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Article

MAKING WINE IN PAÑUL'S CRAFT POTTERY VESSELS: A FIRST APPROACH IN THE STUDY OF THE DYNAMIC OF ALCOHOLIC FERMENTATION AND WINE VOLATILE COMPOSITION

ELABORAÇÃO DE VINHO EM VASILHAS DE CERÂMICA ARTESANAIS DE PAÑUL: UMA PRIMEIRA ABORDAGEM NO ESTUDO DA DINÂMICA DA FERMENTAÇÃO ALCOÓLICA E COMPOSIÇÃO VOLÁTIL DO VINHO

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SUMMARY

Traditional winemaking in amphora-like clay vessels is one of the oldest known methods of wine production. Currently, some wine producers have readopted traditional winemaking methods to generate unique attributes that differentiate their products raising regional wine typicity. The aim of this research was to study the dynamic of alcoholic fermentation and volatile composition of 'Carignan' wines fermented into Pañul's clay vessels and comparing them with the wines vinified into stainless-steel tanks. Density curve of the musts contained in the Pañul's pottery vessels followed a similar trend than in the samples contained in the stainless-steel tanks. The temperatures of the must and the cap during alcoholic fermentation were lower in the Pañul's pottery vessels than in the stainless-steel tanks in most of the evaluated days. Thus, clay vessels may provide temperature-regulating properties beneficing wine fermentation compared to stainless-steel tanks. Pañul's clay vessels produced wines with higher terpenes, β-ionone and 2-phenylethyl alcohol content, and lower values of some individual higher alcohols, isoamyl acetate, lactones, and pH than the stainless-steel tanks. Therefore, the results suggest that Pañul's pottery vessels favored increasing the terpene alcohols and other volatile compounds concentrations, in addition to decreasing certain higher alcohols and acetate esters contents such as benzyl alcohol and iso-amyl acetate. These outcomes may be of interest to ceramic producers and wine producers since they open a range of economic opportunities to diversify their products.

RESUMO

A vinificação tradicional em vasilhas de barro tipo ânfora é um dos mais antigos métodos conhecidos de produção de vinho. Atualmente, alguns produtores de vinho têm readaptado métodos tradicionais de vinificação para gerar atributos únicos que diferenciam os seus produtos, elevando a tipicidade do vinho regional. O objetivo desta investigação foi estudar a dinâmica da fermentação alcoólica e a composição volátil de vinhos 'Carignan' fermentados em vasilhas de barro da Pañul e compará-los com os vinhos vinificados em depósitos de aço inoxidável. A curva de densidade dos mostos contidos nas vasilhas de cerâmica do Pañul seguiu uma tendência semelhante à das amostras contidas nos depósitos de aço inoxidável. As temperaturas do mosto e da manta durante a fermentação alcoólica foram mais baixas nas vasilhas da Pañul do que nos depósitos de inox na maioria dos dias em que foi efetuada testagem. Assim, as vasilhas de barro podem fornecer propriedades reguladoras da temperatura, que beneficiam a fermentação do vinho em comparação com os depósitos de aço inoxidável. As vasilhas de barro de Pañul produziram vinhos com teores mais elevados de terpenos, de β-ionona e de 2-feniletanol, bem como teores mais baixos de alguns álcoois superiores individuais, acetato de isoamilo, lactonas e pH do que os depósitos de aço inoxidável. Portanto, os resultados sugerem que as vasilhas de cerâmica de Pañul favoreceram o aumento da concentração de álcoois terpenos e outros compostos voláteis, diminuindo também o teor de certos álcoois superiores e ésteres de acetato, designadamente o álcool benzílico e o acetato de isoamilo. Estes resultados podem ter interesse para produtores de cerâmica e para produtores de vinho, na medida em que abrem um leque de oportunidades económicas no sentido da diversificação dos seus produtos.

Keywords: winemaking, clay, pottery vessels, Pañul's appellation of origin.

Palavras-chave: vinificação, argila, vasilhas de cerâmica, denominação de origem Pañul.

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INTRODUCTION

Clay minerals have been part of the human existence since antiquity and have been used along history for industrial and commercial uses (Yuan, 2004). Recent discoveries have made it possible to point out that almost every ancient culture, from the Canaanites to the Egyptians, Assyrians, Greeks and Romans, vinified in pottery vessels (McGovern *et al*., 2017). Traditional winemaking in amphora-like clay vessels is one of the oldest known methods of wine production (McGovern *et al*., 2017). "Qvevris" is one example of this, and it was originated in current territories of Georgia in which vessels are buried in the ground to provide natural temperature control during fermentation (Díaz *et al*., 2013a). Another example of this are the "Vinhos de Talha" that are typical of Alentejo, in the south of Portugal (Martins *et al*., 2018). In this traditional method, crushed berries are fermented in clay vessels, regardless of being white, red or a mixture of both grapes, with a minimalist intervention, resulting in white, red or "palhete" wines (Martins *et al*., 2018). Currently, some wine producers and winegrowers have readopted traditional winemaking methods to generate unique attributes that differentiate their products, increasing the diversity of complex flavors that characterize traditional wine typicity (Díaz *et al*., 2013b; del Álamo-Sanza and Nevares, 2017; Montalvo *et al*., 2021). In addition, vessels have a relatively low capacity and allow winemakers to produce single or premium wines by using small batches of high-quality grapes as raw material (Gil i Cortiella *et al*., 2020, 2021). This opens a range of economic opportunities for small producers linked to the wine and pottery industry to diversify their products.

Clay vessels hold porous materials throughout their structure that allow oxygen to permeate towards the contained wine, resulting in a slow rate of wine oxygenation similar to that occurred is wooden barrels used to make wine (del Álamo-Sanza and Nevares, 2017; Gil i Cortiella *et al*., 2020, 2021; Nevares and del Álamo-Sanza, 2021). Oxygen addition at the yeast exponential growth phase during alcoholic fermentation stimulates its rate (Day *et al*., 2015), and the shape and composition of the tank may alter its kinetics. Alternatively, non-oxygenated fermentations provided wines with higher concentration of volatile sulfur compounds, and lower concentration of fermentation products and differences in the concentration of metals than oxygenated fermentations (Bekker *et al*., 2016). During fermentation, oxygen affects the formation of volatile compounds, mainly of esters, higher alcohols, medium-chain fatty acids, branched acids, aldehydes, and ketones (Pozzatti *et al*., 2020; Tarko *et al*., 2020). Oxygen influences lipid composition of the yeast cell, increasing membrane unsaturation index and membrane fluidity (Varela *et al*., 2012). In this way, the concentration of flavor active compounds is affected by the concentration of substrates, which is influenced by yeast growth and environmental conditions (Varela *et al*., 2012). Must oxygenation increases the concentration of ethyl and acetate esters as well as butyl lactate and hexyl lactate resulting in the intensification of fruity aroma (Houtman *et al*., 1980; Tarko *et al*., 2020), however, the research on this subject present some contradictions (Day *et al*., 2015). To our knowledge, there is little available information in the scientific literature that addresses the impacts of clay vessels on fermentation dynamic and wine volatile composition, and much less in vessels made of clays with territorial identity. Thus, it was hypothesized that the wines vinified into craft clay vessels present different fermentation dynamic and content of volatile compounds, reaching higher complexity, than wines made in stainless-steel tanks. Therefore, the aim of this work was to study dynamic of alcoholic fermentation and volatile composition of wines vinified into Pañul's clay vessels (pottery with appellation of origin in Chile) and comparing them with the wines vinified into stainless-steel tanks.

MATERIALS AND METHODS

Characterization of Pañul's pottery vessel used in this trial

Pañul's pottery vessels were hand-made elaborated by a craftsman with plastic clay from a deposit whose mineralization age belongs to the Neogene period (Gajardo and Gutiérrez, 1992; López *et al*., 2004). The deposit is in Pañul, Pichilemu, O'Higgins Region (34º49'S; 71º96' W, 140 meters above sea level). The selected clays are found under the Pañul's pottery origin of appellation, which have plastic and refractory properties that make them ideal for industrial use due to their high resistance (Carrasco *et al*., 2003; Jórdan and Pardo, 2016; Lacoste *et al*., 2017). This origin of appellation arises from a combination of the natural elements of the territory and the peasant knowledge built over the years by the local artisans (Lacoste *et al*., 2017). This pottery is made from three different clays, obtained in the Cordillera de la Costa and combined with each other through their own technique (Lacoste *et al*., 2017). Pañul pottery is distinguished and characterized by its damask beige color, with a burnished surface that provides a soft texture (Such and Borjas, 2019). The potteries are also monochrome and smooth, and they have no decoration as is shown in Figure 1.

The clay fraction was composed of kaolinite, quartz, illite, montmorillonite, vermiculite, and potassium feldspar in a balanced mixed (Lacoste *et al*., 2017). The kaolin fraction corresponded to a combination of quartz, orthoclase, hematite, kaolinite, illite and muscovite (Lacoste *et al*., 2017). Pañul's pottery vessels were hand-made elaborated by a local craftsman and the elaboration of each one of these took about 6 months, depending on the environmental conditions. The clays that arrived from the deposit were stored in an open place for their collection. Then, a portion of clays was taken for the elaboration of the vessels, and they were wet to prepare a paste. The preparation of the paste was carried out from a complex classification of clay and kaolin size by sieves using an auger filler craft machine. The paste obtained was carried in a wheelbarrow to be molded. The molding was performed mainly by casting in plaster molds. The obtained vessels were dried in an open place for 2 to 4 months depending on the climatic conditions of the season. To achieve a good finish of the pottery, an abundant sanding was carried out, which allowed to define its character from the aesthetic point of view. The polishing process provided the surface smoothness and resistance that ended with the closure of the pores of the paste, reducing its absorption and increasing its resistance in general. Molding was performed by two people, and it took place between 25 to 40 days depending on the weather conditions of the season. The firing was made in wood-fired ovens (built by the artisans themselves) at 700-1000 ºC for 10 days, using pine wood as fuel.

Figure 1. Shape of the vessels for winemaking. (a) Pañul's pottery vessels used for vinification in this trial. (b) Pañul's clay vessels used for wine aging.

Research location and plant material selected

The field trial was conducted at the facilities of the University of Talca (Colchagua Campus) (34º 38'S, 71º21'W, 179 meters above sea level; Santa Cruz, O'Higgins region, Chile) in the 2015 season (Figure 1a). It was selected 'Carignan' grapes to be vinified into the Pañul's pottery vessels due to its high aging enological potential in terms of pH and phenolic composition (Gutiérrez-Gamboa *et al*., 2018b). The selected 'Carignan' vineyard was in La Patagua, Santa Cruz, Chile and the grapes were provided by small winegrowers belonging to the Colchagua's Wine Network (Santa Cruz, O'Higgins, Chile). Berry samples were collected weekly from berries pea-size stage for monitoring grape technological maturity and performing the harvest. Grapes were harvested when the content of soluble solids was close to 24 °Brix, titratable acidity remained approximately 4 g/L (sulfuric acid) and the pH was between 3.4 and 3.5.

Vinifications

After the harvest, the grapes were immediately destemmed and crushed to obtain the must which was protected by adding 50 mg/kg of sulfur dioxide. Before using the clay vessels, they were filled with an aqueous solution of tartaric acid (3% v/v) for at least 24 h. The must obtained was weighted and introduced into three stainless-steel tanks (control) and into three Pañul's clay vessels, accounting three replicates for the treatment and control. Stainless-steel tanks consisted of open top cylindrical tanks that has floating lid enables storage of various wine quantities. The treatments were defined as follow; a) Stainless-steel tank: the must was vinified into three stainless-steel tanks; b) Pañul clay vessel: the must was vinified into three Pañul clay vessels. The six deposits were filled approximately with 250 kg of must each one. The treatments were made in triplicate and distributed as a complete randomized design. The deposits were randomly arranged into the wine cellar.

The must was co-inoculated with the commercial yeast *Saccharomyces cerevisiae* strain BO 213 (Laffort, Bordeaux Cedex, France) and with the commercial *Oenococcus oeni* bacterium strain (B28 PreAc, Laffort, Spain) to carry out the alcoholic-malolactic fermentation, which took place at room temperature. This condition allowed knowing the thermal control capacity of each container. To monitor the development of alcoholic fermentation once a day, a hydrometer was used to quantify must density and a thermometer was used to measure cap and must temperature. The temperature of the must and the cap was measured by introducing a laboratory thermometer and was daily monitored in tanks and clay vessels. The alcoholic-malolactic fermentation was considered finished when the wine fermented reached less than 2.5 g/L of residual sugar and less than 100 mg/L of malic acid. The malolactic fermentation finished one week after the end of the alcoholic fermentation. Subsequently, after ten days of maceration–fermentation, the skins and seeds were manually removed, and then the tanks were brought to cold to eliminate the lees. Physicochemical parameters of the wines were determined. Thereafter, analysis of volatile compounds of wine samples from each replicate was carried out.

Determination of physicochemical parameters of wines

The wine classical parameters, such as alcoholic strength, pH, titratable acidity (g/L sulfuric acid) and volatile acidity (g/L acetic acid), total and free sulfur dioxide and reducing sugars were determined according to the OIV methods (OIV, 2003, 2009). Malic acid was analyzed by automated enzymatic test (Biowine 300, Biolan, Bilbao, Spain). Three replicates were obtained from each treatment and control, so the results were the average of three analyses (n = 3).

Analysis of wine volatile compounds by SPE-GC-MS

The volatile compounds of wines were analyzed according to the methodology reported by López *et al*. (2002) with some minor modifications (Gutiérrez-Gamboa *et al*., 2018b; Carrasco-Quiroz *et al*., 2020). Pre-packed cartridges (total volume 3 mL) filled with 200 mg of LiChrolut EN resin (Merck, Darmstadt, Germany) were used for volatile compounds extraction. Before passing the wine through the cartridge, 500 µL of internal standard (2-octanol) were added. The separation, identification and quantification of wine volatile compounds were carried out using an Agilent 7890A gas chromatograph, coupled with a 5975C mass spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA). The unit was equipped with a fused silica capillary column (30 m × 0.25 mm i.d., and 0.5 µm phase thickness, DB-Wax, J & W Scientific, Agilent). The carrier was helium at a flow rate of 1 mL/min. The temperature of the injector was 250 ºC and 2 µL of wine extract were injected. The oven temperature was initially held at 40 ºC for 5 min, then increased linearly at a rate of 2 ºC/min up to 130 ºC and held at that temperature for 5 min; after that, temperature was again increased at a rate of 2 ºC/min up to 180 ºC and held at that temperature for 2 min; finally, the temperature was increased linearly at a rate of 4 ºC/min up to 230 min. The analysis was carried out with two injections: in split mode (50:1) for isoamyl alcohols, benzyl alcohol, 2-phenylethanol, 1-hexanol, ethyl hexanoate, and ethyl octanoate; and in splitless mode (60 min) for the rest of volatile compounds analyzed. Ionization was carried out by electron impact at 70eV. The operating method was full scan mode at *m*/*z* between 30 and 300. Identification was carried out using the NIST library (NIST 08 Software Update). When standards were available, the quantification was based on seven-point calibration curves of the respective standards (Sigma-Aldrich, Steinheim, Germany) (R^2> 0.93) in a 12% (v/v) ethanol solution with 6 g/L of tartaric acid at pH 3.2; otherwise, semi-quantitative analyses were carried out using the calibration curves of the most similar compound. In this way, semi-quantitative analyses were carried out using the calibration curves of the most similar compound for 1-butanol, 3-methyl-3-buten-1-ol, 1-pentanol, 4-methyl-1-pentanol, 3-methyl-1-pentanol, 3-ethoxy-1-propanol, methionol, homovanillyl alcohol, ethyl butanoate, ethyl lactate, ethyl 3-hydroxybutyrate, diethyl succinate, ethyl hexadecanoate, butanoic acid, octanoic acid, decanoic acid, ϒ-nonalactone and 3-hydroxy-2-butanone.

The contribution of volatile compounds to the wine aroma was assessed using the odor activity value (OAV), and was calculated as the ratio between the concentration of the individual compound and the perception threshold found in the literature for the same matrix (Cai *et al*., 2014; Gutiérrez-Gamboa *et al*., 2018a,b).

Statistical analysis

The statistical analyzes on must density, cap and must temperature, physicochemical parameters and volatile compounds were based on variance analysis (one-way ANOVA) using Statgraphics Centurion XVI.I. Differences between samples were compared using the Duncan test at 95% probability level. The treatments were performed in triplicate, so the results of must density, cap and must temperature, physicochemical parameters, and wine volatile compounds were shown as the average of three analyzes (n = 3).

RESULTS AND DISCUSSION

Evolution of must density during fermentation

Must density ranged from 1.100 to 0.995 in the samples contained into Pañul's pottery vessels and it varied from 1.100 to 0.993 in the musts vinified into stainless-steel tanks (Figure 2). Data about fermentation dynamics of musts were very interesting since the density curve of the musts contained in the Pañul's pottery vessels followed a similar trend compared with the samples within the stainless-steel tanks (Figure 2). Besides, the duration of alcoholic fermentation took the same number of days in the Pañul's pottery vessels and stainless-steel tanks regarding must density and sugar consumption by the yeasts (Figure 2). These results contrasted to those reported by Gil i Cortiella *et al*. (2020), who showed that alcoholic fermentation ended five days later in stainless-steel tanks than in clay vessels.

These differences can be ascribed to the fact that the wines were fermented into 225 L clay jars and into 150 L stainless-steel tanks.

Evolution of must temperature during fermentation

Cap temperature ranged from 18 to 26 ºC in the samples contained into Pañul's pottery vessels, and it varied from 18 to 30 ºC in the musts vinified into stainless-steel tanks (Figure 3a). Since the 5 day until the 7 day of alcoholic fermentation, cap temperature was higher in stainless-steel tanks than in Pañul's pottery vessels (Figure 3a). Must temperature ranged from 18 to 23 ºC in the samples contained into Pañul's pottery vessels, and it varied from 18 to 26 ºC in the musts vinified into stainless-steel tanks (Figure 3b). Since the 4 day until the 7 day of alcoholic fermentation, must temperature was higher in stainless-steel tanks than in Pañul's pottery vessels (Figure 3b). The temperatures of the must and the cap during alcoholic fermentation development were lower in the Pañul's pottery vessels than in the stainless-steel tanks, mostly in the middle step of alcoholic fermentation (Figure 3). Thus, it can point out the temperature-regulating properties of clay as one of its primary benefits to wine fermentation compared to stainless-steel tanks.

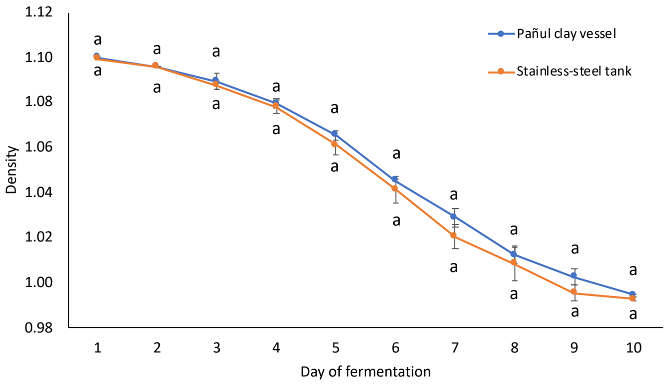

Figure 2. Evolution of must density of the samples vinified into Pañul's pottery vessels and stainless-steel tanks. Data are the overall mean value ± standard error (SE) of treatments (n = 3 replicates by treatment and control).

Clay firing temperature can influence the physical and mechanical properties, such as color, density, porosity, permeability, wave velocity, strength and compactness, elastic modulus and thermal properties of the vessels (Han *et al.*, 2017; Geng and Sun, 2018). Thermal conductivity and diffusivity as well as the bulk density of clay decrease as the firing temperature increases from room temperature to 200 °C, and then become approximately constant (Geng and Sun, 2018). These conditions could provide different levels of thermal conductivity to the clay vessels, favoring cooler alcoholic fermentations than those occurring in stainless-steel tanks.

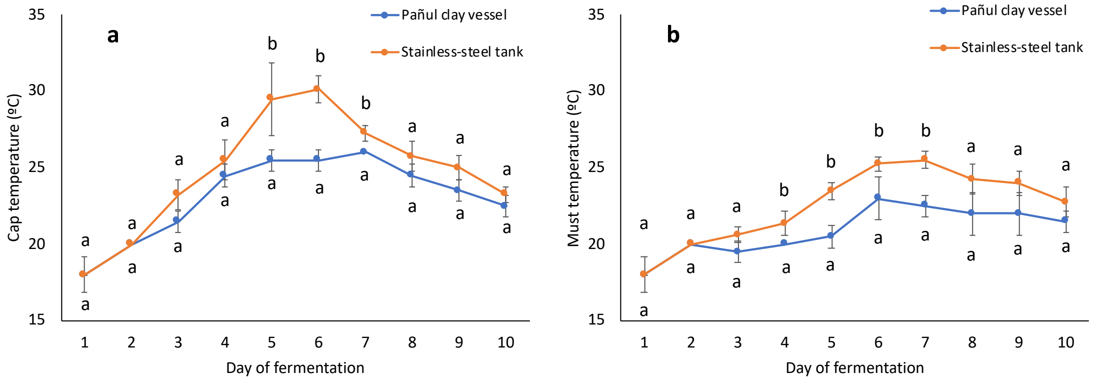

Figure 3. Evolution of (a) cap and (b) must temperature in the samples vinified into Pañul's pottery vessels and stainless-steel tanks. Data are the overall mean value ± standard error of treatments (n = 3 replicates by treatment and control).

Physicochemical parameters of wines

The wines fermented into Pañul's pottery vessels and stainless-steel tanks did not show significant differences in the alcoholic strength, total acidity, and volatile acidity (Table I). The wines fermented into Pañul's pottery vessels presented significantly lower pH than the wines fermented into stainless-steel tanks. Despite these results, the difference on pH is minimal, and from an oenological point of view it probably did not have an important influence on wine quality.

Table I

Physicochemical parameters of wines obtained from Pañul's pottery vessels and stainless-steel tanks

	Alcohol degree (% v/v)	pH	Total acidity (g/L)[1]	Volatile acidity (g/L)
Stainless-steel tank	13.8±0.1a	3.47±0.01b	3.78±0.03a	0.37±0.04a
Pañul's pottery vessel	13.8±0.1a	3.44±0.01a	3.76±0.03a	0.36±0.04a

[1] g sulfuric acid/L. Data are the overall mean value ± standard error of treatments (n = 3 replicates by treatment and control). For each parameter, different letters within a column represent significant differences (Duncan's test $p<0.05$).

Wine volatile compounds

Pañul's pottery vessels produced wines with higher content of limonene, geraniol, β-ionone, 1-hexanol, phenylethyl alcohol, ethyl lactate, ethyl 3-hydroxybutyrate, ethyl hexadecanoate and ϒ-nonalactone than the stainless-steel tanks (Table II). Contrary to these results, Gil i Cortiella *et al*. (2021) reported that the wines fermented and aged in clay vessels showed lower content of some varietal volatile compounds, such as *trans*-nerolidol, *cis-β*-farnesene and linalool formate than the wines fermented on stainless-steel tanks. However, Issa-Issa *et al.* (2021) showed that acids and terpenes were the most abundant families of volatile compounds presented in wines aged in clay vessels, mainly of limonene. Therefore, it is likely that these differences are due to the grapevine variety, however, to date there are still few studies in this regard.

The wines fermented into Pañul's pottery vessels presented lower content of propanol, 1-butanol, 3-hexen-1-ol isomer 1, 3-ethoxy-1-propanol, methionol, benzyl alcohol, homovanillyl alcohol, isoamyl acetate, ethyl hexanoate, hexanoic acid, butyrolactone and 3-hydroxy-2-butanone than the wines fermented on stainless-steel tanks (Table II). Odor activity values (OAVs) showed that limonene, β-ionone, propanol, methionol, phenylethyl alcohol, isoamyl acetate, ethyl hexanoate, hexanoic acid and butyrolactone contributed to wine aroma (Table II). Clay vessels produced wines with higher limonene, geraniol, β-ionone and 2-phenylethyl alcohol content than the stainless-steel tanks. Similarly, Gil i Cortiella *et al*. (2020) showed that the wines elaborated into clay vessels presented lower content of isoamyl acetate than the wines fermented on stainless-steel tanks. The acetate esters biosynthesis by yeasts during wine alcoholic fermentation has been widely studied and related to the activity of acetyltransferases (Plata *et al*., 2003). Plata *et al*. (2005) reported that oxygen inhibited acetyltransferase activity, to a greater extent for isoamyl acetate than for ethyl acetate. Han *et al*. (2017) reported that when the firing temperature of clay rises, the water content decreases and porosity increases. Furthermore, thermal conductivity displays a good linearity with mass loss and a negative exponent with porosity (Han *et al*., 2017). Therefore, the firing temperature allows to make clay vessels with different levels of porosity, affecting gas exchange, oxygen transfer in wines, and by consequence, their isoamyl acetate content.

Total wine volatile composition

Pañul's pottery vessels produced wines with higher content of total terpenes and lower content of total lactones than the stainless-steel tanks (Figure 4). Must fermentation allows the release of terpenoid compounds from their glycosides by yeast and grape glycosidase enzymes (Black *et al*., 2015). At wine cellar, alcoholic fermentation at low temperature is a common practice in white wine elaboration that allows to improve wine aromatic profile by high formation and retention of aromas (Singleton *et al*., 1975). Results found in literature reveal that low temperature favors yeast viability and lengthen the fermentations increasing free terpenes and ethyl esters concentration in Torrontés Riojano wines (Pérez *et al*., 2018). On the contrary, Massera *et al*. (2021) showed that low temperature fermentation was correlated with high esters and low terpene in 'Merlot' wines. On the other hand, low pH increases the rate of ester hydrolysis and the rate of glycoside hydrolysis as well as the rate of isoprenoid rearrangements observed in the resulting aglycone intermediates (Waterhouse *et al*., 2016).

Table II

Volatile compounds content and odor activity values (OAV) of the wines fermented into Pañul's pottery vessels and into stainless-steel tanks

	Steel	**Clay**	**OAV-St**	**OAV-Cy**	**Aromatic descriptor**
Terpenes (μ/L)					
Limonene	44.08±1.69a	53.17±4.55b	2.94	3.54	Fruity, lemon
Linalool	8.72±0.42a	7.98±0.61a	0.58	0.53	Floral and fruity
Citronelool	2.30±0.13a	2.65±0.31a	0.02	0.03	Herbaceous, lemon, species
Geraniol	5.64±0.42a	7.29±0.19b	0.19	0.24	Citric, geranium
C_{13}norisoprenoids (μ/L)					
β-Ionone	2.24±0.20a	3.04±0.10b	24.91	33.81	Pink, purple, balsamic
Higher alcohols (mg/L)					
Propanol	11.65±0.51b	9.51±0.13a	14.03	11.46	Alcohol, ripe fruit
Isobutanol	32.09±1.15a	32.14±0.11a	0.80	0.80	Herbaceous, bitter, alcohol, cloves
1-Butanol	8.35±0.05b	6.31±0.25a	0.06	0.04	Medicinal, alcohol
Isoamyl alcohols	192.26±34.68a	195.53±19.08a	6.41	6.52	Alcohol, burn
3-Methyl-3-buten-1-ol	0.02±0.00a	0.02±0.00a	0.03	0.03	Sweet fruits
1-Pentanol	1.60±0.00a	1.87±0.26a	0.02	0.03	Almond, balsamic, alcoholic
4-Methyl-1-pentanol	1.36±0.09a	1.45±0.20a	0.03	0.03	Almond, toasted
3-Methyl-1-pentanol	7.00±0.06a	7.24±0.26a	14.00	14.48	Earth, mushrooms, cheese, herbaceous and
1-Hexanol	2.22±0.09a	2.91±0.14b	0.28	0.36	Herbaceous, cut grass, wood, spices
3-Hexen-1-ol isomer 1	0.07±0.00b	0.04±0.00a	0.17	0.1	Herbaceous, grass, cypress
3-Hexen-1-ol isomer 2	0.11±0.01a	0.10±0.00a	0.27	0.26	Herbaceous, cut grass
3-Ethoxy-1-propanol	0.22±0.01b	0.03±0.00a	2.24	0.30	Fruity
Methionol	0.79±0.03b	0.64±0.05a	1.59	1.27	Garlic, raw potato, cooked vegetables
Benzyl alcohol	0.24±0.01b	0.13±0.00a	0	0	Almond, citrus
Phenylethyl alcohol	76.90±6.34a	94.05±2.54b	7.69	9.4	Floral, pollen roses, scented
Homovanillyl alcohol	0.10±0.00b	0.08±0.00a	NC	NC	Not found
Acetate esters (mg/L)					
Isoamyl acetate	0.20±0.00b	0.18±0.01a	6.50	5.83	Banana, fruity
Phenylethyl acetate	0.61±0.03a	0.57±0.02a	2.42	2.28	Roses, floral
Ethyl esters (mg/L)					
Ethyl butanoate	0.06±0.00a	0.06±0.00a	2.96	2.93	Fruity, banana, pineapple and strawberry
Ethyl hexanoate	0.32±0.01b	0.28±0.02a	22.91	20.21	Green apple, fruity, strawberry, anise
Ethyl lactate	0.58±0.02a	0.61±0.02b	0	0	Lactic, medicinal
Ethyl octanoate	0.46±0.01a	0.45± 0.02a	91.25	90.22	Floral, pear, pineapple, anise, fruity
Ethyl 3-hydroxybutyrate	0.05±0.00a	0.06±0.00b	0	0	Caramel, toasted
Ethyl decanoate	0.24±0.00a	0.25±0.00a	1.18	1.22	Fruity
Diethyl succinate	1.03±0.12a	0.93±0.01a	0.01	0	Vinous
Ethyl hexadecanoate	0.10±0.01a	0.16±0.00b	0.07	0.11	Fatty, rancid, fruity
Volatile fatty acids (mg/L)					
Butanoic acid	0.73±0.00a	0.74±0.00a	4.21	4.25	Cheese, fatty, rancid
Hexanoic acid	1.81±0.11b	1.63±0.06a	4.33	3.89	Cheese, rancid, fatty
Octanoic acid	1.42±0.11a	1.56±0.06a	2.84	3.12	Hardness, cheese, fatty acids, rancidity
Decanoic acid	0.80±0.00a	0.83±0.05a	0.80	0.83	Greasy, unpleasant
Lactones (mg/L)					
Butyrolactone	5.73±0.45b	3.25±0.04a	163.67	92.76	Caramel, toasted
ϒ-Nonalactone	0.01±0.00a	0.02±0.00b	0.24	0.64	Coconut
Other volatile compounds					
Phenylacetaldehyde	0.64±0.04a	0.69±0.00a	639.71	688.62	Floral, honey
3-Hydroxy-2-butanone	0.17±0.00b	0.10±0.00a	0.01	0	Fruity, wood, moldy, buttery

Cy: Pañul's pottery vessels. St: stainless-steel tanks. NC: not calculated. OAV was calculated from the odor thresholds (μg/L) reported by different authors (Cai *et al*., 2014; Gutiérrez-Gamboa *et al*., 2018a,b). Aromatic descriptors were obtained from these above-mentioned references. Data are the overall mean value ± SE of treatments (n = 3 replicates by treatment and control). For each parameter, different letters within a column represent significant differences (Duncan's test $p \leq 0.05$). Semi-quantitative analyses were carried out using the calibration curves of the most similar compound for 1-butanol, 3-methyl-3-buten-1-ol, 1-pentanol, 4-methyl-1-pentanol, 3-methyl-1-pentanol, 3-ethoxy-1-propanol, methionol, homovanillyl alcohol, ethyl butanoate, ethyl lactate, ethyl 3-hydroxybutyrate, diethyl succinate, ethyl hexadecanoate, butanoic acid, octanoic acid, decanoic acid, ϒ-nonalactone and 3-hydroxy-2-butanone.

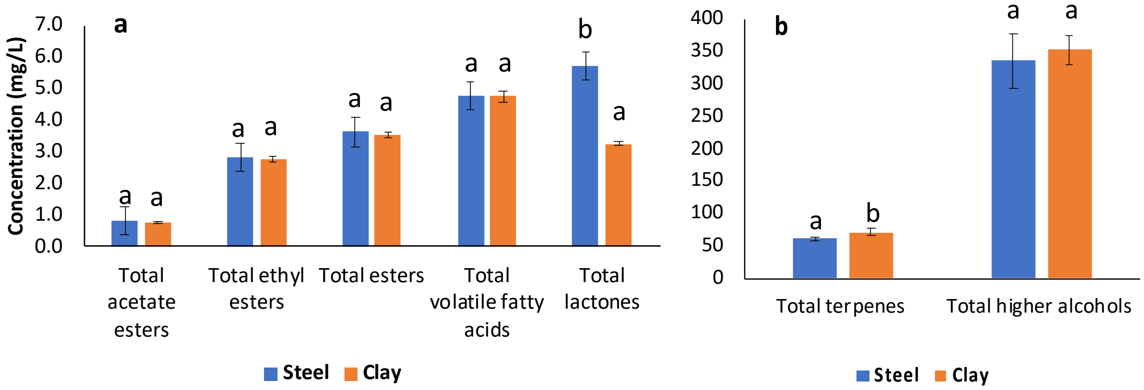

Figure 4. Total concentration of volatile compounds of (a) ethyl acetates (mg/L), ethyl esters (mg/L), esters (mg/L), volatile fatty acids (mg/L), lactones (mg/L); (b) higher alcohols (mg/L) and terpenes (µg/L) of the wines fermented into Pañul's pottery vessels and stainless-steel tanks.

In another perspective, clay vessels may improve wine oxygenation which results in an increase in terpene alcohols and in a general decrease in ethyl and acetate esters, particularly in the content of ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate and isoamyl acetate (Picariello *et al.*, 2020). Must aeration has been shown to improve the production of certain terpene alcohols during yeast sterol biosynthesis (Fujii *et al.*, 1997). Regarding esters, the results are in accordance with those exposed in other studies and in this trial, suggesting that it is possible to increase the production of desirable flavor compounds, such as acetate and esters, by decreasing the availability of oxygen (Moio *et al.*, 2004; Shekhawat *et al.*, 2016). The release of ethyl esters is linked to yeast metabolism of unsaturated fatty acids, and their synthesis is stopped under low oxygen availability (Fujii *et al.*, 1997; Picariello *et al.*, 2020). Moreover, isoamyl acetate decreases as a result of decreased production of alcohol acetyltransferase due to aeration (Fujii *et al.*, 1997). Based on these results, it seems that Pañul's pottery vessels increase must aeration, enhancing the concentration of terpene alcohols, β-ionone and 2-phenylethyl alcohol, also decreasing the content of certain higher alcohols and acetate esters such as benzyl alcohol and isoamyl acetate

CONCLUSIONS

The Carignan musts contained in the Pañul's pottery vessels presented a similar density curve than the stainless-steel tanks. Must and cap temperature during alcoholic fermentation were lower in the Pañul's pottery vessels than in the stainless-steel tanks in most of the evaluated days. For this reason, clay vessels may probably provide temperature-regulating properties compared with stainless-steel tanks. Pañul's clay vessels produced wines with higher levels of terpenes, β-ionone and 2-phenylethyl alcohol, and lower values of some individual higher alcohols, isoamyl acetate, lactones, and pH than the stainless-steel tanks.

Thus, the possible underlying causes (aeration and temperature) modulated by type of vessel and their effects on wine volatile composition are plausibly explained, but naturally remain speculative, since the trial was carried out only in one season and one variety. At last, the revalue of the vulnerable territories that produce clay should be a relevant issue for the future economic diversification of the wine industry.

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