a
C-25-b
C-25-a
SC-25-c
C-25-c
SC-25-a
SC-25-b
Component 2 (5%)
Component 1 (89%)
5
0.0
-5

Figure 5

$$\frac{dAN}{dt} = k_v . AN^n$$

$$\ln v = \ln k_v + n . \ln AN$$

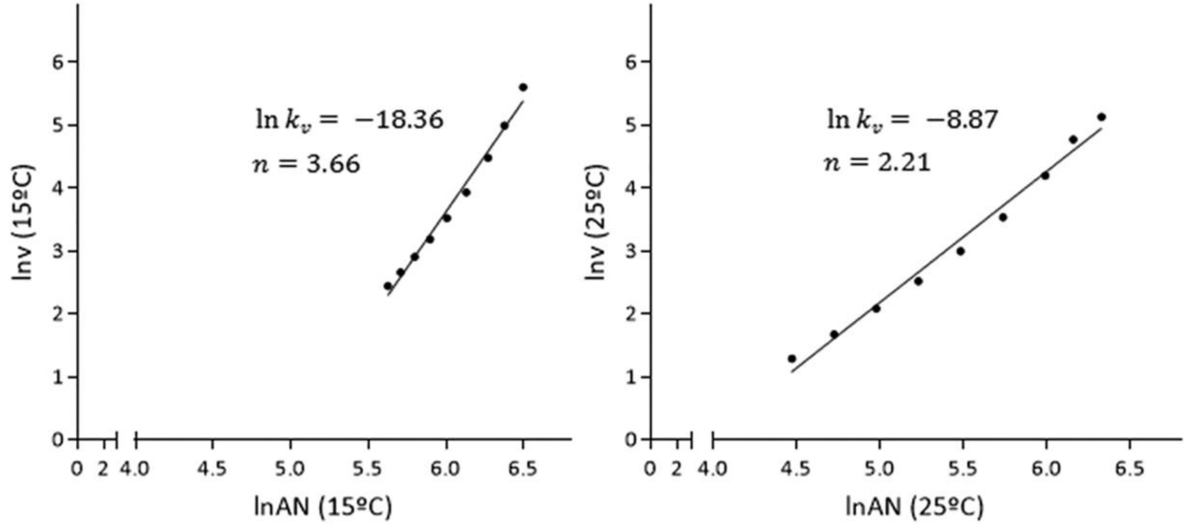

Figure 6