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Chemical, physical, and sensory attributes of Sauvignon blanc wine fermented in different kinds of vessels

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ABSTRACT

The use of vessels that are alternative to conventional cylindrical stainless steel tanks and traditional oak barrels during winemaking has increased in recent years. Examples of these alternative vessels include fashionable, oval-shaped vessels or classic clay jars. A Sauvignon blanc grape juice was fermented (by using a commercial starter made of two different commercial strains from Oenobrands/Anchor Oenology) in four different vessels in triplicate: 150 L cylindrical stainless steel tanks (as a control), 980 L polyethylene oval-shaped tanks, 450 L concrete oval-shaped tanks, and 225 L clay jars. Afterwards, the finished wines were chemically, physically, and sensorially characterized. The wine fermented in the concrete vessels had a slightly higher pH (about 0.05 pH units) and lower titratable acidity (about 0.20 g/L of tartaric acid equivalents) value than those of the control wine. The wine fermented in the clay jars showed the lowest amounts of C10, C12, and C14 volatile compounds (containing about 69% of those compounds when compared with the control wine). The wine fermented in the polyethylene oval-shaped tanks was described as the most bitter, while the wine fermented in the concrete oval-shaped tanks was described as the least fruity when wines were sensory analyzed by a panel of 11 wine experts. The wines fermented in the oval-shaped vessels showed lower volatile acidity (about 25% reduction of volatile acidity when compared with wines fermented in non-oval-shaped vessels), higher residual sugars (wines fermented in the oval-shaped vessels contained about 1.7 g/L of residual sugars, while wines fermented in the non-oval-shaped vessels contained about 1.4 g/L of residual sugars), higher phosphorous content (with an increase of about 12%), and required a lower dose of bentonite to achieve protein stability (about 64 g/hL of bentonite) than those of the other wines (about 75 g/hL of bentonite). Even though some statistical differences were found among wines fermented in the different vessels, it should be noted that the differences were very small. Thus, it seems that the impact of the fermentation vessel type on final wine features is lower than expected.

1. Introduction

The wine market is one of the most competitive beverage markets worldwide, and it was estimated that 31.4 billion euros were spent on global wine trading in 2018 (International Organisation of Vine and Wine, 2019), of which bottled wines accounted for approximately 21.9 billion euros. Winemakers need to distinguish their products to successfully compete in this difficult market (Menghini, 2015), taking into account the heterogeneity of consumer preferences (Hughson, Ashman, De La Huerga, & Maskowitiz, 2004; Lockshin & Corsi, 2012; Pomarici, Lerro, Chrysochou, Vecchio, & Krystallis, 2017). One of the strategies of wineries for distinguishing wines and promoting their brands in the market is to avoid the use of homogenizing techniques during winemaking to maximize the typicality of their wines, which are sold as either the terroir or varietal typicality. The use of vessels that are alternative to oak barrels has increased in wineries in recent decades to maximize the intrinsic attributes of wines from a specific geographical origin, since the oak from barrels releases a set of well-known compounds that confers typicality to a wine due to aging in contact with the wood (del Alamo-Sanza & Nevares, 2018; Garde-Cerdán & Ancín-Azpilcueta, 2006). Some of the vessels gaining prominence on wineries include vessels made of concrete or clay, which are materials that have
not been used for several decades or even centuries. Moreover, the sale of technical plastics with well-defined oxygen permeability and of food grade by winery suppliers has also increased in recent years and also represent an accessible choice for winemakers as alternatives to oak vessels. In addition to exploring the use of these forgotten and new materials, the use of oval-shaped vessels has also increased (Goldfarb, 2008), because of the reasons pointed in the results and discussion section. These kinds of vessels are currently being used in wineries in combination with conventional stainless steel tanks as well as traditional oak barrels.

Although all of these vessels have been implemented in wineries in recent years, no scientific data exists in the literature (at least to our knowledge) about the impact of the vessel on the finished wine composition and attributes. To address this issue, the present study contains a complete chemical, physical, and sensory characterization of wines made from Sauvignon blanc grapes fermented in (1) cylindrical stainless steel tanks, (2) oval polyethylene tanks, (3) oval concrete tanks, and (4) clay jars in triplicate to determine the impact of the use of these vessels during winemaking on the final composition and attributes of the wines.

2. Methods and materials

2.1. Winemaking

The grapes used for this study were grapes of Sauvignon blanc cv. from the Leyda Valley (Chile) [33°54′4.568″S; 71°14′0.841″W], coming from the Bucallemu vineyard (fruit yield 12 ton/ha). Grapes were hand-harvested on April 16 (2018) and transported to the Miguel Torres Chile Winery in Curicó (Maule, Chile). Grapes were destemmed, crushed and pressed (around 65% juice yield). Grape juice was subjected to a settling period of 24 h (12–14 °C) in a stainless steel tank of 50,000 L. Then, the grape juice was racked to the different tanks used for this trial and inoculated with 0.2 g/L of a commercial starter of yeasts (ANCHOR VINO, Kyungki-Do, Korea). The Sauvignon blanc juice had a sugar content of 22.1°Brix, 6.75 g/L (tartaric acid equivalents) titratable acidity, 0.38 g/L (acetic acid equivalents) volatile acidity, a pH of 3.4, and 174 mg/L yeast assimilable nitrogen (YAN). Four different commercially available tanks were used in triplicate (hence, a total of twelve tanks were used for this assay). The tanks used were classified as follows: [CYLINOX], 150 L stainless steel tanks; [OVO PE], 980 L oval-shaped polyethylene tanks (Apollo FLEXTANK, Partner Ltd., Chile); [OVO CNCR], 450 L oval-shaped concrete tanks (De Navarra Ltd., Chile); and [JAR CLAY], 225 L clay jars (Value Juices Ltd., Chile). All tanks were placed in an underground cellar at a controlled temperature (18 ± 1 °C), and the fermentation process was controlled daily by measuring the density and temperature of the tanks. The alcoholic fermentation was considered complete when the density remained constant for at least two days and the residual sugar concentration was below 2 g/L. Once the alcoholic fermentation was finished, a sample of 5 L of wine from each tank was taken, sulfited (200 mg K2S2O5/L), and kept at 2 °C for 15 days to achieve tartaric stabilization. Then, the wines were bottled in green glass bottles (750 mL) and capped with crown caps. Bottles were stored in a dark cellar (16 ± 1 °C) until analysis. Wine samples were chemically and sensorily analyzed six months after bottling.

2.2. General analyses

General analyses of the grape juice (soluble solids, titratable acidity, YAN, volatile acidity, pH), and wines (alcohol content, titratable acidity, volatile acidity and pH) were performed according to the analytical methods recommended by the International Organisation of Vine and Wine (International Organisation of Vine and Wine, 2016). pH measurements were performed by using a METTLER TOLEDO Seven Compact pH/ion meter (Mettler Toledo, Columbus, OH, USA). The color intensity and CIELAB coordinates of the wines were analyzed as previously reported (Gil, Cortiella, Úbeda, Del Barrio-Galán, & Peña-Neira, 2019) by using a SHIMADZU UV-1900 UV–Vis spectrophotometer (Shimadzu Latin America S.A., Montevideo, Uruguay). The same spectrophotometer was used for the determination of the phenolic index I280 as previously reported (Ribereau-Gayon, Glories, Maujean, & Dubourdieu, 2006). Turbidity measurements were performed by using a HANNA HI 88731-ISO turbidimeter (HANNA Instruments Chile, Chile), and conductivity measurements were performed by using a HANNA HI 5321 conductometer (HANNA Instruments Chile, Chile). The fast heat test (Estérelas et al., 2009) was used to determine the protein stability of the wines and the required dose of bentonite to achieve protein stability for each wine.

2.3. Organic acid analysis

Wine samples were diluted 1:5 with an aqueous solution of 0.10% phosphoric acid (v/v) prior to injecting the samples in an Agilent 1260 Infinity Series chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump, an autosampler, a column oven, and a diode array detector. For the separation of organic acids for HPLC-DAD analysis, a Supelcogel H column (25 cm × 4.6 mm, Sigma Aldrich, Chile) was used under isocratic conditions by using an aqueous solution of 0.10% phosphoric acid (v/v) as the mobile phase. Twenty microliters of each sample was injected, and the elution of acids was monitored at the detection wavelength of 210 nm. Tartaric acid (Sigma Aldrich, Chile), malic acid (Sigma Aldrich, Chile), and citric acid (Sigma Aldrich, Chile) were identified and quantified with external standards.

2.4. Elemental analysis

The wine composition for macro- (K, P, Mg, Ca, Si, Na and B) and microelements (Fe, Mn, Zn and Cu) was determined by MP-AES 4200 microwave plasma atomic emission spectroscopy (Agilent Technologies, Santa Clara, CA, USA) after acid digestion of the wine samples. Five milliliters of wine sample was mixed with 3 mL of 65% HNO3 (Merck, Chile) and 1 mL of 30% H2O2 (Sigma Aldrich, Chile) and subjected to a cycle in an autoclave (LabTech, Daiban Labtech CO. LTD. Kyungki-Do, Korea). After the digestion process, samples were diluted with ultrapure water (PURELAB Ultra, Elga Labwater, Arquimede, Chile) to a final volume of 10 mL, and this solution (dilution 1) was used for microelement quantification. An aliquot of 2 mL of dilution 1 was diluted again with ultrapure water to a final volume of 10 mL (dilution 2), and this solution was used for macroelement quantification.

2.5. Individual low-molecular-mass phenolic compound determination

Low-molecular-mass phenolic compounds were extracted by solid-phase extraction (SPE) using Oasis® MCX 6 cc (500 mg) cartridges (Waters, Ireland). Nine milliliters of wine were mixed with 9 mL of aqueous 0.1 M hydrochloric acid and adsorbed to a preconditioned cartridge. Samples were washed with 9 mL of aqueous 0.1 M hydrochloric acid and 9 mL of ultrapure water. After that, the phenolic compounds were eluted with 18 mL of absolute ethanol. The eluate was dried using a rotary evaporator (Buchi Rotavapor R-210, equipped with a Buchi B-491 heating bath and coupled to a Buchi V-300 vacuum pump equipped with an Buchi I-300 interface for pressure control, Cientec Instrumentos Científicos, Chile), and the dried extracts were dissolved in 900 μL of a water-methanol mixture (80:20). The resulting extracts were filtered with 0.22 μm syringe-driven filter units (Milllex® - GV, Merck Millipore Ltd., Ireland) and injected into an Agilent 1200 Series chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a degasser, a quaternary pump, an autosampler, a column oven, and a diode array detector. The chromatographic method used has been

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**References**

Goldfarb, S. (2008). The fast heat test (ESTERELAS et al., 2009) was used to determine the protein stability of the wines and the required dose of bentonite to achieve protein stability for each wine.

Ribereau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2006). Turbidity measurements were performed by using a HANNA HI 88731-ISO turbidimeter (HANNA Instruments Chile, Chile), and conductivity measurements were performed by using a HANNA HI 5321 conductometer (HANNA Instruments Chile, Chile). The fast heat test (ESTERELAS et al., 2009) was used to determine the protein stability of the wines and the required dose of bentonite to achieve protein stability for each wine.

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previously published (Schwarz, Picazo-Bacete, Winterhalter, & Hermosín-Gutiérrez, 2005), and phenolic compounds were identified by comparing their retention times with those of available commercial standards and by comparing their spectra with bibliographic data. The quantification of hydroxycinnamic acid was performed with the external standard method by using caffeic acid (Sigma Aldrich, Chile) as a standard.

2.6. Volatile compounds

The volatile compound profiles of wines were obtained by GC–MS after SPME as previously reported (Gil et al., 2019) using an Agilent 7890B gas chromatograph coupled to an Agilent 5977 inert quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) equipped with a Gerstel MP2 autosampler (Mülheim an der Ruhr, Germany). A DB-WAX capillary column (60 mm × 0.25 mm, 0.25 μm film thickness; Agilent Technologies) was used with helium as the carrier gas at a flow rate of 1 mL/min.

2.7. Soluble polysaccharide content of wines

Polysaccharides were estimated by means of HRSEC-RID after precipitation with cold acidified ethanol as previously reported (Fanzone et al., 2012).

2.8. Sensory analysis of wines

A descriptive analysis of samples were performed by a panel of 11 winemakers and wine experts familiarized with Chilean Sauvignon blanc wines. Nine attributes were scored by tasters using a 10 cm unstructured line (with an expression denoting the intensity and direction above each end of the line). Among them, one visual attribute (color intensity), four smell attributes (aroma intensity, typicality, vegetal scents and fruity scents), and four taste attributes (sour, mouthfeel, bitterness, and persistence) were scored. Wines were tasted at 12–14 °C serving temperature by using ISO official tasting glasses (ISO 3591.1977). The obtained scores of tasted wines were computed by using the software PanelCheck (v. 1.4.2).

2.9. Statistical analysis

All results are expressed as the arithmetic mean ± standard deviation of three replicates. One-factor analysis of variance (ANOVA) (P < 0.05) was performed with Infostat software (v. 2018), and the Student-Newman-Keuls post hoc test was used for multiple comparisons and computed with the same software for physical and chemical data. One-factor analysis of variance (ANOVA) (P < 0.05) was performed with PanelCheck V1.4.2 software for sensory data.

3. Results and discussion

3.1. Effect of vessel type on the alcoholic fermentation process

As can be observed in Table 1, all fermentations finished properly, since the final residual sugars were lower than 2 g/L for each vessel type and the volatile acidity of all finished wines was below their sensory threshold (stabilized around 1.1 g/L for white wines (Cliff & Pickering, 2006)). Moreover, no significant differences were observed among the alcohol content of the finished wines. These data indicate that all vessels used for this trial are suitable for white grape juice fermentation during white wine production. Although all vessels used allowed for the correct depletion of sugars in the grape juice, some differences in fermentation development and general chemical attributes of the finished wines were observed among the different vessels.

The completion of alcoholic fermentation was five days longer in the CYL INOX tanks than in all the other vessels despite the residual sugar of the finished CYL INOX wine being lower than that of the wines finished in the other tanks (Table 1). Slight differences in fermentation kinetics were observed and are shown in Fig. 1: while the tumultuous phase of the alcoholic fermentation was similar for all of the vessels, differences in the stationary phase could be observed, with the CYL INOX tanks showing a slower consumption rate of sugar than that of the other vessels. Furthermore, the fermentation performed in the CYL INOX tanks needed more time to reach a residual sugar level below 2 g/L when the density of the wine remained stable (last days of alcoholic fermentation). Thus, it seemed that for the CYL INOX tanks, the fermentation became more sluggish when compared with the fermentations performed in the other vessels.

Several causes for sluggish fermentations during winemaking have been proposed, such as nutrient deficiency (mainly nitrogen limitation but also mineral or vitamin deficiency), a low pH, microbial incompatibility, the presence of inhibitory substances (such as ethanol, medium chain fatty acids, sulfites, toxins, or acetic acid), extreme temperatures, and oxygen deficiency (Alexandre & Charpentier, 1998; Bisson, 1999). Since the grape juice fermented in all of the vessels was the same and all fermentations were performed in the same way, the slower fermentation rate of the CYL INOX tanks could not be attributable to compositional features of the grape juice (deficiency of nutrients or presence of toxic compounds from the grapes). The temperature of grape juices/wines during alcoholic fermentation was monitored, and it ranged between 12 °C and 20 °C for all of the vessels throughout the fermentation trials. Thus, it seemed that extreme temperatures did not explain the differences in fermentative kinetics among the vessels. In contrast, due to the tank geometry and the impermeability of stainless steel, it is possible that the grape juices in the CYL INOX tanks retained more carbon dioxide, and, as a consequence, a lower dissolution of oxygen could take place in this kind of tank. Although alcoholic fermentation is an anaerobic process, it is well known that yeasts need some oxygen to synthesize sterol and fatty acids (survival factors) to regulate plasma membrane permeability and ethanol tolerance (Bisson, 1999; du Toit, Marais, Pretorius, & du Toit, 2006; Ribereau-Gayon, Dubourdieu, Doneche, & Lonvaud, 2006). Hence, when fermenting grape juice is aerated, enhanced yeast growth and viability are observed, especially at the end of alcoholic fermentation (Alexandre & Charpentier, 1998). These data agree with the hypothesis that oxygen deficiency could have delayed the fermentation in the CYL INOX tanks.

3.2. Effect of vessel type on the color and general chemical parameters of wine

No differences in alcohol content were observed among the wines fermented in the different vessels. In contrast, both oval-shaped tanks used in this trial (OVO PE and OVO CONCR) show lower volatile acidity and higher residual sugar levels than those of the other tanks, which could indicate an influence of tank geometry on alcoholic fermentation behavior.

Regarding the volatile acidity of finished wines, it was observed that the CYL INOX wines contained higher amounts of volatile acidity, although it is below their sensory threshold (1.1 g/L of acetic acid equivalents for white wines (Cliff & Pickering, 2006)) and their legacy threshold (1.5 g/L of acetic acid equivalents for dry white wines according to Chilean law (Ley 18.445)). This fact also supports the idea that the CYL INOX fermentations were carried out in the presence of lower amounts of oxygen, since strict anaerobiosis increases acetic acid production by yeasts (Aceituno et al., 2012; Ribereau-Gayon, Dubourdieu, et al., 2006; Ribereau-Gayon, Glories, et al., 2006). Moreover, it has been described that yeasts metabolize a large proportion of the acetic acid secreted in grape juice at the beginning of alcoholic fermentation (via acetyl CoA in the lipid-producing pathways), while they do not metabolize acetic acid during the last stages of fermentation (Ribereau-Gayon, Dubourdieu, et al., 2006; Ribereau-
Gayon, Glories, et al., 2006). Thus, since fermentation in the CYLINOX tanks was longer than that in the other vessels, it seems quite logical that the accumulation of acetic acid in this kind of tank was greater than that in the other tanks.

The color of the finished wines (Table 1) showed some slight significant differences in color intensity as well as in the yellow-blue CIELab coordinate (b*). The wine fermented in the JARCLAY vessels showed a relatively high color intensity, which could indicate phenolic oxidation due to a greater exposure to oxygen during the fermentation process. Similarly, b* was higher for the wines fermented in the JARCLAY and OVO PE vessels, which indicated a greater yellow component in wines fermented in these kinds of vessels. Despite these differences, it should be noted that the color differences (although statistically significant) were very small and therefore not noticeable by the human eye. Thus, it seemed that the vessel type used during fermentation under our conditions had no impact on the color of the finished wines.

### 3.3. Effect of vessel type on the acidity of wine

Changes in pH and titratable acidity (TA) during alcoholic fermentation result from the balance between the formation of weak acids by the yeasts and the precipitation of tartrate salts. In general, TA always decreases during alcoholic fermentation, while the pH could increase or decrease depending on the initial pH of the grape juice. When
the pH is above 3.5, the pH increases due to tartrate precipitation, and when the pH is below 3.5, it decreases during alcoholic fermentation due to tartrate precipitation (Boulton, 1980). Our results agree with this, since the grape juice had an initial pH below 3.5 and both the TA and pH decreased during fermentation.

In view of the obtained results (Table 1), it seems that the vessels used during alcoholic fermentation impacted the pH and TA of the resulting wines. Specifically, the wine fermented in the OVO CNCR vessel showed the highest pH value. In addition, the wine fermented in the OVO CNCR tanks showed the lowest TA value, while the wine fermented in the OVO PE tanks showed the highest TA value. The effect of the vessel type on the acidity of the finished wines was probably related to the material of which the tanks are made, since concrete could release several inorganic compounds into the wine, altering its acidic balance.

To better understand the impact of the vessel type on the acidity of the finished wines, the main organic acids (tartaric acid, malic acid, and citric acid) were determined by HPLC-DAD, and the results are shown in Table 1. The malic acid concentration of the wines was higher than that of tartaric acid. This fact is probably related to the origin of the grapes used for winemaking, which came from a coastal vineyard that is highly influenced by the Pacific Ocean (i.e., the Humboldt Current is very cold at Chile’s latitude), and as such, the vineyard is locate in what is considered a cool-climate valley in Chile. The predominance of malic acid in grape juices from cool-climate vineyards (Ribereau-Gayon, Dubourdieu, et al., 2006; Ribereau-Gayon, Glories, et al., 2006) has been described due to less combustion directly correlated with vineyard temperature during the ripening period.

Wines fermented in the JAR CLAY and OVO CNCR vessels showed lower amounts of tartaric acid. These data seem to indicate a higher precipitation of tartrate salts during alcoholic fermentation and subsequent cold stabilization of these wines, since tartaric acid is not metabolized by yeasts during alcoholic fermentation.

Differences in the malic acid concentration of the finished wines were also significant, with the wine fermented in the CYL INOX tanks showing the highest amount and the wine fermented in the JAR CLAY vessels showing the lowest amount. To our knowledge, malate salt precipitation has not been described to occur during winemaking as does tartrate salts (Chidi, Bauer, & Rossouw, 2018; Volschenk, van Vuuren, & Viljoen-Bloom, 2017). In contrast, malic acid consumption (Ramon-Portugal et al., 1999) and malate production (Yéramian, Vuuren, & Viljoen-Bloom, 2017) have been described in yeasts during alcoholic fermentation. Thus, the small differences observed in the malic acid concentration of wines could be attributed to differences in yeast metabolism during alcoholic fermentation. Although the differences in malic acid concentration were statistically significant, they were quite low (the maximum difference among malic acid concentrations of the wines was 0.17 g/L, which represents a difference of approximately 5% of the malic acid content), and they probably do not have an appreciable sensory impact.

Statistical differences in the citric acid content were also observed among wines fermented in the different vessels. Specifically, wines fermented in the OVO CNCR and JAR CLAY vessels showed higher amounts of citric acid, with 2-fold higher citric acid concentrations than that of the wine fermented in the CYL INOX tanks and 25% more citric acid than that in the wine fermented in the OVO PE tanks. Citric acid is an intermediate of the tricarboxylic acid cycle, and, as such, it is commonly present in nature: citric acid plays a critical role in the metabolism of grape cells as well as yeasts cells (Chidi et al., 2018). Several fermentative conditions impact citric acid production by yeasts: carbon source, nitrogen source, yeast strain, medium pH, fermentation temperature, trace metal ions, and the presence of oxygen (Yalcin, Bozdemir, & Ozhas, 2010). Since the grape juice fermented in all vessels was the same and the yeast starter culture was also the same for all fermentations, the observed differences in citric acid content among the finished wines could not be related to the initial grape juice composition. Considering the fermentative conditions, two different reasons could explain the higher content of citric acid in the wines fermented in the JAR CLAY and OVO CNCR vessels. On the one hand, as mentioned before when fermentation and general parameters of wines were discussed, it could be hypothesized that the JAR CLAY fermentation took place in the presence of a greater amount of dissolved oxygen than that of the CYL INOX fermentation. The role of oxygen as a factor influencing citric acid production has been previously described: the higher the dissolved oxygen content is, the higher the citric acid production by yeasts during alcoholic fermentation (Yalcin et al., 2010). Thus, it seems quite logical that the JAR CLAY wine contained a higher amount of citric acid than that of the CYL INOX wine. On the other hand, the presence of metal ions, such as manganese, iron and zinc, has been found to promote citric acid production by yeasts (Yalcin et al., 2010). As discussed below (elemental composition of wines, Table 2), the concrete of the OVO CNCR vessels released some inorganic compounds into the fermentation medium (by dissolution), including the aforementioned cations, and, consequently, the presence of higher amounts of metal ions in the grape juice fermented in the OVO CNCR vessels could explain the relatively high amount of citric acid in the resulting wine.

### 3.4. Effect of vessel type on the elemental composition of wine

The concrete (OVO CNCR) and clay (JAR CLAY) vessels used in this trial were not sealed (coated with epoxy resins or other polymer), and consequently, the grape juice/wine was in direct contact with the vessel material. It is well known that concrete (Allahverdi & Skvara, 2000) and clay (Jozefaciuk & Bowanko, 2002) can be attacked by acidic solutions (such as grape juice with a pH of 3.4), and, as a result, an enrichment of grape juice/wine with inorganic constituents from the vessel material could take place. To ascertain whether this phenomenon took place during winemaking, the conductivity (Table 1) and the elemental composition (Table 2) of the finished wines were determined.

### Table 2

Elemental analysis of wines determined by means of MP-AES. The results (mean ± standard deviation) are expressed as mg/L, and different letters in a row indicate significant differences (p < 0.05) between vessel types using the Student-Newman-Keuls multiple comparison test.

<table>
<thead>
<tr>
<th>Element</th>
<th>CYL INOX</th>
<th>OVO PE</th>
<th>OVO CNCR</th>
<th>JAR CLAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manganese (Mn)</td>
<td>0.560 ± 0.026ab</td>
<td>0.577 ± 0.032ab</td>
<td>0.623 ± 0.050b</td>
<td>0.623 ± 0.050b</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>0.410 ± 0.105a</td>
<td>0.361 ± 0.036a</td>
<td>0.623 ± 0.050b</td>
<td>0.507 ± 0.021a</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>53.1 ± 6.1 ab</td>
<td>62.2 ± 10.3 b</td>
<td>43.0 ± 3.5 a</td>
<td>53.8 ± 5.2 ab</td>
</tr>
<tr>
<td>Silicon (Si)</td>
<td>27.4 ± 2.3</td>
<td>34.5 ± 11.2</td>
<td>42.7 ± 9.8</td>
<td>29.5 ± 3.3</td>
</tr>
<tr>
<td>Sodium (Na)</td>
<td>20.5 ± 1.1</td>
<td>21.3 ± 2.1</td>
<td>21.5 ± 2.9</td>
<td>19.8 ± 1.4</td>
</tr>
<tr>
<td>Boron (B)</td>
<td>4.20 ± 0.12</td>
<td>4.65 ± 0.55</td>
<td>4.22 ± 0.08</td>
<td>4.01 ± 0.18</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>0.513 ± 0.071 a</td>
<td>0.593 ± 0.029 ab</td>
<td>0.650 ± 0.017 b</td>
<td>0.537 ± 0.050 a</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>0.103 ± 0.040</td>
<td>0.097 ± 0.031</td>
<td>0.070 ± 0.010</td>
<td>0.083 ± 0.032</td>
</tr>
</tbody>
</table>
The conductivity of the wine fermented in the OVO CNCR vessels was statistically greater than that of the wines fermented in all other tested vessels. These data clearly indicate that the OVO CNCR wines contained more ionic compounds than the other wines. Hence, it seems clear that fermenting grape juice on concrete vessels causes the resulting wine to become rich in inorganic ionic compounds. In contrast, no evidence for the enrichment of the wine fermented in the JAR CLAY vessels with ionic compounds was observed.

The elemental composition of the wines are shown in Table 2 and supports the idea that the OVO CNCR wine was enriched with inorganic compounds from the concrete, since the OVO CNCR wine contained the highest amounts of potassium, magnesium, iron, manganese, and zinc. Moreover, the OVO CNCR wine contained more phosphorus than that in the CYL INOX and JAR CLAY wines. Hence, under our conditions, the concrete vessels clearly released inorganic compounds into the wine. In addition to demonstrating the release of inorganic compounds from the concrete to the wine, the elemental composition of the wines can also help to better understand the differences in wine acidity described above.

It is well known that calcium and potassium tartrate salts have low solubility in hydroalcoholic solutions (such as wine) and thus precipitate (Correa-Gorospe, Polo, & Hernandez, 1991; Correa-Gorospe, Polo, Rodriguez-Badiola, & Rodriguez-Clemente, 1991; Vernhet, Pellerin, Belleville, Planque, & Moutounet, 1999). As commented before, the tartaric concentration was lower in the JAR CLAY and OVO CNCR wines than in the CYL INOX and OVO PE wines. These data seem to indicate that higher precipitation of tartrates took place in the OVO CNCR and JAR CLAY wines. The OVO CNCR wine showed the highest potassium amount and the lowest amount of calcium (Table 2), while no significant differences in potassium and calcium content were observed for the JAR CLAY wine when compared with those of the CYL INOX and OVO PE wines. In view of these results, it seems that the wine fermented in the OVO CNCR tanks underwent greater precipitation of calcium tartrate than that of the wines fermented in the other vessels, while no evidence for greater precipitation of potassium tartaric salts was observed. It is possible that the concrete released calcium salts into the grape juice/wine, increasing the calcium ion concentration of the wine and, in turn, increasing the instability and precipitation rate of calcium tartaric salts either during alcoholic fermentation or during the subsequent cold treatment of the wine. Moreover, the increased precipitation of calcium tartrate could justify the aforementioned higher pH value and lower titratable acidity of the OVO CNCR wine than those of the other wines.

It should be noted that the inner surface of the OVO CNCR and JAR CLAY vessels were coated in salts after the fermentation trial; thus, it could be hypothesized that those vessels favored the nucleation of tartaric salts probably because they have a more irregular surface, and the accumulated tartrate crystals on the inner surface of the tank could have acted as seeds for further crystallization. This hypothesis agrees with the lower amount of tartaric acid in the OVO CNCR and JAR CLAY wines than that in the other wines. In contrast, there was no evidence of tartrate deposits on the inner surface of the CYL INOX and OVO PE tanks, which is in accordance with the higher amount of tartaric acid for these wines than that of the other wines considering the aforementioned hypothesis.

3.5. Effect of vessel type on the phenolic composition of wine

The wine fermented in the OVO CNCR vessels showed the highest total phenolic content (I280), as determined spectrophotometrically (Table 1). However, these data are not consistent with the observed low-molecular-mass phenols detected via HPLC-DAD after SPE from the wines (Table 3). Thus, it is possible that the higher absorbance at 280 nm of the wine fermented in the OVO CNCR vessels could have been caused by interference of the inorganic salts released from the concrete into the wines instead of by an increased content of phenolic compounds.

All of the phenolic compounds identified and quantified were hydroxycinnamic acids (and their derivatives), while no flavonols, flavan-3-ols, or benzoic acids were detected. This fact seems to indicate that the concentrations of flavonols and benzoic acids in the wines were very small and were probably below the detection limit of our analytical method. Considering that hydroxycinnamic acids can be found in the flesh of grapes and that other phenolic compounds are mainly found in the seeds and skins (Kennedy, 2006), it is not surprising that our wines contained appreciable amounts of only hydroxycinnamates since the grapes were immediately pressed after they were hand-harvested and transported to the winery. Indeed, hydroxycinnamates have been described as the major phenolic compounds of white wines, which agrees with our results (Kennedy, 2006; Ribereau-Gayon, Dubourdieu, et al., 2006; Ribereau-Gayon, Glories, et al., 2006).

As the JAR CLAY wine showed a slightly deeper color, a lower content of hydroxycinnamates could be expected due to a loss of these compounds by oxidation phenomena. However, no significant differences in the total content of hydroxycinnamic acids (and their derivatives) were found between the wines fermented in the different vessels. Statistical differences were found for some individual compound contents as well as for that of the total content of free hydroxycinnamic acids, tartaric ester derivatives, and ethyl ester derivatives. However, the differences were quite low and probably had no impact on the sensory perception of the wines. Thus, it seems that the shape and material of the vessels used during alcoholic fermentation have a low impact on the phenolic composition of white wines.

3.6. Effect of vessel type on the macromolecular composition of wine

One of the reasons why the use of oval-shaped vessels has been widespread in wineries is the hypothesis that the oval shape favors the formation of convection currents inside the liquid, which, in turn, promotes the movement of suspended solids (from grapes or from yeast lees) within the liquid, enhancing the solubilization of macromolecular compounds. However, no scientific evidence exists to confirm this hypothesis, and physically, it is difficult to explain the generation of convection currents inside the liquid if there is no temperature gradient within it. To address this issue, the turbidity and polysaccharide content of the finished wines were measured.

No significant differences in turbidity were observed between the wines fermented in the different kinds of vessels used in this study (Table 1). Thus, it seems that, at least during alcoholic fermentation, the wines fermented in the oval-shaped vessels were not enriched with higher amounts of suspended solids. However, as shown in Table 1, the wines fermented in the oval vessels (OVO PE and OVO CNCR) required lower doses of bentonite to achieve wine protein stability (determined according to the fast heat test). Therefore, it seems to be that the shape of the vessels could impact the stabilization of white wine proteins.

For wine turbidity, no significant differences were found for the polysaccharide content of the wines fermented in the different vessels or for the total content of polysaccharides of the different quantified size fractions (Table 4). Thus, once again, no evidence of increased macromolecular compound enrichment was found for the wines fermented in the oval vessels under our conditions.

3.7. Effect of vessel type on the volatile composition of wine

The volatile profile of the finished wines was determined by SPME-GC-MS. Forty-five volatile compounds (25 esters, 8 alcohols, 7 terpenes (among them, 5 monoterpenes and 2 sesquiterpenes), 3 acids, 1 aldehyde, and 1 non-megastigmane C13-norisoprenoid) were identified and quantified, and the results can be found in Table 5. In general, significant differences were mainly found for ester compounds among the wines fermented in the different vessels in this study.

Higher alcohols correspond to alcohols with more than two carbon
Table 3
Hydroxycinnamic acid composition of wines. The results (mean ± standard deviation) are expressed as mg/L caffeic acid equivalents. Different letters in a row indicate significant differences (p < 0.05) between vessel types using the Student-Newman-Keuls multiple comparison test.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CYL INOX</th>
<th>OVO PE</th>
<th>OVO CNCR</th>
<th>JAR CLAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid</td>
<td>2.41 ± 0.09 a</td>
<td>2.41 ± 0.02 a</td>
<td>2.59 ± 0.04 b</td>
<td>2.42 ± 0.02 a</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>0.26 ± 0.02 a</td>
<td>0.30 ± 0.01 b</td>
<td>0.28 ± 0.01 ab</td>
<td>0.26 ± 0.00 a</td>
</tr>
<tr>
<td>trans-Coumaric acid</td>
<td>0.84 ± 0.02 a</td>
<td>1.03 ± 0.10 b</td>
<td>0.96 ± 0.04 ab</td>
<td>0.85 ± 0.02 a</td>
</tr>
<tr>
<td>cis-Coumaric acid</td>
<td>0.24 ± 0.05</td>
<td>0.21 ± 0.00</td>
<td>0.21 ± 0.00</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>Caftaric acid</td>
<td>8.63 ± 0.07 b</td>
<td>8.66 ± 0.06 b</td>
<td>8.28 ± 0.06 a</td>
<td>8.92 ± 0.20 c</td>
</tr>
<tr>
<td>Coutaric acid</td>
<td>3.86 ± 0.04 a</td>
<td>3.85 ± 0.02 a</td>
<td>3.78 ± 0.03 a</td>
<td>3.97 ± 0.06 b</td>
</tr>
<tr>
<td>Fentaric acid</td>
<td>0.35 ± 0.02 a</td>
<td>0.39 ± 0.01 b</td>
<td>0.38 ± 0.01 ab</td>
<td>0.38 ± 0.00 ab</td>
</tr>
<tr>
<td>Ethyl Caffeate</td>
<td>0.55 ± 0.03 b</td>
<td>0.53 ± 0.01 ab</td>
<td>0.64 ± 0.02 c</td>
<td>0.50 ± 0.01 a</td>
</tr>
<tr>
<td>Ethyl trans-Coumarate</td>
<td>0.13 ± 0.02</td>
<td>0.11 ± 0.00</td>
<td>0.12 ± 0.00</td>
<td>0.12 ± 0.00</td>
</tr>
<tr>
<td>Ethyl cis-Coumarate</td>
<td>0.27 ± 0.01 a</td>
<td>0.28 ± 0.01 b</td>
<td>0.29 ± 0.00 b</td>
<td>0.25 ± 0.01 a</td>
</tr>
<tr>
<td>Total free hydroxycinnamic acids</td>
<td>3.75 ± 0.10 a</td>
<td>3.94 ± 0.13 ab</td>
<td>4.04 ± 0.07 b</td>
<td>3.74 ± 0.04 a</td>
</tr>
<tr>
<td>Total tartaric ester derivatives</td>
<td>12.84 ± 0.07 b</td>
<td>12.91 ± 0.06 b</td>
<td>12.44 ± 0.09 a</td>
<td>13.26 ± 0.27 c</td>
</tr>
<tr>
<td>Total ethyl ester derivatives</td>
<td>0.95 ± 0.05 b</td>
<td>0.93 ± 0.02 b</td>
<td>1.06 ± 0.02 c</td>
<td>0.87 ± 0.02 a</td>
</tr>
<tr>
<td>Total hydroxycinnamic acids and derivatives</td>
<td>17.54 ± 0.12</td>
<td>17.78 ± 0.19</td>
<td>17.54 ± 0.16</td>
<td>17.88 ± 0.25</td>
</tr>
</tbody>
</table>

(1): High-molecular-weight fraction of polysaccharides (Number average molecular weight Mn = 158.7 ± 2.4 KDa). (2): Medium-molecular-weight fraction of polysaccharides (Number average molecular weight Mn = 34.3 ± 0.6 KDa). (3): Low-molecular-weight fraction of polysaccharides (Number average molecular weight Mn = 16.3 ± 0.6 KDa). (4): Oligosaccharide fraction (Number average molecular weight Mn = 5.9 ± 0.2 KDa).

Table 4
Polysaccharide content of wines determined by means of HRSEC-RID. The results (mean ± standard deviation) are expressed as mg/L. Different letters in a row indicate significant differences (p < 0.05) between vessel types using the Student-Newman-Keuls multiple comparison test.

<table>
<thead>
<tr>
<th>Polysaccharide fraction</th>
<th>CYL INOX</th>
<th>OVO PE</th>
<th>OVO CNCR</th>
<th>JAR CLAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMWf(1)</td>
<td>73.2 ± 0.8</td>
<td>79.0 ± 2.2</td>
<td>76.7 ± 2.5</td>
<td>71.9 ± 5.1</td>
</tr>
<tr>
<td>MMWf(2)</td>
<td>143.3 ± 2.9</td>
<td>145.1 ± 3.4</td>
<td>138.1 ± 6.6</td>
<td>139.1 ± 4.5</td>
</tr>
<tr>
<td>LMWF(3)</td>
<td>204.2 ± 2.5</td>
<td>221.1 ± 4.3</td>
<td>170.0 ± 3.6</td>
<td>203.3 ± 3.5</td>
</tr>
<tr>
<td>OLIGf(4)</td>
<td>40.3 ± 12.5</td>
<td>45.6 ± 4.3</td>
<td>42.0 ± 7.8</td>
<td>39.2 ± 1.6</td>
</tr>
<tr>
<td>TOTAL</td>
<td>277 ± 14.3</td>
<td>291.8 ± 1.7</td>
<td>273.9 ± 0.1</td>
<td>270.5 ± 10.8</td>
</tr>
</tbody>
</table>

(1): High-molecular-weight fraction of polysaccharides (Number average molecular weight Mn = 158.7 ± 2.4 KDa). (2): Medium-molecular-weight fraction of polysaccharides (Number average molecular weight Mn = 34.3 ± 0.6 KDa). (3): Low-molecular-weight fraction of polysaccharides (Number average molecular weight Mn = 16.3 ± 0.6 KDa). (4): Oligosaccharide fraction (Number average molecular weight Mn = 5.9 ± 0.2 KDa).

Esters in wine have two different origins: enzymic esterification during the fermentation process and chemical esterification during long-term aging. Moreover, the same esters may be synthesized in either way (Ribereau-Gayon, Dubourdieu, et al., 2006; Ribereau-Gayon, Glories, et al., 2006). Under our conditions, since long-term aging did not take place, differences in ester composition among the wines was attributed to their differential formation during alcoholic fermentation. As can be observed in Table 6, significant differences were observed for total esters, as well as for total acetate esters and total ethyl esters. Acetate esters and ethyl esters are mainly produced by yeast metabolism through the fatty acid acyl- and acetyl-coenzyme A (CoA) pathways (Boss et al., 2015). Thus, regarding the differences in ester content of the finished wines, it seems that there were differences in yeast metabolism depending on the vessel in which the fermentation took place.

As shown in Table 6, no significant differences among wines fermented in the different tanks were observed for total terpenes, total monoterpenes or total sesquiterpenes. However, both quantified sesquiterpenes (farnesene and nerolidol) showed significant differences, with the wine fermented in the CYL INOX tanks containing the highest concentrations. In addition, significant differences were also found for the α-terpineol content among wines, with the wine fermented in the OVO PE tanks having the lowest concentration. Although some yeast strains can biosynthesize monoterprenoid compounds (Swiegers, Bartowsky, Henschke, & Pretorius, 2005), the terpenes present in wine come mostly from grapes. Thus, given the observed differences in the farnesene, nerolidol, and terpineol contents, it is possible to hypothesize that the fermentation vessel could contribute to modulation of the aromatic character of finished wines, increasing or decreasing the varietal (grape-originated) aromatic compounds of wines.

Finally, to better understand the differences in volatile compounds as a function of the vessel used during alcoholic fermentation, as well as the possible character of the finished wines, the summation of certain volatile compounds as a function of their carbon backbone length is shown in Fig. 2, where it can be observed that the JAR CLAY wine contained lower amounts of C10, C12, and C14 compounds. These data suggest again that the fermentation conducted in JAR CLAY vessels took place with a greater presence of dissolved oxygen since the C10, C12, and C14 compounds are related to the fatty acid metabolism of yeasts and, as discussed above, the lower the dissolved oxygen there is, the higher the release of medium-chain fatty acids (Bisson, 1999; Bisson & Butzke, 2000; du Toit et al., 2006). For the total C9 compound content (i.e., hexyl and hexenyl acetates, hexanoate esters and the respective grape precursors hexanol and hexenol (Boss et al., 2015)), significant differences were observed, with the wine fermented in the OVO PE tanks showing the lowest amount. C9 alcohols (hexanols and hexenols) have been described as compounds that impart characteristic...
herbaceous smells to wines made from unripe grapes (Ribereau-Gayon, Dubourdieu, et al., 2006; Ribereau-Gayon, Glories, et al., 2006). Thus, given that the fermented grape juice was the same for all the tanks, the differences observed in the amount of C6 compounds also seem to indicate that the use of different vessels during alcoholic fermentation could modulate the aromatic character of the finished wines. This hypothesis could be further reinforced by the total C7 compound content of the finished wines: the wines fermented in the CYL INOX and JAR CLAY vessels exhibited the highest concentration of those compounds (largely heptyl acetate and ethyl heptanoate). It has been described that yeasts mainly produce esters with an even number of carbons within their structure, while the production of esters with an odd number of carbon atoms is more common in wine.
odd number of carbons is known to be related to the use of grape precursors by yeasts (Bosset al., 2015).

3.8. Effect of vessel type on the sensory attributes of wine

To understand the impact of the chemical and physical differences observed among the wines on the overall quality of the wine, a sensory analysis of the finished wines was performed. The sensory profiles obtained for the wines fermented in the different tanks are shown in Fig. 3. For the CYL INOX wine, the mouthfeel, sour and fruity characters were the prominent. In the case of the JAR CLAY wine, the tasters highlighted its fruitiness. Regarding the OVO PE wine, bitterness obtained the highest score among the evaluated attributes, while it was determined to be the wine with the lowest varietal typicality. Finally, for the OVO CNCR wine, the tasters highlighted the vegetal aroma of the wine. Although the vegetal character of the OVO CNCR wine was not statistically higher than that of the wines fermented in the other tanks, it should be noted that this wine contained the greatest amount of total C6 compounds (Fig. 2), especially when compared with that in the OVO PE wine, which was described as the least vegetal.

Despite the different sensory profile obtained for each wine, significant differences were only found among wines for two of the nine evaluated attributes: aromatic fruitiness and gustatory bitterness. The wine fermented in the OVO PE tanks was described as the most bitter, while the OVO CNCR wine was described as the least bitter. Regarding this result, it seems that the tank material has a greater influence on wine bitterness than that of its shape. The wines described as the fruitiest were those fermented in the CYL INOX and JAR CLAY vessels, while the OVO CNCR wine was described as the least fruity. Regarding the volatile compound profiles discussed above, the CYL INOX and JAR CLAY wines contained higher amounts of the C7 esters ethyl heptanoate and heptyl acetate. The presence of both compounds have been reported in Cabernet Sauvignon wines (Liang, Chen, Reeves, & Han, 2013). Although its odor thresholds in wine matrix remain unknown, these esters have been described as fruity scents (Clarke & Bakker, 2004) and thus, they may be related to the high fruity character of CYL INOX and JAR CLAY finished wines.

4. Conclusions

In view of the presented results, it could be concluded that the type of vessel used during alcoholic fermentation of white wine has a low impact on final wine color neither their phenolic nor polysaccharide contents. In contrast, greater impact of vessel on wine acidity, elemental composition, volatile compounds content, potential stability, and sensory perception of wines were observed.
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Declaration of Competing Interest

None.

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