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CRediT authorship contribution statement

E. Romina Castellanos: Validation, Formal analysis, Investigation, Data Curation, Visualization, Writing - original draft. **Viviana P. Jofre:** Conceptualization, Writing, Review & Editing, Formal analysis, Visualization, Supervision, Project administration, Funding acquisition, Writing - original draft. **Martín L. Fanzone:** Conceptualization, Funding acquisition. Review. **Mariela V. Assof:** Conceptualization, Formal analysis, Validation. Review. **Anibal A. Catania:** Formal analysis, Validation. **A. Mariela Diaz-Sambueza:** Formal analysis, Validation. **Francisco J. Heredia:** Formal analysis. Review. **Laura A. Mercado:** Formal analysis, Validation.

Title:

Effect of different closure types and storage temperatures on the color and sensory characteristics development of Argentinian Torrontes Riojano white wines aged in bottles.

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Running title:

Changes in Torrontes Riojano wines under different aging conditions.

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Abstract

During aging, most bottled white wines lose their distinctive organoleptic characteristics according to their storage conditions (closure, temperature, time). However, the effect of these factors on organoleptic characteristics of Torrontes Riojano wines (TRw) has not been studied yet. This study aimed at evaluating the combined effect of closure type and storage temperature on the organoleptic properties development of TRw over a 18-month aging period, during which wine was bottled with natural (C) and synthetic (SyC) corks, and screwcaps (SC). Bottles were kept 18 months in thermostatized chambers (15°C; 25°C). At different aging times, consumed oxygen (CO), SO_2, total phenols (TP), color, and sensory properties were evaluated. CO, TP, and browning index evolutions depended on the interaction between closure and temperature, whereas CIELAB parameters (lightness, chroma, hue) depended on closure-time and temperature-time interactions. At both storage temperatures, SC had a lower hue decrease and a lower chroma increase than C and SyC. The highest temperature prompted a more yellow and darker color of TRw. Thus, it allowed discrimination throughout the process. Considering the aging process, their aromatic intensity, fresh fruit, and yellow and green nuances decreased, while brown hue, color intensity, linalool, and oxidized character rose as their storage time was increased. At the end of their aging, TRw kept to 15°C were not differenced by closure, and they were characterized by their fresh fruit, floral, and high aromatic intensity attributes. At 25°C, SyC presented higher color intensity and herbaceous characters, while C and SC showed a more oxidized character. In conclusion, the interaction closure type-storage temperature is critical for organoleptic stability properties of bottled TRw during aging. Thus, screwcaps and low-temperature storage conditions can preserve the TRw varietal characteristics, increasing their shelf-life significantly.

Keywords: Torrontes Riojano white wines, storage conditions, consumed oxygen, sulfur dioxide, wine color, sensory analysis.

1. Introduction[·]

Torrontes Riojano (*Vitis vinífera* L.) is an Argentinian white variety that arose from a natural cross between Listan Prieto and Moscatel de Alejandría (Agüero et al., 2003). This variety, which is noted because of its plasticity of implantation in different agro-ecological regions, produces wines with distinctive organoleptic characteristics (Fanzone et al., 2019). Traditionally, these wines are consumed within their year of production; although, based on their experience, winemakers state that they have a great potential to be aged. However, there are no scientific criteria to prove which the optimal storage conditions are for Torrontes Riojano wines to keep their typicality during long periods of aging.

As it is well known, the chemical composition and sensory attributes of bottled young wines change during aging, depending mainly on their storage conditions (e.g. closure type, temperature, time) (Tarko et al., 2020; Giuffrida-de-Esteban et al., 2019). The closure type has direct implications on the development of wine's color and aromas during aging. That is why, depending on its air permeability, it allows the entry of differential amounts of oxygen into bottles, facilitating the progress of degradative reactions on compounds related to the wine's organoleptic properties (Lagorce-Tachon et al., 2016; Ugliano, 2013). Wine post-bottling development is complex and it differs between red and white wines. Whereas red wines benefit from a small degree of oxygenation as it contributes to color stabilization, astringency reduction, and aroma improvement (Ćurko et al., 2021); white wines are less resistant to oxygen, leading to oxidative off-flavors and browning that reduce wine's quality (Coetzee et al., 2016). However, tight-sealing and lack of oxygen can also lead to negative sensory attributes (Karbowiak et al., 2010), as it can contribute to the development of complex sulfur aromas (Ugliano et al., 2011). On the other hand, during aging, there are changes in the balance of the SO_2 active forms depending on the oxygen level entering through the closure and storage temperatures, among others (Karbowiak et al., 2019; Arapitsas et al., 2014). The changes of these factors

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[·] **Abbreviations:**

TRw: Torrontes Riojano wines. **C:** natural cork. **SyC:** coextruded-synthetic stopper. **SC:** screwcap. **mx-CO:** maximum consumed oxygen. **HSP:** headspace pressure. **OTR:** oxygen transmission rate. **C-15, SyC-15, SC-15:** TRw with different stoppers aged at 15°C. **C-25, SyC-25, SC-25:** TRw with different stoppers aged at 25°C. **GH:** green hue. **YH:** yellow hue. **FF:** fresh fruits. **LL:** linalool. **OX:** oxidized character. **BT:** bitterness. **AC:** acidic taste. **AI:** aromatic intensity. **OB:** orange blossom. **CA:** chemical aromas. **CI:** color intensity. **SW:** sweet taste. **FL:** floral. **HB:** herbaceous. **BH:** brown hue.

result in a decrease of free SO_2, causing the loss of its antioxidant capacity on the reactive oxygen species and favoring the development of oxidative reactions (Danilewicz, 2011; Elias & Waterhouse, 2010).

During the oxidation process, wines' o-diphenols are oxidized to o-quinones and semiquinone radicals, whereas the oxygen is reduced to H_2O_2. These radical species may undergo further reactions, e.g. condensation reactions, causing the formation of colored compounds with high molecular weight (Waterhouse & Laurie, 2006; Danilewicz, 2003). Furthermore, as quinones are electrophilic compounds, they react quickly with some phenols, producing dimers or polymers that may rearrange their structure to form new o-diphenols. These regenerated o-diphenols will be oxidized again and, consequently, they will accelerate their polymerization reactions of phenolic compounds (Li et al., 2008). On the other hand, the H_2O_2 formed during the phenols oxidation process, in combination with Fe^{+2} through the Fenton reaction, generates hydroxyl radicals (Elias & Waterhouse, 2010). These last radical species are not selective and react with ethanol and tartaric acid to form acetaldehyde and glyoxylic acid, respectively. These carbonylic compounds are good nucleophiles and they can intervene in ulterior reactions associated with wine's color development. For instance, glyoxylic acid reacts with catechin to produce a (+)-catechin/glyoxylic acid adduct, which reacts with a further (+)-catechin to form a carboxymethine linked to (+)-catechin dimers. The dehydration of these dimers forms xanthenes, which can undergo oxidation generating yellow xanthylium salts that have a maximum absorption between 440 and 460 nm (Tarko et al., 2020; Bührle et al., 2017; Laurie & Waterhouse, 2006). Also, during storage, volatile substances are modified due to different reactions, including hydrolysis, esterification, and oxidation. Likewise, the loss of freshness and fruity aromas of young wines are mainly through ester hydrolysis (Coetzee et al., 2016; Coetzee, 2014; Oliveira et al., 2011); and these reactions are accelerated because of high storage temperatures (Cejudo-Bastante et al., 2013; Hopfer et al., 2012). On the other hand, the oxidation of white wines is characterized by the loss of their varietal characteristics and secondary aromas and by the formation of atypical aromas associated with wine deterioration, such as honey-like, cooked vegetable, farm food, among others (Coetzee et al., 2016; Karbowiak et al., 2010).

Therefore, the aging process and its suitable management are crucial to preserve the varietal characteristics and to obtain wines with a style and quality wanted. To the best of our knowledge, there is no published information on the interactive effect of different aging factors on the evolution of organoleptic properties of Torrontes Riojano wines. Thus, this work aimed at studying the joint effect of closure type and storage temperature on the color and sensory characteristics development of Torrontes Riojano wines (Mendoza, Argentina) throughout 18 months of bottle aging.

2. Materials and Methods

2.1. Wine samples and experimental design

Torrontes Riojano grapes were harvested (22,500 kg, 22.2°Bx, season 2016, Ugarteche, Mendoza, Argentina), destemmed using a crusher destemmer, and pneumatically pressed up to 1.4 bar. To avoid oxidation during these processes, 100 $mg.kg^{-1}$ sodium metabisulfite was added. The obtained grape juice had a pH 3.18, titratable acidity (TA) 7.16 $g.L^{-1}$ tartaric acid, and 80 nephelometric turbidity units (NTU). Vinification was carried out in Fincas Patagonicas winery using standard protocols. The alcoholic fermentation (AF) was conducted by commercial yeasts (20 $g.hL^{-1}$ *Saccharomyces cerevisiae*, Uvaferm CGC62, Lallemand-Inc., Canada) into a 150 hL stainless-steel tank. The AF took 21 days, and the fermentation temperature was maintained at 12-17°C. At the end of AF, to suppress the malolactic fermentation, molecular SO_2 was adjusted to 0.55 $mg.L^{-1}$. The initial wine had 2.79 $g.L^{-1}$ of malic acid. After stabilization treatments, the Torrontes Riojano wine was filtered through a 0.45μm plate filter (NTU 0.45). Prior to bottling, free SO_2 was adjusted to 22.8 mg L^{-1} to ensure 0.8 mg L^{-1} of molecular SO_2. Wine was fractionated (15±2°C) in 750-mL transparent bottles (300 units). One hundred bottles were closed with natural cork (C, special flower, 45x24mm, Portocork, USA), 100 with coextruded-synthetic stopper (SyC, 45x24mm, SelectSeriesTM, Nomacorc, USA), and 100 with screwcap (SC, 60×30mm, tin-foil-Saran/Tin, Arpex-Internacional, Argentina). Bottle headspace volumes were 6 cm^3 for C and SyC, and 16.6 cm^3 for SC. Bottling manipulations (filling, purging, etc.) were performed using high-purity nitrogen (Praxair-TechInc., Argentina). Bottles were divided into 2 sets, each consisting of 50-C, 50-SC, and 50-SyC, which were placed vertically in cardboard boxes and kept in thermostatized chambers (T15:15±2.4°C, T25:25±1.9°C) for 18 months. Sampling (bottles in triplicate) was at 0, 2, 3, 4, 6, 9, 12, 15, and 18 months. The microbiological stability was evaluated by OIV protocols (OIV, 2010).

2.2. Oxygen measurements

Twenty-four bottles were adapted for oxygen measurements (4 per treatment). Inside each bottle, using food-grade silicone, 2 sensors (Planar-Oxygen-Sensitive-Spot-PSt3, PreSens, Germany) were glued: one in the middle of the bottleneck, and another in the middle of the bottle-body (Supplementary Figure 1). Dissolved (DO) and headspace oxygen (HSO) concentrations were measured by luminescence non-destructive technology Fibox3-Trace fiber-optic oxygen-meter (PreSens, Germany). Initial measurements (zero time) were considered at 20 hours after bottling when percentual relative standard deviations (%RSD) of measurements were below 20%. Subsequent measurements were every 3 days during the first 6 months, 1 time/week during the second semester, and 1 time/15-day from 12 to 18 months. Consumed-oxygen (CO) was estimated according to the procedures detailed in Vidal et al. (2014). Oxygen transmission rate values (OTR, provided by cap-suppliers) were 4.56, 4.07, and 0.61 mg.L^{-1}/year for C, SyC, and SC, respectively.

2.3. Measurements of chemical and physical parameters

Standard enological parameters were measured by infrared spectroscopy using a platinum-diamond ATR single-reflection sampling module-cell coupled to a Bruker-Alpha instrument-OPUS software (Bruker-Optics, Germany). Free (fSO_2) and total (tSO_2) sulfur dioxides were measured with a FIAstar™5000 analyzer (Foss Analytical, Denmark). The UV-vis-spectrophotometer (Lambda25, PerkinElmer, USA) was used for the absorptiometric measurements. Total phenols (TP) were determined by the Folin-Ciocalteau assay (Singleton et al. 1999). CIELAB parameters and color differences were determined by Giuffrida-de-Esteban et al. (2019). Table 1 shows the chemical and physical parameters of the initial wine.

Table 1

2.4. Sensory analyses

A trained sensory panel (9 women and 4 men, aged 30 to 50) was constituted to carry out sensory studies. In each session, wine samples (30-40 mL, 8°C-10°C) were dispensed in tasting glasses (IRAM, 1999). Each panellist worked in an isolated booth (lit by full-spectrum 6500 K Candil-lamps). During the trial, 14 sessions were conducted (panel training sessions and sample evaluation: 1st-4th and 5th-14th session, respectively). In the first one, commercial Torrontes Riojano wines were tasted, and sensory evaluation criteria were standardized. In the second and third sessions, pattern blind identifications (acidity, bitterness, aromas, Supplementary Table 1) were done. In the fourth one, following Cadena et al. (2014), the visual, taste, and olfactory attributes of the young- Torrontes Riojano wines were evaluated and selected. In the fifth session, Triangular tests were done with 3-month-old Torrontes Riojano wines. In order to do so, consecutive sessions corresponding to each treatment were carried out. In those sessions, three samples with different combinations of treatments were assessed (e.g., C15-C25-C15; C15-SC15-C15), and the tasters had to select the odd sample. Judges rested five minutes between sets to minimize the tiredness of sense-organs. In these studies, it was considered that the analyzed samples were significantly different from the control ones, at a

95% confidence level, when 10/13 judges made the correct choice over the whole set of wines (Roessler et al., 1978). In the remaining sessions, before descriptive sensorial analyses (6, 12, and 18 months), judges agreed on descriptors used to characterize treatments. Three sessions were conducted to evaluate each treatment (using a non-structured linear scale from 0 to 10), where judges were only informed about wines' variety.

2.5. Statistical analyses

Data was analyzed by carrying out a multifactorial analysis of variance (Multifactorial ANOVA). Tuckey-HSD-test, Levene-test, and Pearson-test (α=0.05) were used to analyze the differences among each treatments, homogeneity of variances, and the correlation between variables, respectively. Principal Component (PCA) and Linear Discriminant (LDA) Analyses were performed on sensory data. In PCAs, the confidence ellipses indicating 95% confidence intervals was obtained from the multivariate distribution of the Hotelling test (p-value< 0.05), and was constructed using panellipse function on R (Husson et al., 2005). Sensory data was collected with Sodelsa's free software (ISETA, Buenos Aires, Argentina). Statgraphics Centurion-XVI (StatPoint-Technologies-Inc., USA), GraphPad Prism-7 (GhaphPad-Software-Inc., USA), and the free R-SensoMineR-package were employed for statistical analyses.

3. Results and discussion

3.1. Wine composition

At bottling, the Torrontes Riojano wines presented pH 3.20, 13.40 %V/V ethanol, 0.30 g.L^{-1} volatile acidity, and 6.00 g.L^{-1} titratable acidity. During wines' aging, these parameters were not significantly affected by closure types, or storage temperatures (Supplementary Table 2). For all treatments, at 18 month of aging, ethanol contents and pH values did not show any differences from the initial wine. Throughout the aging, total acidity decreased to 5.08 ±0.62 g.L^{-1} and volatile acidity increased to 0.58±0.03 g.L^{-1}. These results are in agreement with other studies focused on the aging of white wines from different varieties (Ricci et al., 2017; Liu et al., 2015; Hopfer et al., 2012; Lopes et al., 2009). This increase in volatile acidity, which remained below the acceptable level of 0.7–0.9 g.L^{-1} (Goode & Harrop, 2011), could be linked to chemical oxidative processes (Bakker & Clarke, 2012) and not to microbiological spoilage (Supplementary Table 4).

On the other hand, TP content declined as aging time rose. Over the first year, TP evolution was conditioned by the interaction between closure type and storage temperature (p-value=0.0388). During this period, Torrontes Riojano wines stored at 15°C and sealed with screwcap (SC) showed higher TP levels compared to those closed with corks (C) and synthetic stopper (SyC); but at 25°C wines were not differentiated by closure types. However, from months 12 to 18, there were not any statistical differences among treatments (Supplementary Table 2).

The TP drops observed during aging could be associated with chemical reactions (polymerization, complexation, condensation) where wine phenolic compounds are involved (Pati et al., 2019; Kallithraka et al., 2009). Even though phenolic constituents are slightly dissociated (pKa ~ 9 to 10) at wine's pH and they do not react directly with oxygen, they are the key substrate for non-enzymatic oxidations during aging (Oliveira et al., 2011; Waterhouse & Laurie, 2006; Danilewicz, 2003). Some authors proposed that oxidation, based on the Fenton reaction, would result in the formation of semiquinone radicals, which would later oxidize to quinones (Elias & Waterhouse, 2010; Danilewicz, 2003). And these reactions, which facilitate further condensation leading to the formation of phenolic polymers (Oliveira et al., 2011), could be favored by high storage temperatures (Recamales et al., 2006).

3.2. Oxygen and sulfur dioxide

From bottling to the end of storage, the total oxygen consumed (mg/bot) for each treatment was: C-15, 11.59 (±0.37); SyC-15, 9.96 (±0.15); SC-15, 5.12 (±0.37); C-25, 11.23 (±0.70); SyC-25, 9.33 (±0.18); and SC-25, 5.72 (±0.32). But, during the fourth month of aging, the evolution of oxygen depended on the interaction closure-storage temperature (p-value=0.0024). Thus, in this period, the total oxygen consumed (mg/bot) for each treatment was: C-15, 9.27 (±0.37); SyC-15, 7.86 (±0.13); SC-15, 4.77 (±0.25); C-25, 8.91 (±0.68); SyC-25, 7.22 (±0.18); and SC-25, 5.49 (±0.12). Besides, there was a maximum decrease in DO, falling 70 times below its initial value. But, from 120 days until the end of the aging process, the oxygen evolution changed due to the effect of closure type and it did not depend on storage temperature (Figure 1, Supplementary Figure 2, Supplementary Table 3).

Figure 1

Throughout the first four month of aging, C-25 showed a fast increase of CO, reaching its maximum value (mx-CO, 1.008±0.006 mg/bot) between days zero and 15, as DO was slowly decreasing during that time. At the beginning of the trial, the system could be considered to be in a pseudo stationary state, since in that period the HSO remains high and could offset the DO lowering (Supplementary Figure 2), which is being consumed in different chemical processes where it is involved (Perez-Benito, 2017; Ugliano, 2013; Navarro-

Laboulais et al, 2012). The same behavior was observed in SyC-25 (mx-CO, 0.939±0.111 mg/bot), and SC-25 (mx-CO, 0.648±0.1373 mg/bot).

Furthermore, Torrontes Riojano wines conserved at 15°C showed mx-CO in the period between the 30th and the 50th days of aging, while DO had a significant fall (Supplementary Figure 2). For C and SyC, the mx-CO was, on average, 1.194±0.213 mg/bot and 1.029±0.295 mg/bot, respectively. In this same period, DO lowered from 1.750±0.210 mg/bot to 0.018±0.007 mg/bot for C, and from 1.128±0.028 mg/bot to 0.024±0.006mg/bot for SyC. This could be because the system, outside of the stationary state, could not be able to compensate for the DO loss neither by the concentration of oxygen into headspace nor by the oxygen that entering through the closure (Navarro-Laboulais et al, 2012). In turn, SC presented an mx-CO of 0.709 ±0.059 mg/bot, while DO did not vary significantly. Likewise, during this aging time at both storage temperatures, C and SyC showed about 1.4 times more mx-CO than SC. This might be associated with the differences in headspace pressure (HSP) they had. At the onset of the experiment, C, and SyC presented HSP values twofold superior to SC (data not shown). Consequently, at a constant temperature, and according to Henry's law, C and SyC could have higher DO which could be more available to react with wines' reducing compounds (Peters, 2017; Dimkou et al., 2011). Additionally, when bottles are sealed with cylindrical caps, an additional amount of oxygen can enter into the system during the first weeks of aging. This incoming oxygen could be associated with the compression generated in the bottleneck at bottling or with the gas transfer through the glass/stopper interface (Chanut et al., 2021; Lagorce-Tachon et al., 2016; Ugliano et al., 2011). This could increase the partial pressure in the headspace, which could promote dissolution and consumption of oxygen.

Besides, the period of maximum oxygen consumption depended on storage temperatures. During the first four-month of aging, Torrontes Riojano wines stored at 15°C showed a delay (15-20 days) in reaching mx-CO compared to wines kept at 25°C. This might be because higher storage temperature could have facilitated the conversion of triplet oxygen (oxygen molecular form) to radical species (oxidizing agents), increasing the oxygen consumption rate (Ugliano, 2013; Oliveira et al., 2011; Karbowiak et al., 2010; Danilewicz, 2003). After the period described above, the differences found in oxygen consumption were related to the closure type (p-value<0.05), but not to storage temperatures. From the 4th to 18th month, the CO declined in all treatments (Fig. 1); however, for SC it was 21% higher than for C and SyC. These results were similar to those shown by Dimkou et al. (2011) in their studies on CO evolution for Riesling wines closed with screwcaps and coextruded cork aged for 2 years. The CO differences observed in this trial could be associated with the stopper' physical structures. While corks and screwcaps are practically impermeable to air, the trapped air within the cork structure could be slowly transferred into wines throughout aging (Karbowiak et al., 2019; Lopes et al., 2007). Also, OTRs are linked to the differential permeability of stoppers to the surrounding air which could lead to different amounts of oxygen (SC<SyC<C) being diffused through the closures into wines during storage (Lagorce-Tachon et al., 2016).

Related to sulfur dioxide evolution throughout the trial, Torrontes Riojano wines showed a reduction in fSO_2 and tSO_2 concentration (Supplementary Table 2, Supplementary Figure 3). During the first four months, regardless of the treatments, the fSO_2 fell near to 50% of their initial value; and from then on to the end of aging, it reached values below 5 $mg.L^{-1}$. The fSO_2 diminution could be related to different chemical processes in which it participates during aging. As mentioned previously, to react with wine components, the dissolved oxygen must be converted into $^{\cdot}OOH$. This radical species reacts with phenolic compounds generating hydrogen peroxide (H_2O_2) when there is Fe^{+2} and/or Cu^{+} in the medium, and this reaction product is scavenged by fSO_2 (Danilewicz, 2007; Boulton et al., 1996). Thus, fSO_2 prevents the unspecific oxidation of H_2O_2 on organic compounds of wines, favoring the chemical and organoleptic stabilities of these beverages over time (Danilewicz, 2011; Elias & Waterhouse, 2010). Also, fSO_2 may decrease due to its participation in the reduction of quinones (derived from the oxidation of phenolic compounds) to form sulphonic acids (Oliveira et al., 2011; Danilewicz, 2007). In this regard, a strong correlation between fSO_2 decrease and TP diminution was observed ($r_{15°C}= 0.906$; $r_{25°C}= 0.934$). Besides, throughout the aging process, the decrease of fSO_2 at 25°C was almost 6% more than it was at 15°C. It is well known that higher storage temperatures enhance H_2O_2 production and they increase oxidative reaction rates (Karbowiak et al., 2010). This could lead to a greater loss of fSO_2 because its consumption could be increased to reduce the harmful effects of radical species on easily oxidizable compounds in wines.

Nevertheless, a decline in tSO_2 concentration depended on storage temperature ($p\text{-value}<0.05$). Throughout the study, regardless of the closure type used, the wines aged at 15°C showed higher tSO_2 values than those kept at 25°C (Supplementary Figure 3). At the end of the aging, C-15, SyC-15, C-25, and SyC-25 showed, on average, 1.3% tSO_2 less than SC-15 and SC-25. Moreover, wines stored at 15°C had nearly 2 times more tSO_2 than those aged at 25°C (Supplementary Table 2). These results might indicate that when fSO_2 decreases, the bounded SO_2 forms (HSO_3^-/carbony-compounds hydrolyzable adducts) could begin to dissociate to restore the broken fSO_2/tSO_2 equilibrium, with a consequent drop in tSO_2 concentration (Sacks et al., 2020; Waterhouse et al., 2016; Danilewicz, 2016). Furthermore, at both storage temperatures, the differences in tSO_2 found between wines with porous stoppers and those sealed with screwcaps could be related to the amount of oxygen entering the bottle during aging. When the oxygen input is moderate as it occurs in SC, fSO_2 is preferentially consumed; however, when the O_2 input is high, as in C and SyC, not only free forms are mobilized, but also the SO_2 reversibly bound forms (Karbowiak et al., 2019). Therefore, the OTR high values of porous stoppers compared to screwcaps would favor the tSO_2 loss, as it has been observed in this study (Supplementary Figure 3).

3.3. Wine color

The browning during wine aging, among other mechanisms, might be associated with the oxidation of phenolic compounds and the later polymerization of their oxidized products, or with the polymerization reactions between phenols and other compounds such as acetaldehyde or glyoxylic acid (Li et al., 2008). The phenolic compounds of white wines as the o-diphenols (gallic and caffeic acids and its esters, catechin, epicatechin, and their derivatives) are considered as the most susceptible compounds to non-enzymatic oxidation. Also, the flavan-3-ols had shown significant correlations with the browning degree of white wines (Scrimgeour et al., 2015; Motta, 2013; Waterhouse & Laurie, 2006; Danilewicz, 2003; Fernández-Zurbano et al., 1998). During aging, absorbance at 420 nm (A420) has been employed as a useful index to evaluate the browning degree of white wines due to non-enzymatic oxidation (Pati et al., 2019). The A420 evolution depended on closure type-aging time and storage temperature-aging time interactions (Table 2). The wines' pale yellow color changed to intense yellow as storage time increased, and the A420 increase rate rose with increasing temperature and these changes are more noticeable in wines sealed with natural and synthetic corks. In the period between the 4th and the 18th months of aging, ΔA420 (AU) was: C-15, 0.022; SyC-15, 0.016; SC-15, 0.011; C-25, 0.014; SyC-25, 0.015; and SC-25, 0.013. To observe the correlation of the A420 rise with the TP diminution during storage time, a regression study was carried out. The correlation coefficient between A420 and TP decreased with increasing storage temperature ($r_{15°C}$= -0.850; $r_{25°C}$= -0.945). Also, A420 at 25°C increased 1.3 times more than 15°C (Supplementary Figure 4). This might indicate that a higher color development could be linked to the TP reduction, which can be accelerated by elevated aging temperatures (Ricci et al., 2017; Scrimgeour et al., 2015; Li et al., 2008). Besides, throughout the storage, C-25 and SyC-25 presented a superior browning degree to the rest of the wines; but between them, there were not any significant differences. Also, the A420 values for SC-25 were greater in comparison to C-15 and SyC-15; however, among these treatments, there were not any statistical differences (p-value>0.05). In addition, the A420 for SC-15 increased slowly along time and it was smaller than in the other treatments. Moreover, from the beginning to the end of the storage, the A420 mean percentual rise was 38.70% for C-25 and SyC-25, 32.58% for SC-25, 31.51% for C-15 and SyC-15, and 24.98% for SC-15. Furthermore, Torrontes Riojano wines sealed with screwcaps had the lowest CO concentration during aging (Figure 1), which might mean that the use of this closure type and low storage temperatures might provide better color preservation for these white wines over time.

Table 2

Figure 2

The color of Torrontes Riojano wines was also assessed employing the CIELAB coordinates. During aging, lightness (L*), chroma (C^*_{ab}), and hue (h_{ab}) evolutions depended on closure type-aging time and storage temperature-aging time interactions, whereas the green/red (a*) and yellow/blue (b*) color components were mainly affected by the storage temperature (Table 2 and Supplementary Table 2). Figure 2B shows the distribution of wines from different aging treatments throughout the trial on the CIELAB a*b* color plane. In this plot, samples were located in the h_{ab} region defined between 91° and 99°, related to yellow tones with a very slight tendency to green. In general, over the 18 months of storage, wines showed characteristic color changes associated with aging (decreases of h_{ab} and L* and increases of C^*_{ab}). During the first months of storage, wines kept at 15°C were grouped closer to the origin of coordinates and they had lower C^*_{ab} values, which determined the final color of these wines to be in the pale-yellow category. On the contrary, at the same aging period, wines stored at 25°C presented higher b* and C^*_{ab} values, related to the major browning degree that they showed. Also, as the b* and C^*_{ab} coordinates increased during time storage (C-25 and SyC-25 had the highest values, SC-15 the lowerest, and the other wines intermediate values), the color of the Torrontes Riojano wines became yellower and darker (lower L*). Besides, during the aging at both storage temperatures, SC treatment displayed a lower decrease of h_{ab} compared to C and SyC, showing that screwcaps could favor the preservation of wines´ yellow-green nuances. Furthermore, for SC-15, the L* values were not significantly affected in the evolution; and, at the end of the trial, they remained higher than in the rest of the treatments, which were not differentiated (p-value>0.05) neither by closures nor storage temperatures.

Additionally, the color difference ($\Delta E^*_{ab}{}^{(f-i)}$) between specific aging times (6-0, 12-0, 18-0) was evaluated. The $\Delta E^*_{ab}{}^{(f-i)}$ 3 has been quoted as a minimum value to discriminate between wines by an average-observer (Giuffrida-de-Esteban et al., 2019; Martínez et al., 2001). However, such estimates must be done with caution, as those $\Delta E^*_{ab}{}^{(f-i)}$ limit values were used to differentiate red wines. Other authors employed $\Delta E^*_{ab}{}^{(f-i)}$ 1 and $\Delta E^*_{ab}{}^{(f-i)}$ 2 for white wines comparisons (Šottníková et al., 2014; Lopes et al., 2009), and these values might be more appropriate to evaluate the color evolution of Torrontes Riojano wines during aging. All wines conserved at 25°C showed $\Delta E^*_{ab}{}^{6-0)}$, $\Delta E^*_{ab}{}^{(12-0)}$, and $\Delta E^*_{ab}{}^{(18-0)}$ values above 2; despite having the $\Delta E^*_{ab}{}^{(f-i)}$ values for SC-25 were always 1.5 times less than the C-25 and SyC-25 at each point. While C-15 and SyC-15 exceeded that reference value from 12 month onwards, SC-15 only showed $\Delta E^*_{ab}{}^{18-0)}>2$ (Supplementary Table 2). These results confirm that the Torrontes Riojano white wine color changes during storage, being particularly important from 6 month onwards, especially for those closed with natural cork and coextruded synthetic cork. Thus, the color differences observed for the C and SyC treatments could be related to the higher oxygen consumption they had during aging (which facilitated the phenolic compounds' oxidation) compared to wines sealed under more airtight conditions (Fig. 1).

3.4. Sensory analyses

In general, Torrontes Riojano young wines are sensory characterized by a pale golden-yellow color, a slightly bitter aftertaste, and a high aromatic intensity, regardless of the agro-ecological zone where grapes come from (Fanzone et al., 2019). Those sensory attributes could be related to the non-volatile ((+)-catechin, caffeic acid, quercetin-3-glucuronide) and volatile (holotrienol, linalool, geraniol, nerol, β-citronellol, β-cyclocitral, β-damascenone) varietal composition identified in these young wines, and to fermentative compounds (tyrosol, ethyl esters, higher alcohol acetates, higher alcohols) arising from winemaking (Pérez et al., 2018; Romano, 2013). In the present study, at 3 months of storage, the Triangular test was performed to evaluate if the different treatments affected the sensory profile of Torrontes Riojano wines. In these ABA tests, only C-15 from C-25 and SyC-15 from SyC-25 were discriminated against, showing that closure types and storage temperatures started to affect the sensory characteristics of Torrontes Riojano wines. Then, at 6, 12, and 18 months of storage, the sensory profiles of the wines were carried out through descriptive sensory analyses. The attributes selected by the judging panel to describe the wines depended on the evaluated aging time. At 6 months of wines storage, the consensus attributes were green hue (GH), yellow hue (YH), fresh fruits (FF), linalool (LL), oxidized character (OX), bitterness (BT), acidic taste (AC), and aromatic intensity (AI). At 12 months, there were GH, YH, FF, LL, OX, BT, AC, AI, orange blossom (OB), chemical aromas (CA), color intensity (CI), and sweet taste (SW). And, at 18 months, there were YH, FF, OX, BT, AC, AI, CI, floral (FL), herbaceous (HB), and brown hue (BH). To analyze the influence of different factors over the sensory attributes of Torrontes Riojano wines across aging, the Multifactorial ANOVA was conducted (Table 3). The factors storage temperature, aging time, judge (randomly enumerated in each session), and session (replicates) were nested within closure type factor. It was observed that the judge and replicate factors had no significant effect (p-value>0.05) on the sensory differences found among the treatments. Likewise, throughout aging, the sensory attributes OX and BT depended on the closure type-temperature-aging time interaction; FF, YH and CI on the temperature-aging time interaction; LL, GH and YH on the closure type-aging time interaction; while the others showed no significant differences due to the interaction effect of the evaluated factors (Table 3).

Table 3

Then, at each evaluated aging time, to highlight the similarity of the samples, and to determine the main attributes that contributed to differentiating them, the Principal Component Analysis (PCA, unsupervised pattern recognition technique) was performed. The PCA biplot and confidence ellipses were constructed with 95% certainty based on Hotelling’s test (Husson et al., 2005). The sizes of the confidence ellipses were related to the variability of each wine (Supplementary Figure 5 A.1, B.1 and C.1), while the color dots in the loading plot showed a variability depending on sensory attributes (Supplementary Figure 5 A.2, B.2 and C.2). At 6 months of storage, the PCA analysis explained 94.45% of data variance. The Principal Component 1 (PC1, 87.19%), related to the high GH, LL, FF, AI, OX, and YH eigenvalues, led to discriminate wines by the storage temperature effect; and the PC2 (7.26%) was mainly correlated to AI and BT eigenvalues, which contributed to separate wines according to closure type effect. Torrontes Riojano wines kept at 15°C were characterized by GH, LL, and FF attributes, but C-15 and SyC-15 presented a lower aromatic intensity than SC-15. On the other hand, C-25 and SyC-25 were associated with oxidized characters (caramelized, honey aromas) and yellow nuances; but SC-25 presented higher AI and lower OX and YH scores than the other wines stored at 25°C. At 12 months of aging, the PCA explained 90.95% of the data variance and showed that the judging panel was able to differentiate wines according to the temperature effect. The C-15, SyC-15, and SC-15 were not discriminated by closure types and were associated with FF, LL, and OB attributes. In opposite, C-25, SyC-25, and SC-25 were showed higher scores of color intensity, oxidized character, and yellow hue. At this aging time, wines kept at 25°C also presented rubbery, sulfurous, and metallic flavors, which the panel described as chemical aromas. At 18 months of storage, the PCA analysis explained 86.46% of the total variance. Judges were unable to discriminate among C-15, SyC-15, and SC-15, and associated them with high FL, FF, and AI attributes. At this aging time, the yellow hues nuances evolved into browning hues evidencing that the oxidation process was advanced, especially at higher temperatures. The SyC-25 had the highest BH and CI scores, whereas C-25 and SC-25 presented a more oxidized character; thus at 25°C, the Torrontes Riojano wines could be differentiated by the closure type effect.

On the other hand, to evaluate the sensory attributes of Torrontes Riojano wines, a Linear Discriminant Analysis (LDA) was performed. LDA is a supervised pattern recognition method based on the determination of linear discriminant functions, which maximizes the ratio of between-class variance and minimize the ratio of within-class variance (Berrueta et al., 2007). In order to achieve this and to ensure independence among the variables (a mandatory requirement for the LDA technique), the information obtained from the exploratory analyses (PCA) and the Multifactorial ANOVA (Table 3), was submitted to conduct some correlation studies (Pearson's test, α=0.05). Considering the correlation coefficients among variables in the correlation matrix (data not shown), the attributes CI, LL, BH, OX, FF, AI, GH, and YH were selected as predictor variables.

From the selected predictor variables, three discriminant functions were obtained (Supplementary Table 5), which together, represented 99.01% of the total variance (α=0.01). The Discriminant Functions 1 and 2 explained 87.25% and 9.36% of the total variance, respectively, and they had Wilks Lambda values less than 1.10^{-5}, which shows that Torrontes Riojano wines could be discriminated among them through the aging. Thus, considering the aging process as a whole, the aromatic intensity of wines alongside fresh fruit, yellow and green nuances decreased, while brown hue, color intensity, linalool, and oxidized character rose as storage time went by. Figure 3 shows that wines were sensory discriminated mainly by their time and storage temperature; although at the end of the process (18 months), the closure type effect allowed us to separate those aged at 25°C.

Figure 3

4. Conclusion

During the aging stage, different physical and chemical processes take place that modify the organoleptic characteristics of bottled Torrontes Riojano white wines. The permeability of stoppers to the surrounding air affects the intake and consumption of oxygen and, therefore, the stability of compounds associated with the wines' color and sensory properties. The higher the permeability degree, the more oxygen and sulfur dioxide are consumed, since oxidizing and antioxidant reactions are promoted, respectively. As a consequence, during aging, Torrontes Riojano wines sealed with corks and synthetic stoppers show an increase in the browning index, hue, and chroma, and a decrease in lightness, freshness, fruity aromas, and aromatic intensity in a shorter period than those sealed with screwcaps. Also, the storage temperature affects the rate of the reactions mentioned above. The 25°C aging temperature facilitates the development of oxidative reactions, increasing the occurrence velocity of organoleptic defects, and decreasing the quality of the products. In this sense, the interaction between closure type and storage temperature is critical to the stability of the organoleptic properties of these wines during their bottle aging. Thus, to preserve and increase the shelf-life of Torrontes Riojano wines, the best aging conditions are the use of screwcaps and low storage temperatures.

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Table Captions

Table 1: Chemical and physical parameters of Torrontes Riojano wine at bottling.

Table 2: Multifactorial ANOVA for color parameters of Torrontes Riojano wines across the aging process.

Table 3: Multifactorial ANOVA for sensory attributes of Torrontes Riojano wines across the aging process.

Supplementary Table 1: Reference standards for sensory analyses of aged Torrontes Riojano wines.

Supplementary Table 2: Chemical and physical parameters of Torrontes Riojano wines from aging treatments at different storage times.

Supplementary Table 3: Probability values (multifactor ANOVA) for closure type (A), aging temperature (B), and aging time (C) factors to the oxygen concentration in Torrontes Riojano wines.

Supplementary Table 4: Microbiological composition of Torrontes Riojano wines during aging.

Supplementary Table 5: Standardized coefficients for discriminant functions based on sensory attributes of Torrontes Riojano wines aged under different conditions for 18 months.

Figure Captions

Figure 1: Evolution of consumed oxygen throughout 18 months of storage for Torrontes Riojano wines aged under different conditions. **A.** CO evolution at 25°C. **B.** CO evolution at 15°C.

CO consumed oxygen (mg/bot equivalent to mg/750 mL). C, SyC, and SC are Torrontes Riojano wines sealed with natural cork, coextruded synthetic cork, and screwcap, respectively. 15 and 25 are storage temperatures, 15°C, and 25°C, respectively.

Figure 2: Evolution of color parameters throughout 18 months of storage for Torrontes Riojano wines aged under different conditions. **A**. A420 nm (absorbance at 420 nm) evolution during aging. **B**. The CIELAB a*b* color plane (a* green/red color component; b* yellow/blue color component; h_{ab} hue angle).

C, SyC, and SC are Torrontes Riojano wines sealed with natural cork, coextruded synthetic cork, and screwcap, respectively. 15 and 25 are storage temperatures, 15°C, and 25°C, respectively.

Figure 3: Supervised pattern recognition (LDA) based on sensory attributes of Torrontes Riojano wines aged under different conditions for 18 months.

LDA Linear Discriminant Analysis, DF1, and DF2 are Discriminant Function 1 and 2, respectively. Dotted lines are confidence ellipses (statistical significance at 95%). C, SyC, and SC are Torrontes Riojano wines sealed with natural cork, coextruded synthetic cork, and screwcap, respectively. 15 and 25 are storage temperatures, 15°C, and 25°C, respectively.

Supplementary Figure 1: Sensors, sensor positioning tool, and measurement instrument.

Each bottle had a QR code corresponding to the sensor that was used to calibrate the equipment during the measurements. The same bottles were used throughout the aging to measure headspace oxygen (HSO) and dissolved oxygen (DO) values.

Supplementary Figure 2: Oxygen evolution during the firsts 4 months of storage for Torrontes Riojano wines aged under different conditions. **A.** Oxygen evolution for C-15. **B.** Oxygen evolution for SyC-15. **C.** Oxygen evolution for SC-15. **D.** Oxygen evolution for C-25. **E.** Oxygen evolution for SyC-25. **D.** Oxygen evolution for SC-25.

DO dissolved oxygen, HSO headspace oxygen, and CO consumed oxygen (units: mg/bot equivalent to mg/750 mL). C, SyC, and SC are Torrontes Riojano wines sealed with natural cork, coextruded synthetic cork, and screwcap, respectively. 15 and 25 are storage temperatures, 15°C, and 25°C, respectively.

Supplementary Figure 3: Evolution of sulfur dioxide throughout 18 months of storage for Torrontes Riojano wines aged under different conditions.

tSO_2 and fSO_2 are total and free sulfur dioxide ($mg.L^{-1}$), respectively. C, SyC, and SC are Torrontes Riojano wines sealed with natural cork, coextruded synthetic cork, and screwcap, respectively. 15 and 25 are storage temperatures, 15°C, and 25°C, respectively.

Supplementary Figure 4: Regression analyses between A420 and total phenols of Torrontes Riojano wines aged at 15°C (**A**), and 25°C (**B**) during 18 months.

A420 absorbance at 420 nm, TP total phenols concentration expressed in $g.L^{-1}$ gallic acid equivalents. Regression parameters: r correlation coefficient (Pearson test), R^2 determination coefficient, DW Durbin-Watson test, model from ANOVA test, LOF lack of fit test.

Supplementary Figure 5: Unsupervised pattern recognition (PCA) based on sensory attributes of Torrontes Riojano wines aged under different conditions for 18 months. **A.1.**, **B.1.**, and **C.1.** are wine factor maps at 6, 12, and 18 months of storage, respectively. **A.2.**, **B.2.**, and **C.2.** are sensory attributes loadings (95 % confidence ellipses based on the multivariate distribution of Hotelling's test, p-value<0.05) at 6, 12, and 18 months of storage, respectively.

PCA Principal Component Analysis, PC1, and PC2 are Principal Components 1 and 2, respectively. Lines are confidence ellipses (statistical significance at 95%). C, SyC, and SC are Torrontes Riojano wines sealed with natural cork, coextruded synthetic cork, and screwcap, respectively. 15 and 25 are storage temperatures, 15°C, and 25°C, respectively. Sensory attributes: green hue (GH), yellow hue (YH), fresh fruits (FF), linalool (LL), oxidized character (OX), bitterness (BT), acidic taste (AC), and aromatic intensity (AI), orange blossom (OB), chemical aromas (CA), color intensity (CI), sweet taste (SW), floral (FL), herbaceous (HB), and brown hue (BH).

Table 1: Chemical and physical parameters of Torrontes Riojano wine at bottling.

Standard enological parameters		*Color parameters*	
pH	3.15	A420 (absorbance at 420 nm)	0.076
ethanol (%V/V)	13.40	L* (lightness)	99.20
volatile acidity (acetic acid, g/L)	0.30	C^*_{ab} (chroma)	4.05
titratable acidity (tartaric acid, g/L)	6.00	h_{ab} (hue)	99.48
free sulfur dioxide (mg/L)	22.80	a* (green/red component)	-0.63
total sulfur dioxide (mg/L)	35.30	b* (yellow/blue component)	3.99
total phenols (gallic acid, g/L)	1.59		

Table 2: Multifactorial ANOVA for color parameters of Torrontes Riojano wines across the aging process.

	Factors[a]						
	c	T	t	c-T	c-t	T-t	c-T-t
A420 (absorbance at 420 nm)	**	***	***	ns	*	***	ns
L* (lightness)	***	**	***	ns	**	**	ns
C*ab (chroma)	***	***	***	ns	***	***	**
h_{ab} (hue)	***	***	***	ns	***	***	**
b* (yellow/blue component)	ns	***	*	ns	ns	ns	ns
a* (green/red component)	ns	***	*	ns	ns	ns	ns
degree of freedom to F test	143						

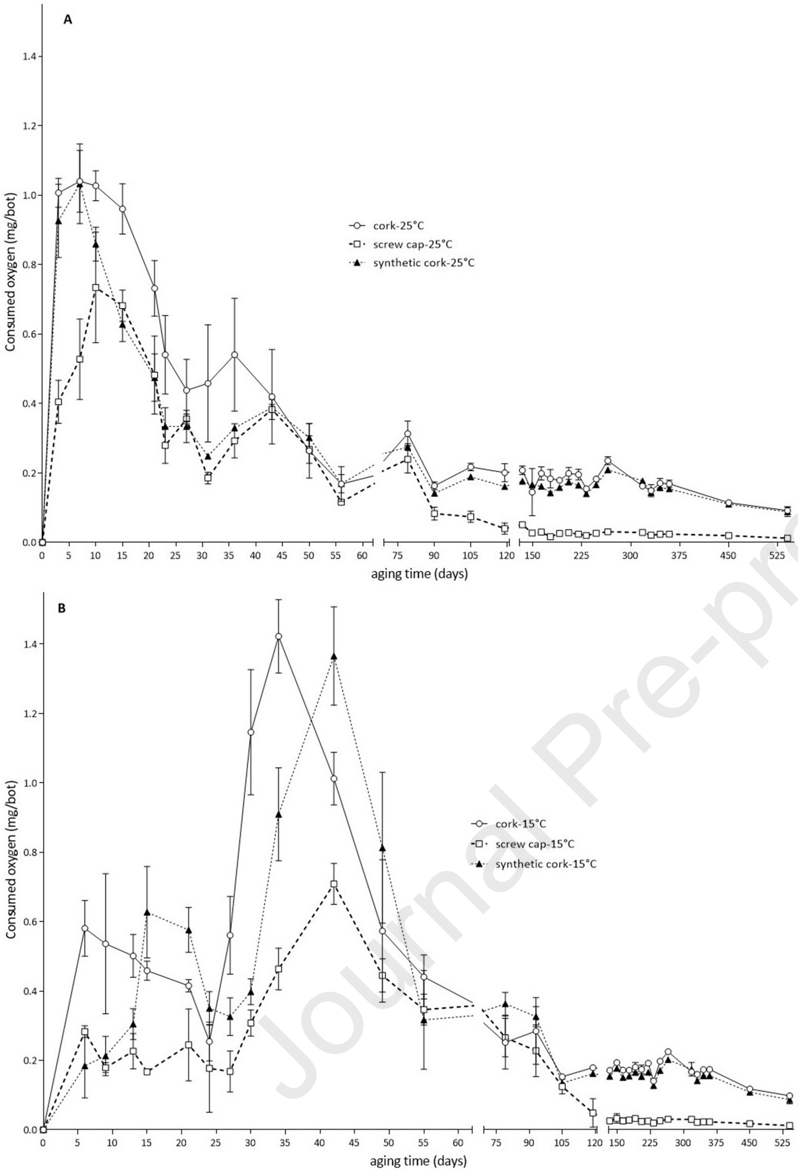
[a] c closure types; T storage temperatures; t aging times; c-T, c-t, T-t, c-T-t are the factor interactions.

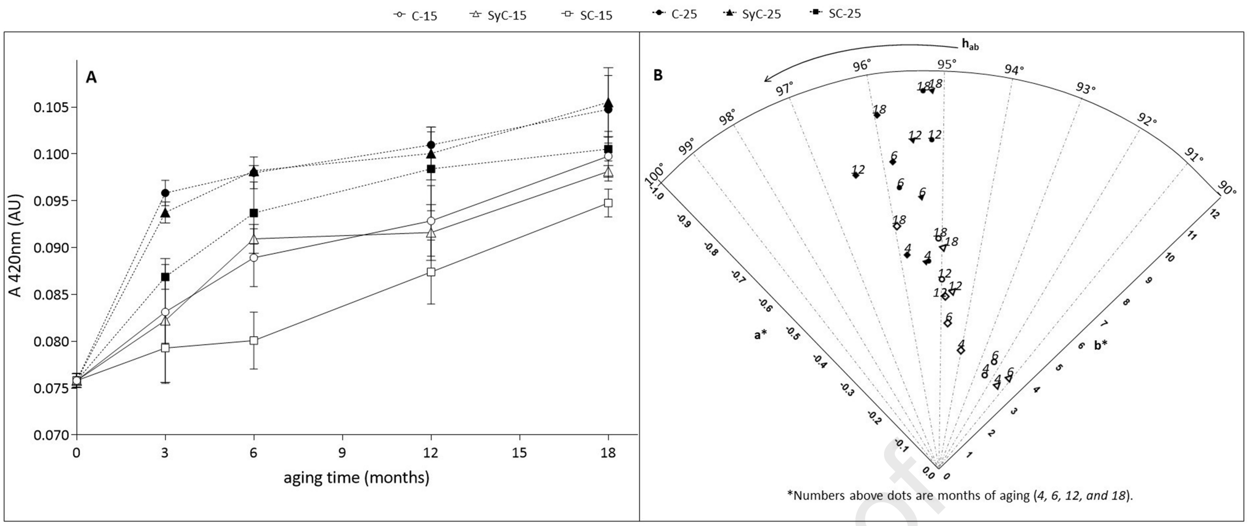
ns not significant at p-value> 0.05; * significant at p-value< 0.05; ** significant at p-value< 0.01; *** significant at p-value< 0.001

Table 3: Multifactorial ANOVA for sensory attributes of Torrontes Riojano wines across the aging process.

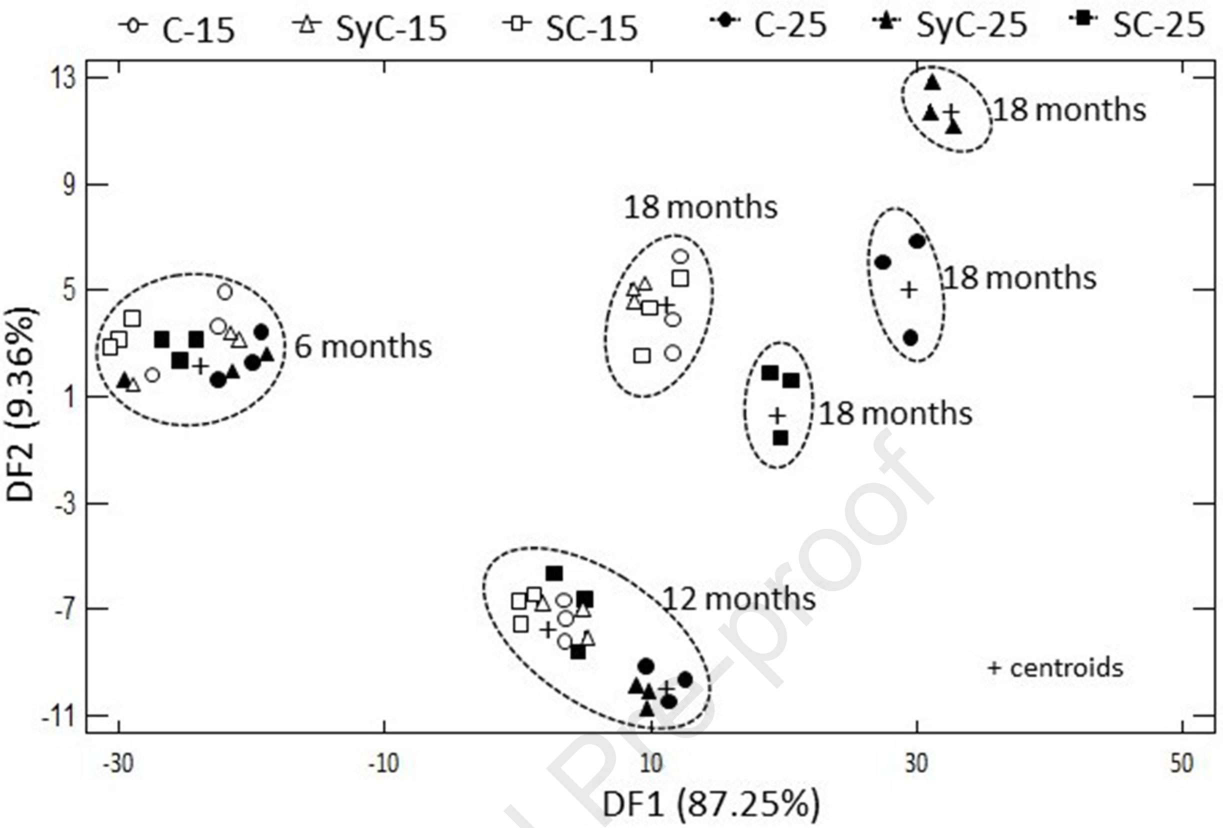
	Factors[a]						
	c	T	t	c-T	c-t	T-t	c-T-t
fresh fruits (FF)	ns	***	***	ns	ns	**	ns
linalool (LL)	ns	***	***	ns	*	ns	ns
floral (FL)	ns	ns	ns	ns	ns	ns	ns
orange blosson (OB)	ns	ns	ns	ns	ns	ns	ns
herbaceous (HB)	ns	ns	ns	ns	ns	ns	ns
oxidized character (OX)	**	***	***	*	***	ns	**
chemical aroma (CA)	ns	ns	ns	ns	ns	ns	ns
green hue (GH)	**	*	***	ns	**	ns	ns
yellow hue (YH)	*	***	***	ns	*	*	ns
brown hue (BH)	ns	**	***	ns	ns	ns	ns
aromatic intensity (AI)	ns	*	ns	ns	ns	ns	ns
color intensity (CI)	**	***	ns	**	ns	*	ns
bitterness (BT)	*	***	ns	**	**	***	**
acidity (AC)	ns	ns	ns	ns	ns	ns	ns
sweet taste (SW)	ns	ns	ns	ns	ns	ns	ns
degree of freedom to F test	*589*						

[a] c closure types; T storage temperatures; t aging time; c-T, c-t, T-t, c-T-t factor interactions. ns not significant at p-value> 0.05; * significant at p-value< 0.05; ** significant at p-value< 0.01; *** significant at p-value< 0.001.


A
Consumed oxygen (mg/bot)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
cork-25°C
screw cap-25°C
synthetic cork-25°C
0 5 10 15 20 25 30 35 40 45 50 55 60
75 90 105 120
150 225 300 375 450 525
aging time (days)
B
Consumed oxygen (mg/bot)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
cork-15°C
screw cap-15°C
synthetic cork-15°C
0 5 10 15 20 25 30 35 40 45 50 55 60
75 90 105 120
150 225 300 375 450 525
aging time (days)



*Numbers above dots are months of aging (*4, 6, 12, and 18*).


C-15
SyC-15
SC-15
C-25
SyC-25
SC-25
13
9
5
1
-3
-7
-11
-30
-10
10
30
50
DF2 (9.36%)
DF1 (87.25%)
6 months
18 months
18 months
18 months
18 months
12 months
+ centroids

Highlights

. Torrontes Riojano wines aged in bottles modified their color and sensory properties.

. Color changes were significant from 6 months of aging.

. Wines sealed with natural and synthetic corks showed the most noticeable color changes.

. During aging, wines were sensory discriminated by closure type and storage temperature.

. Aging at 15°C preserves the varietal characteristics of Torrontes Riojano wines.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.