



# Assessment of colour and tannin extraction in Tempranillo and Cabernet-Sauvignon using small-scale fermentation vessels

 Antoni Sánchez-Ortiz<sup>1,\*</sup>, Montserrat Nadal-Roquet Jalmar<sup>1</sup>, Miriam Lampreave-Figueras<sup>1</sup> and Josep Maria Mateo-Sanz<sup>2</sup>

<sup>1</sup> Departament de Bioquímica i Biotecnologia. Facultat d'Enologia de Tarragona. Universitat Rovira i Virgili. Campus Sescelades, Marcel·lí Domingo, s/n, 43007 Tarragona, Spain

<sup>2</sup> Departament d'Enginyeria Química. Escola Tècnica Superior d'Enginyeria Química. Universitat Rovira i Virgili, Av. Paisos Catalans 26, 43007, Tarragona, Spain

 \*corresponding author: antonio.sanchezo@urv.cat

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## ABSTRACT

Researchers typically perform winemaking experiments using small volumes of grapes. This study examined which small-vessel volume (10, 25, 50 and 100 L) gives better repeatability during red winemaking extraction of colour and tannin in research studies. Few studies have actually evaluated the repeatability of small-scale fermentations using two varieties of different phenolic potential: Tempranillo and Cabernet-Sauvignon. We investigated how volume size may affect the composition of colour and tannins for these two varieties and result in potentially different phenolic contents. Furthermore, for each variety, we compared the small scale vessel with a commercial fermentation using a 2.500 L capacity. 50 L tanks resulted in optimum extraction of phenols and colour. High repeatability was observed for alcohol content, pH and total acidity, anthocyanins, and procyanidins for both varieties amongst vessel sizes. Kinetics of fermentation performed faster in big berry driven grapes (Tempranillo) regardless of the volume. Instead, for small berry grapes (Cabernet-Sauvignon), vessel size affected the kinetics of fermentation and therefore the extraction can be altered. Very high repeatability for the alcohol by volume, pH and total acidity ( $CV \leq 7\%$ ) as well as anthocyanins and procyanidins by HPLC ( $15\% \leq CV \leq 20\%$ ) for both varieties in all volume sizes. This research provides a solid basis for validating the reproducibility of small-scale fermentations of two red grapevines with different phenolic potential and sheds new light on the potential and limitations of small-scale winemaking.

## KEYWORDS

small-scale fermentation, Cabernet-Sauvignon, colour and phenolic extraction, Tempranillo

## ABBREVIATIONS

ABV: Alcohol by volume;  
AG: Acetyl glucosides;  
ANOVA: Analysis of variance;  
CG: Cumaryl-glucoside;  
CS: Cabernet-Sauvignon;  
CV: Coefficients of variation;  
DAD: Diode Array Detector;  
GC: coumaroyl glucoside;  
HPLC: High-performance liquid chromatography;  
MS: Mass spectrometry;  
PC: Principal component;  
PCA: Principal Component Analysis;  
RRLC: Rapid Resolution Liquid Chromatography;  
TE: Tempranillo;  
TOF: Time of flight;  
TOFMS: Time of Flight Mass Spectrometer

## INTRODUCTION

Research in viticulture relies mostly on measuring yield and grape composition to assess which management practices convey an improvement in vine performance and, as a consequence, could be worth implementing in the field (Ferreira *et al.*, 2014). Although useful, this approach does not allow a completely satisfactory evaluation, since researchers cannot assess the extent to which the effects observed are transferred to the composition of the wine; i.e., to the quality of the final product (Pascual *et al.*, 2016).

To overcome this limitation, some researchers introduce small-scale fermentations in their experiments to obtain a more complete evaluation, which is widely recognized as a positive step forward in the applicability of research (Sampaio *et al.*, 2007). However, despite its relevance, little attention has been paid to evaluating the extent to which reducing grape processing volume in small-scale winemaking affects fermentation dynamics, wine composition, and reproducibility. On the one hand, authors such as Baker (1978), Mirabel *et al.* (1999), Dallas *et al.* (2003), González-Manzano *et al.* (2004) and Kroll *et al.* (1956) have published results addressing grape-seed extraction in model wine solutions, and others such as Lopes *et al.* (2002) and Rossouw *et al.* (2012) report on yeast performance as influenced by commercial and small-scale tanks. On the other hand, in a direct comparison of small-scale to commercial winemaking, Casalta *et al.* (2010) compared the aromatic compounds of Chardonnay and noted that only three experiments used different fermentation volumes in red varieties. Schmid *et al.* (2007) compared three wine volumes (20, 50 and 300 kg) of a blend of Cabernet-Sauvignon and Cabernet franc, in an experiment that focused on evaluating the suitability of frozen must, and reported that winemaking outcomes were comparable among the three volumes compared. In the same research team, Jiranek (2010) and Schmid and Jiranek (2011) compared fresh, frozen and blast-frozen grape fermentation using two different volumes (80 and 500 kg), and concluded that the wines were similar under wine tasting conditions. Finally, Sampaio *et al.* (2007) compared a small volume of Pinot noir (3.5 kg) to a commercial fermentation (4540 kg) and observed that it was possible to effectively control oxidation and spoilage at this volume, although significant

differences were observed in wine composition between both scales.

From this information, it can be seen that the existing research in this field is scarce. Therefore, while taking into account that small-scale winemaking conditions vary between experiments, as regional or winemaker preferences and protocol modifications may affect any stage of winemaking (i.e., yeast inoculation, cap management regime, and malolactic fermentation), there is a clear need to understand how conditions (particularly tank size) affect the composition of the wines obtained (Cerpa-Calderon and Kennedy, 2008). Moreover, the above-mentioned research did not consider repeatability, which is particularly relevant since high variability may limit potential buyer interest in purchasing from small-scale wineries, and an additional source of variation could interfere with data analysis.

This work aimed to evaluate the repeatability and reproducibility of small-scale winemaking. The differential aspect of this research was that four replicates were used with four different volumes of two distinct red varieties (Lasanta *et al.*, 2014) Tempranillo and Cabernet-Sauvignon and that the small-scale fermentation protocols used mirrored typical winemaking techniques commonly used in small wineries producing premium red wines worldwide.

## MATERIALS AND METHODS

### 1. Experimental design

This research was conducted in the experimental winery of the Enology Faculty in Tarragona, Spain, using grapes from the faculty experimental vineyards (41°8'54"N, 1°11'54"E, Altitude: 50 m). The vineyards are located near the coast in the Designation of Origin Tarragona (Spain), which has a Mediterranean climate. The soils are typically fertile and dense and are managed according to standard practices in the region. Grapes from two distinct varieties were used – Tempranillo (TE) and Cabernet-Sauvignon (CS) – with the former based on large berries and low-to-medium phenolic potential, and the latter based on small-sized berries and high phenolic content. Field trials often include a small number of vines in each basic plot of study. This does not facilitate the collection of a significant crop to use in specific research. Wine researchers typically use very small vats to achieve their study results. These small vats often do not facilitate sufficient extraction of polyphenols, to mimic the results

that would occur in a real winemaking program, where larger vats would be employed. This is because either the laboratory does not have vats large enough to do microvinifications, or because the sample quantity that we are permitted to test from a vineyard is not sufficient or not available. Our research here, focusing on small-scale vessel results, aims to help researchers verify the minimum amount of grapes required to perform a vinification with results comparable to standard (larger) fermentations. In total, 8 treatments resulted, using a combination of two varieties – Tempranillo and Cabernet-Sauvignon – and four different small-scale volumes (10, 25, 50 and 100 L stainless steel vats) with 4 replicates for each treatment.

## 2. Small-scale vessel description

For both varieties, four different small-scale volumes (10, 25, 50 and 100 L) were compared. All vessels had a ratio height/diameter of 0.6. All tanks were made of stainless steel, with a rubber gasket to help keep the lid tight. For each variety and tank volume, four replicates were done. Additionally, a commercial-sized large fermentation was performed in a 2500 L stainless steel tank. Diameter (cm) × height (cm) of the small stainless steel tanks used to ferment the grapes (Table 1): 10 L (20 × 32), 25 L (27 × 45), 50 L (35 × 53) and 100 L (43 × 69). The tank covers have a diameter of 15 cm (10, 25 and 50 L volume) and 30 cm (100 L volume) to protect the wine from oxidation. A rubber gasket helped keep the lid tight, keeping the post-alcoholic-fermentation wine protected and maintained until it could be stabilized and bottled. Additionally, a commercial-sized large fermentation (C-2500 L) was performed in a 2500 L stainless steel tank, to compare results with the small-scale fermentations described above.

## 3. Winemaking procedure

Grapes were handpicked at full ripeness into 20 kg boxes and stored at 21 °C in a cold room

before crushing. Grapes were de-stemmed and crushed individually for each tank volume using a Bucher Vaslin Delta E2 destemmer. Tanks were filled one-by-one to three-quarters capacity to ensure an upper appropriate fermentation cap management. Room temperature during fermentation was kept at 23 °C, and 40 ppm sulphur dioxide was added to the must. All tanks were inoculated with 20 g/hl yeast (ICV GRE Selection Inter Rhône, Lallemant®, Canada). The pomace was gently hand-punched down twice a day until an alcoholic fermentation was achieved. During the tumultuous stage, must density and temperature were both measured daily, sugar consumption was controlled, and extremely high temperatures were avoided (higher than 28 °C) during the winemaking process. The pomace was pressed once fermentation was completely exhausted (reducing sugars < 2 g/L), which took 8 days in TE and 12 in CS. Free-run wines were then obtained using a cone-shaped funnel (Lacor inox 18/20; diameter 22 cm) to separate the pomace from the wine. Press wine was obtained using a 40 L Hydropress with a capacity of juice yield of up to 20–25 L per pressing, depending on the variety and ripeness of fruit (<http://www.vigopresses.co.uk>). After pressing, the juice was settled overnight and racked to the same tank to promote clarity. Potassium metabisulphite was added (Winy Sepsa Enartis) to reach 20 ppm of sulphur dioxide to prevent microbial spoilage. Wines were stabilized at 4 °C for 2 months, followed by racking before bottling in December and kept at 4 °C for further storage. Finished wines were bottled without fining or filtering. The wines did not undergo malolactic fermentation to avoid unwanted apparent malolactic deviations, and no oak treatment or ageing was undertaken.

## 4. Grape and wine analysis

All grape batches were analysed before they were introduced into each tank. One hundred berries from each variety were used to determine the sugar level, acidity, and pH, and another 300 berries were used to analyse phenolic maturity.

**TABLE 1.** Size of fermentation vats.

Volume (L)	Diameter (Ø-cm)	Height (H-cm)	Weight (g)	Ratio Ø/H
10	20.0	32.0	2.2	0.63
25	27.0	45.0	2.9	0.60
50	35.0	53.0	9.9	0.66
100	43.0	69.0	21.0	0.62
C-2.500	130.0	190.0		0.68

Sugar content (Brix) was determined using a handheld portable refractometer (Model 102/112/102bp). Total titratable acidity (TTA; g/L) was measured by titration with sodium hydroxide, and pH was measured using a pH meter (Crison Micro CM 2201). The modified Glories method, consisting of berry samples macerated at pH 3.6 instead of pH 3.2 (Nadal, 2010) was used to analyse phenolic maturity. Berries were blended (Oster Blender Classic 3 Model 4655) and macerated in an agitator (Edmund Bühler GmbH<sup>TM</sup> SM-30) to determine total anthocyanin (T Ant) and tannin content (Ribéreau-Gayon *et al.*, 2003) (Ribéreau-Gayon and Stonestreet, 1965). After fermentation of the juice, alcohol by volume (ABV), pH, TTA (Total titratable acidity), T Ant (Total Anthocyanins) and tannins within each combination were analysed.

### 5. HPLC anthocyanin and procyanidin analyses

Anthocyanin identification followed the methodology detailed in Valls *et al.* (2009) and adapted from DeVilliers *et al.* (2004) through high-performance liquid chromatography (HPLC) using a Hewlett Packard Liquid Chromatograph (Waters Corporation, Milford, MA, USA) equipped with

a Zorbax Eclipse Plus C18 Column (150 × 2.1 mm; 3.5 μm) and a Zorbax Eclipse Plus-C18 Precolumn (12.5 × 4.6 mm; 5 μm). Injection volume was 5 μL; elution was performed with a mobile phase A of HPLC-grade water (0.2 % trifluoroacetic acid) and a mobile phase B using methanol (0.2 % trifluoroacetic acid). The column temperature was set at 50 °C and the HPLC was coupled to a Diode Array Detector (DAD). A mass spectrometry (MS) detector was used to assist in the identification. Anthocyanidin-3-monoglucosides and respective acetylated and coumaroylated glycosides were identified based on their ultraviolet-visible (UV-vis) spectra and retention times (Table 2).

Anthocyanidins were quantified making a comparison with internal standards. Calibration curves were obtained by injecting standards with different concentrations of malvidin 3-glucoside (Extrasynthese, Genay, France). The range of the linear calibration curves was 0.1 to 1.0 mg/L for the lower ( $R^2 > 0.996$ ), 0.1–5.0 mg/L for intermediate ( $R^2 > 0.987$ ), and 10.0–200.0 mg/L for the higher concentration compounds ( $R^2 > 0.987$ ). Unknown concentrations were determined from the regression equations, and the results were expressed as milligrams of malvidin 3-glucoside.

**TABLE 2.** Peak assignments, retention times, and mass spectral data of anthocyanidins.

Peak #	Analytes	Retention time	(m/z)	Code Id.
1	Delphinidin 3-O-glucoside	10.8	465	Dp3G
2	Cyanidin 3-O-glucoside	11.8	449	Cy3G
3	Petunidin 3-O-glucoside	12.5	479	Pt3G
4	Peonidin 3-O-glucoside	13.4	463	Pn3G
5	Malvidin 3-O-glucoside	13.8	493	Mv3G
6	Delphinidin 3-O-acetilglucoside	15.3	507	Dp3AG
7	Cyanidin 3-O-acetilglucoside	16.2	491	Cy3AG
8	Petunidin 3-O-acetilglucoside	16.7	521	Pt3AG
9	Peonidin 3-O-acetilglucoside	17.6	505	Pn3AG
10	Malvidin 3-O-acetilglucoside	17.8	535	Mv3AG
11	Delphinidin 3-O-cumarilglucoside	17.6	611	Dp3CG
12	Cyanidin 3-O-cumarilglucoside	18.5	595	Cy3CG
13	Petunidin 3-O-cumarilglucoside	18.7	625	Pt3CG
14	Peonidin 3-O-cumarilglucoside	19.3	609	Pn3CG
15	Malvidin 3-O-cumarilglucoside	19.4	639	Mv3CG

Code assignments: Dp3G (Delphinidin 3-O-glucoside); Cy3G (Cyanidin 3-O-glucoside); Pt3G (Petunidin 3-O-glucoside); Pn3G (Peonidin 3-O-glucoside); Mv3G (Malvidin 3-O-glucoside); Dp3AG (Delphinidin 3-O-acetilglucoside); Cy3AG (Cyanidin 3-O-acetilglucoside); Pt3AG (Petunidin 3-O-acetilglucoside); Pn3AG (Peonidin 3-O-acetilglucoside); Mv3AG (Malvidin 3-O-acetilglucoside); Dp3CG (Delphinidin 3-O-cumarilglucoside); Cy3CG (Cyanidin 3-O-cumarilglucoside); Pt3CG (Petunidin 3-O-cumarilglucoside); Pn3CG (Peonidin 3-O-cumarilglucoside); Mv3CG (Malvidin 3-O-cumarilglucoside).

Free anthocyanin content was determined using a calibration curve (based on the peak area,  $y = 0.7968x + 7.5756$ ;  $R^2 = 0.9774$ ), which was established using malvidin 3-glucoside standard solutions submitted to the same procedure. The repeatability of HPLC analysis gave a coefficient of variation of  $< 7\%$ .

Procyanidins were analysed by injecting 3  $\mu\text{L}$  of wine samples through Rapid Resolution Liquid Chromatography (RRLC) using a Zorbax Eclipse XDB-C18 ( $50 \times 30$ ;  $1.8 \mu\text{m}$ ) followed by an RRLC in-line pre-column ( $4.6 \text{ mm}$ ,  $0.2 \mu\text{m}$ ) at  $30^\circ\text{C}$ . The HPLC injection volume was  $1.4 \mu\text{L}$ , with a  $0.7 \text{ mL/min}$  flux; mobile phase A: water ( $0.1\%$  formic acid), mobile phase B: methanol ( $0.1\%$  formic acid). Phenolic compounds were identified according to their order of elution, retention times of pure compounds (gallic acid, catechin, procyanidin dimer B2, mono gallate dimer, procyanidin trimer C1, and epicatechin gallate) and their molecular masses. Table 3 shows the retention time and  $m/z$  for each compound. A different calibration curve was used for gallic acid ( $R^2 = 0.9957$ ), catechin ( $R^2 = 0.9779$ ), Procyanidin B2 ( $R^2 = 0.9851$ ),

Epicatechin ( $R^2 = 0.9884$ ), Epicatechin gallate ( $R^2 = 0.9935$ ) and Trimer C1 ( $R^2 = 0.9848$ ).

## 6. Sensory Analyses

Descriptive analysis is a sensory method by which the attributes of a food or product are identified and quantified, using human subjects who have been specifically trained for this purpose. The analysis can include all parameters of the product, or it can be limited to certain aspects, for example, aroma, taste, texture, and aftertaste. Many descriptive analysis methods and method variations are currently employed by sensory professionals: flavour profile, quantitative descriptive analysis (QDA), spectrum and texture profile (Hootman, 1992).

Unifying criteria from experts are of the utmost importance in cases where a new model of microvinification is being established, as in this case. Before carrying out the tasting of different vessels, a random sample from each of the varieties (both Tempranillo and Cabernet) was taken, to define the criteria for the tasting sheet that was later used in the panel.

**TABLE 3.** Peak assignments, retention times, and mass spectral data of procyanidins.

Peak #	Analytes	Retention time	$m/z$	Code Id.
1	Procyanidin trimer C	0.6	865.1989	ptC
2	Gallic acid	0.8	169.0147	GA
3	Procyanidin dimer B3	1.9	577.1364	pdB3
4	Procyanidin dimer B1	2.1	577.1364	pdB1
5	Procyanidin trimer T2	2.4	865.1989	ptT2
6	(+)-Catechin	2.8	289.0722	Cat
7	Procyanidin dimer B4	3.4	577.1364	pdB4
8	Procyanidin dimer B2	3.7	577.1364	pdB2
9	Procyanidin dimer B2-3-O-gallate (Dimer monogallate)	4.5	729.1469	PdB2MG1
10	Procyanidin dimer B2-3'-O-gallate (Dimer monogallate)	4.7	729.1469	PdB2MG2
11	(-)-Epicatechin	5.0	289.0722	EC
12	Procyanidin trimer C1 (-)-epicatechin-3-O-gallate	5.0	865.1989	ptECG
13	Procyanidin dimer B1-3-O-gallate	5.1	577.1364	pdB1G1
14	Dimer digallate	5.7	881.1683	DDG
15	(-)-Epicatechin-O-gallate	6.2	441.0835	ECG
16	Procyanidin dimer B1-3'-O-gallate	6.6	577.1364	pdB1G2

Code assignments: ptC (Procyanidin trimer C); GA (Gallic acid), pdB3 (Procyanidin dimer B3); pdB1 (Procyanidin dimer B1); ptT2 (Procyanidin trimer T2); Cat ((+)-Catechin); pdB4 (Procyanidin dimer B4); pdB2 (Procyanidin dimer B2); PdB2MG1 (Procyanidin dimer B2-3-O-gallate); PdB2MG2 (Procyanidin dimer B2-3'-O-gallate); EC ((-)-Epicatechin); ptECG (Procyanidin trimer C1 (-)-epicatechin-3-O-gallate); pdB1G1 (Procyanidin dimer B1-3-O-gallate); DDG (Dimer digallate); ECG ((-)-Epicatechin-O-gallate); pdB1G2 (Procyanidin dimer B1-3'-O-gallate).

The panel of experts, five men and five women aged 25–50 years, was composed of different tasters and winemakers, each with more than 10 years of experience participating on tasting panels. All of these professional tasters previously participated in many international wine competitions and ratings of AOC (Appellation of Origin) wines. The selected panellists tasted two samples as a standard reference (one of each variety) before the set trial. They were instructed to concentrate on the sensations they could identify and to align the criteria to reflect the corresponding sensory attributes.

Our research group created a specific panel chart for this trial. To verify whether each different vessel permitted a similar extraction of the phenolics into the wine, a chart tasting was performed specifying the types of wine attributes. Each sample was evaluated by: (1) Visual intensity; (2) Nose quality; (3) Taste: acidity, astringency, mature tannins, body and unctuousness; (4) Overall impression (including overall mouthfeel of a wine, provided by acidity, tannin, alcohol, sugar and the way these components are balanced). The tasting panel was well trained for perfectly understanding the rating criterion, and therefore an ideal panel to describe each attribute uniformly; i.e., green tannins describes wines that leave a coarse, rough, furry or drying sensation in the mouth, usually attributed to high tannin levels found in some red wines. Excessive, unbalanced tannins can taste bitter and leave the same drying, furry sensation in the mouth as a very strong tea. Mature tannin descriptors include smooth and velvety. Body, a textural description for a wine that feels full and weighty on the palate, typically associated with wines with relatively high alcoholic content; and unctuousness, an adjective to describe a thick, rich and glycerine-laden wine with an equally rich aroma.

Profile development was carried out in two-hour sessions (1 for each), and each assessor evaluated eight wines (4 Tempranillo and 4 Cabernet) and compared four samples (10, 25, 50 and 100 L) at a time. Each resulting sample was the blend/average of the former four replicates. The first set of samples was Tempranillo with less colour and tannins, followed by Cabernet-Sauvignon samples. The resting time between both sets was 15 min. Samples (20 mL) were presented at 12–13 °C in tulip type transparent glasses covered with glass Petri dishes and identified by specific codes. All samples were expectorated and mineral water was provided for oral rinsing.

Intensity values were measured from 1 to 5 (from low and weak to more powerful), and the parameters calculated for each attribute were: average, minimum, maximum and standard error of the intensity mean. Analysis of variance (ANOVA) was carried out to assess attributes significantly different across wines, using the General Linear Model command in SPSS v.17.0. The quantitative descriptive analysis method was used (Stone *et al.*, 1974; Hootman, 1992; Stone and Sidel, 1993).

## 7. Data analysis

The effect of tank size on wine composition was evaluated through one-way analysis of variance (ANOVA);  $P < 0.05$ , and the *Tukey post-hoc* test were used. The comparison of small-scale wines to commercial-sized tanks was performed using Principal Component Analysis (PCA), considering the 2500 L tank as a supplementary item; i.e., they were not included to calculate the principal components (PC), but to evaluate its performance. Statistical analyses were performed using R (R Core Team ; Foundation for Statistical Computing; <http://www.R-project.org/>) and the FactoMineR (Husson *et al.*, 2020) and “factoextra” packages (Kassambara and Mundt, 2016) for personal computer (PC) calculation and a graphical representation, respectively.

## RESULTS

### 1. Grape composition

Grape composition before fermentation was very similar for all tank sizes (Table 4), and low variability occurred between tanks of the same size (coefficients of variation [CV] < 5 %). This finding was essential, to guarantee that the differences eventually observed in wine composition were not due to differences in grape composition, but were associated with the winemaking process.

### 2. Fermentation performance

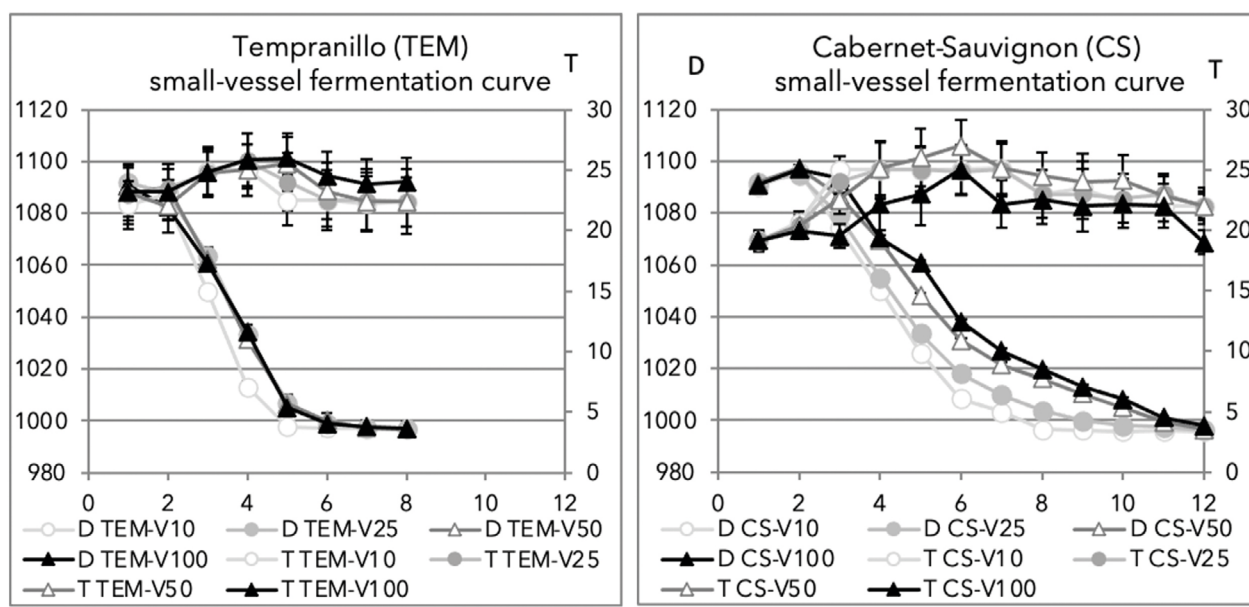
Winemaking conditions allowed adequate fermentation dynamics in the 32 tanks included in the experiment, achieving a complete transformation of sugars into ethanol.

Density rapidly decreased after the second day of fermentation for both varieties, and 5 and 9 days after the start of fermentation, with only a small quantity of sugar remaining in TE and CS, respectively. At this point, the final stage of fermentation (slow fermentation process) began and, after 3 days, the remaining sugars were completely transformed into alcohol.

**TABLE 4.** Must composition and berry weight of each tank.

Volume	Brix	pH	TA (g/L)	Bw (g)
TE-10	23.1 ± 0.1 <sup>a</sup>	3.42 ± 0.02 <sup>a</sup>	6.46 ± 0.12 <sup>a</sup>	2.21 ± 0.11 <sup>a</sup>
TE-25	23.1 ± 0.1 <sup>a</sup>	3.40 ± 0.01 <sup>ab</sup>	6.53 ± 0.15 <sup>a</sup>	2.30 ± 0.14 <sup>a</sup>
TE-50	23.2 ± 0.1 <sup>a</sup>	3.44 ± 0.02 <sup>b</sup>	6.60 ± 0.10 <sup>a</sup>	2.20 ± 0.13 <sup>a</sup>
TE-100	23.2 ± 0.1 <sup>a</sup>	3.41 ± 0.01 <sup>a</sup>	6.54 ± 0.11 <sup>a</sup>	2.33 ± 0.08 <sup>a</sup>
CS-10	23.8 ± 0.1 <sup>b</sup>	3.26 ± 0.02 <sup>b</sup>	5.00 ± 0.10 <sup>a</sup>	1.39 ± 0.14 <sup>b</sup>
CS-25	24.1 ± 0.1 <sup>a</sup>	3.21 ± 0.01 <sup>c</sup>	5.20 ± 0.10 <sup>a</sup>	1.43 ± 0.06 <sup>b</sup>
CS-50	24.1 ± 0.1 <sup>a</sup>	3.26 ± 0.02 <sup>b</sup>	5.18 ± 0.07 <sup>a</sup>	1.51 ± 0.12 <sup>b</sup>
CS-100	23.8 ± 0.1 <sup>b</sup>	3.26 ± 0.01 <sup>b</sup>	5.21 ± 0.06 <sup>a</sup>	1.56 ± 0.12 <sup>b</sup>

<sup>a</sup>Values with different letters denote a statistically significant difference ( $p < 0.05$ ). Results show the mean value and standard deviation. TA: titratable acidity. Bw: berry weight.



**FIGURE 1.** Evolution of fermentation in small-tanks. Evolution of density during fermentation.

In general terms, the complete alcoholic fermentation of TE and CS could be divided into two different stages: tumultuous and slow. The duration of tumultuous fermentation varied according to the composition of the must and the temperature at which it was carried out. Grapes were stored at 21 °C in a cooler before crushing. The cellar temperature was set at 22 °C and the temperature in the tank was held at 28 °C at the tumultuous stage to ensure a good extraction of polyphenols. This step should be carefully considered to avoid uncontrolled fermentation and make this methodology reliable. The yeasts developed comfortably, thus ensuring the total transformation of all sugar into alcohol for both grape varieties. Density rapidly decreased after

the second day of fermentation for each variety and vessel (Figure 1).

Approximately 5 and 9 days after the start of fermentation (for TE and CS, respectively), a small quantity of sugar corresponding to a density of  $\rho = 1010 \text{ kg/m}^3$  remained in the must. At this point, the second stage of fermentation (slow fermentation process) began, which transformed the remaining final grams of sugar into alcohol over the following 3 days. TE showed a rapid decrease until the 5<sup>th</sup> day of fermentation, when it reached  $\rho = 997.8, 1007.0, 1006.5$  and  $1005.0 \text{ kg/m}^3$ , respectively, for each increasing small-scale volume (25, 50, 75 and 100 L). Fermentation kinetics in TE required 8 days to ferment all the reducing sugars, showing a slow decrease for the last 3 days.

The CS required 12 days to complete the fermentation process. Temperatures did not exceed 28 °C for both kinetics under the same conditions of controlled room temperature and vessel size. After fermentation, the temperature decreased to 22 °C in both cases.

Modelling data using linear functions proved easier for predicting the kinetics of the fermentation processes of both varieties/volume studies. As tumultuous fermentation occurred with a different duration for each variety compared with the slow stage, two regression curves were calculated for each combination variety/volume. As expected, in the tumultuous phase (when maximal fermentation activity occurred) and slow fermentation stage (after tumultuous fermentation), two slopes were clearly distinguished on the fermentation curves for both varieties (Table 5). Linear regression slopes of the tumultuous stage ranged between  $-21.933$  and  $-24.850$  for TE and  $-12.286$  and  $-17.321$  for CS, indicating faster

kinetics for TE in the tumultuous fermentation stage. The coefficient of determination was also higher in the tumultuous stage. Next, considering all volume vessels, the TE slopes from the tumultuous stage did not indicate substantially different kinetics between volumes, although, for the 10 L capacity vessel, it appeared to decrease faster, with a curve described by  $y = -24.8x + 1121$ , compared with the 25, 50, and 100 L vessels ( $y = -22.4x + 1124$ ;  $y = -22.1x + 1122$ , and  $y = -21.9x + 1120$ , respectively), showing very similar slopes. However, the slow stage revealed a similar tendency, having the lowest slope for the 10 L vessel. CS showed a proportional relationship between slope and volume. The 10 L tank had the highest slope in the tumultuous stage ( $y = -17.3x + 1119$ ), and the lowest slope on the slow stage ( $y = -1.9x + 1016$ ), indicating that the tumultuous part of fermentation proceeded faster in the 10 L vessel than any other vessel evaluated.

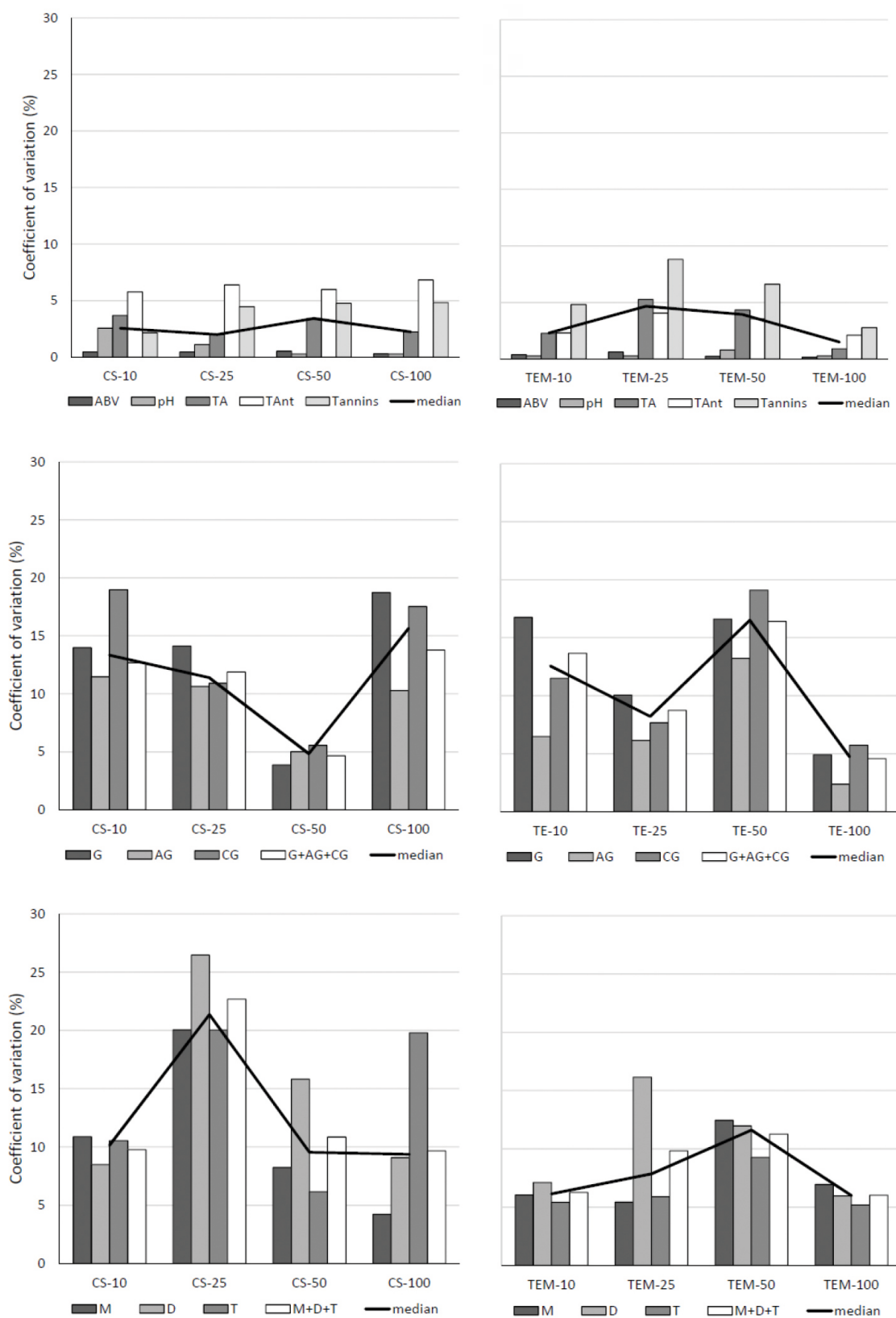
**TABLE 5.** Kinetics of fermentation, tumultuous and slow stages.

Treatment	Tumultuous fermentation stage	R <sup>2</sup> value	Slow fermentation stage	R <sup>2</sup> value
TE 10 L	$y = -24.8x + 1121$	0.95	$y = -0.25x + 999$	0.83
TE 25 L	$y = -22.4x + 1124$	0.95	$y = -3.15x + 1020$	0.76
TE 50 L	$y = -22.1x + 1121$	0.93	$y = -3.2x + 1021$	0.85
TE 100 L	$y = -21.9x + 1120$	0.97	$y = -2.53x + 1016$	0.80
CS 10 L	$y = -17.3x + 1119$	0.95	$y = -1.90x + 1016$	0.68
CS 25 L	$y = -15.9x + 1118$	0.96	$y = -3.41x + 1034$	0.85
CS 50 L	$y = -13.6x + 1118$	0.93	$y = -5.66x + 1062$	0.98
CS 100 L	$y = -12.3x + 1117$	0.90	$y = -6.53x + 1074$	0.98

**TABLE 6.** Wine analysis of tanks after fermentation of TE (Tempranillo) and CS (Cabernet-Sauvignon).

Volume	ABV	pH	TA (g/L)	T Ant (mg/L)	Tannins (g/L)
TE-10	$12.82 \pm 0.05^b$	$3.63 \pm 0.01^b$	$5.25 \pm 0.12^c$	$311 \pm 7^c$	$2.3 \pm 0.2^{bc}$
TE-25	$12.90 \pm 0.08^{ab}$	$3.68 \pm 0.01^b$	$5.32 \pm 0.28^{bc}$	$367 \pm 15^a$	$1.9 \pm 0.5^{bc}$
TE-50	$12.77 \pm 0.03^b$	$3.75 \pm 0.03^a$	$5.54 \pm 0.24^b$	$385 \pm 14^a$	$2.7 \pm 0.5^{ab}$
TE-100	$12.91 \pm 0.02^a$	$3.76 \pm 0.01^a$	$5.60 \pm 0.05^b$	$342 \pm 7^b$	$3.0 \pm 0.1^a$
CS-10	$13.18 \pm 0.06^a$	$3.51 \pm 0.09$	$5.97 \pm 0.22^{ab}$	$341 \pm 20^c$	$1.3 \pm 0.6^{bd}$
CS-25	$13.23 \pm 0.06^a$	$3.57 \pm 0.04$	$6.00 \pm 0.12^{ab}$	$402 \pm 26^a$	$1.1 \pm 0.3^{cd}$
CS-50	$13.18 \pm 0.07^a$	$3.55 \pm 0.01$	$6.14 \pm 0.21^{ab}$	$379 \pm 23^{ab}$	$1.9 \pm 0.3^b$
CS-100	$13.25 \pm 0.04^a$	$3.53 \pm 0.01$	$6.27 \pm 0.14^a$	$363 \pm 25^{bc}$	$2.0 \pm 0.2^b$

<sup>a</sup> Values with different letters denote a statistically significant difference ( $p < 0.05$ ). ABV: Alcohol by volume. pH: Potential hydrogen; TA: Titratable acidity in tartaric. T Ant: Total anthocyanin and tannins. Results show the mean value and standard deviation.



**FIGURE 2.** Coefficient of variations (%) for Cabernet-Sauvignon (CS) and Tempranillo (TE).

ABV: Alcohol by volume. pH, . TA: titratable acidity. T Ant: Total anthocyanins. G: glucosides. AG: acetyl glucosides. CG: coumaroyl-glucosides. M: monomers. D: dimers. T: trimers.

### 3. Effect of small-scale tank volume on wine composition

With regards to the basic parameters of wine composition, tank size was observed not to influence ABV, pH, or TA in either CS or TE (Table 6), but it did affect phenolic composition (T Ant, and tannins). The highest T Ant values were observed in the intermediate sizes (25 and 50 L), whereas for tannin content, the highest values were found in the larger tanks (50 and 100 L) in both varieties. One of the most relevant effects of tank size from a research perspective is increasing or decreasing the variability of the composition of the wine obtained from replicates. When the CV obtained for each variable, tank size, and variety were compared (Figure 2), all values were low, especially for ABV, pH, and TA ( $CV < 4\%$ ), but also for T Ant and tannin content ( $CV < 8\%$ ). Taking into account that the observed CVs were satisfactory for all tank sizes and varieties (less than 5%), there was slightly greater variability in the intermediate sizes (25 and 50 L) with TE. This observation supported the repeatability of wine quality at any of the tank sizes concerning the major wine composition parameters.

T Ant composition (Tables 7 and 8) in the medium-sized tanks (25 and 50 L) was higher than any other volumes (10 and 100 L) in TE. Malvidin glucosides (G) were more highly extracted (up to one-third) than acetyl glucosides (AG). Furthermore, the latter showed almost the same concentration of coumaroyl glucosides (GC). In CS, the greatest anthocyanin contents were found in the biggest volumes (Table 9). CS tanks measuring 10 and 25 L showed delayed extraction of anthocyanins (Table 9), giving 117.7 mg/L of T Ant in 10 L, 128.5 mg/L in 25 L, 361.9 mg/L in 100 L, and 384.4 mg/L in 50 L. Thus, in the case of CS, it appears that the larger the tank, the greater the extraction (50, 100). In CS, the difference between G and AG total concentration was not remarkable, with lower extractions observed in the smaller volumes in all cases. Reproducibility in terms of anthocyanin content can be said to be satisfactory, since the CVs for all anthocyanin families were below 20%, with the median CV being 13% for CS and 10% for TE (Figure 2c and 2d). Tank size appeared to affect the reproducibility, although the observed effect was different for each variety. In TE, the lowest CVs were found for the 100 L and 25 L tanks, whereas in CS this occurred in the 10 L and 50 L tanks.

**TABLE 7.** Anthocyanin wine profile (glucoside, acetyl glucoside and coumaroyl glucoside) for Tempranillo.

Analytes	C-2500 L	10 L	25 L	50 L	100 L
Mv3G	70.3 ± 8.1 <sup>ab</sup>	70.9 ± 12.2 <sup>ab</sup>	84.5 ± 8.4 <sup>a</sup>	85.1 ± 13.4 <sup>a</sup>	60.8 ± 2.3 <sup>b</sup>
Pt3G	8.4 ± 1.8 <sup>c</sup>	15.1 ± 2.6 <sup>ab</sup>	17.6 ± 2.1 <sup>a</sup>	18.5 ± 4.0 <sup>a</sup>	13.2 ± 1.0 <sup>b</sup>
Dp3G	5.6 ± 0.4 <sup>a</sup>	6.3 ± 1.3 <sup>a</sup>	7.3 ± 1.0 <sup>a</sup>	8.1 ± 2.2 <sup>a</sup>	5.8 ± 0.6 <sup>a</sup>
Pn3G	11.0 ± 0.5 <sup>b</sup>	12.7 ± 1.5 <sup>a</sup>	14.2 ± 0.9 <sup>a</sup>	16.1 ± 1.7 <sup>a</sup>	12.0 ± 0.6 <sup>b</sup>
Cy3G	0.8 ± 0.2 <sup>a</sup>	0.8 ± 0.1 <sup>a</sup>	0.9 ± 0.2 <sup>a</sup>	1.2 ± 0.2 <sup>a</sup>	0.8 ± 0.1 <sup>a</sup>
Total G	96.1 ± 1.0 <sup>b</sup>	105.9 ± 17.7 <sup>ab</sup>	124.4 ± 12.5 <sup>a</sup>	129.0 ± 21.4 <sup>a</sup>	92.7 ± 4.5 <sup>b</sup>
Mv3AG	30.1 ± 0.5 <sup>b</sup>	29.4 ± 1.7 <sup>b</sup>	38.9 ± 2.2 <sup>a</sup>	40.2 ± 5.1 <sup>a</sup>	26.8 ± 0.5 <sup>c</sup>
Pt3AG	0.9 ± 0.2 <sup>a</sup>	1.0 ± 0.2 <sup>a</sup>	1.4 ± 0.2 <sup>a</sup>	1.5 ± 0.3 <sup>a</sup>	0.9 ± 0.0 <sup>a</sup>
Dp3AG	0.2 ± 0.0 <sup>a</sup>	0.2 ± 0.0 <sup>a</sup>	0.3 ± 0.0 <sup>a</sup>	0.3 ± 0.1 <sup>a</sup>	0.2 ± 0.0 <sup>a</sup>
Pn3AG	2.9 ± 0.2 <sup>b</sup>	2.8 ± 0.3 <sup>b</sup>	3.9 ± 0.3 <sup>a</sup>	4.1 ± 0.5 <sup>a</sup>	2.6 ± 0.2 <sup>b</sup>
Cy3AG	0.1 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>
Total AG	33.7 ± 0.6 <sup>b</sup>	33.5 ± 2.2 <sup>b</sup>	44.7 ± 2.7 <sup>a</sup>	46.2 ± 6.1 <sup>a</sup>	30.5 ± 0.7 <sup>b</sup>
Mv3CG	25.8 ± 0.9 <sup>ab</sup>	27.6 ± 2.6 <sup>ab</sup>	29.6 ± 2.0 <sup>a</sup>	28.9 ± 4.6 <sup>a</sup>	21.6 ± 0.9 <sup>b</sup>
Pt3CG	4.1 ± 0.4 <sup>b</sup>	4.9 ± 0.9 <sup>ab</sup>	5.8 ± 0.7 <sup>a</sup>	5.8 ± 1.6 <sup>ab</sup>	3.6 ± 0.4 <sup>b</sup>
Dp3CG	1.8 ± 0.2 <sup>b</sup>	1.5 ± 0.4 <sup>b</sup>	1.8 ± 0.2 <sup>ab</sup>	1.8 ± 0.6 <sup>b</sup>	1.2 ± 0.2 <sup>b</sup>
Pn3CG	5.7 ± 0.2 <sup>ab</sup>	5.1 ± 0.5 <sup>a</sup>	6.2 ± 0.4 <sup>a</sup>	6.6 ± 1.3 <sup>a</sup>	4.2 ± 0.2 <sup>b</sup>
Cy3CG	1.2 ± 0.1 <sup>b</sup>	1.6 ± 0.3 <sup>a</sup>	1.7 ± 0.2 <sup>a</sup>	1.9 ± 0.4 <sup>a</sup>	1.2 ± 0.1 <sup>b</sup>
Total CG	38.6 ± 1.9 <sup>ab</sup>	40.7 ± 4.7 <sup>a</sup>	45.1 ± 3.5 <sup>a</sup>	44.9 ± 8.6 <sup>a</sup>	31.8 ± 1.8 <sup>b</sup>

<sup>a</sup> Values with different letters denote a statistically significant difference ( $p < 0.05$ ). Results show the mean value and standard deviation. Dp3G (Delphinidin 3-O-glucoside); Cy3G (Cyanidin 3-O-glucoside); Pt3G (Petunidin 3-O-glucoside); Pn3G (Peonidin 3-O-glucoside); Mv3G (Malvidin 3-O-glucoside); Dp3AG (Delphinidin 3-O-acetylglucoside); Cy3AG (Cyanidin 3-O-acetylglucoside); Pt3AG (Petunidin 3-O-acetylglucoside); Pn3AG (Peonidin 3-O-acetylglucoside); Mv3AG (Malvidin 3-O-acetylglucoside); Dp3CG (Delphinidin 3-O-cumarilglucoside); Cy3CG (Cyanidin 3-O-cumarilglucoside); Pt3CG (Petunidin 3-O-cumarilglucoside); Pn3CG (Peonidin 3-O-cumarilglucoside); Mv3CG (Malvidin 3-O-cumarilglucoside).

**TABLE 8.** Anthocyanin wine profile (glucoside, acetyl glucoside and coumaroyl glucoside) for Cabernet-Sauvignon.

Analytes	C-2500 L	10 L	25 L	50 L	100 L
Mv3G	95.6 ± 13.7 <sup>a</sup>	31.3 ± 3.9 <sup>b</sup>	34.1 ± 5.2 <sup>b</sup>	114.1 ± 3.8 <sup>a</sup>	103.6 ± 19.4 <sup>a</sup>
Pt3G	6.7 ± 1.5 <sup>a</sup>	1.0 ± 0.1 <sup>b</sup>	1.1 ± 0.2 <sup>b</sup>	7.8 ± 0.2 <sup>a</sup>	7.4 ± 1.8 <sup>a</sup>
Dp3G	2.4 ± 0.9 <sup>a</sup>	0.2 ± 0.1 <sup>b</sup>	0.3 ± 0.1 <sup>b</sup>	2.7 ± 0.3 <sup>a</sup>	2.6 ± 0.9 <sup>a</sup>
Pn3G	7.1 ± 0.7 <sup>ab</sup>	8.2 ± 1.6 <sup>ab</sup>	9.7 ± 0.9 <sup>b</sup>	8.2 ± 0.8 <sup>ab</sup>	7.7 ± 0.7 <sup>a</sup>
Cy3G	0.1 ± 0.0 <sup>a</sup>	0.0 ± 0.0 <sup>a</sup>	0.0 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>
Total G	111.9 ± 16.8 <sup>a</sup>	40.6 ± 5.7 <sup>b</sup>	45.3 ± 6.4 <sup>b</sup>	132.9 ± 5.1 <sup>a</sup>	121.4 ± 22.7 <sup>a</sup>
Mv3AG	173.0 ± 15.4 <sup>a</sup>	69.2 ± 7.7 <sup>b</sup>	74.4 ± 7.9 <sup>b</sup>	209.7 ± 10.2 <sup>a</sup>	200.7 ± 20.4 <sup>a</sup>
Pt3AG	3.5 ± 0.7 <sup>a</sup>	0.5 ± 0.1 <sup>b</sup>	0.5 ± 0.1 <sup>b</sup>	4.5 ± 0.2 <sup>a</sup>	4.4 ± 0.7 <sup>a</sup>
Dp3AG	0.8 ± 0.2 <sup>a</sup>	0.1 ± 0.0 <sup>b</sup>	0.1 ± 0.0 <sup>b</sup>	0.9 ± 0.1 <sup>a</sup>	0.8 ± 0.2 <sup>a</sup>
Pn3AG	1.4 ± 0.3 <sup>a</sup>	1.9 ± 0.4 <sup>a</sup>	1.9 ± 0.2 <sup>a</sup>	1.8 ± 0.3 <sup>a</sup>	1.8 ± 0.1 <sup>a</sup>
Cy3AG	0.2 ± 0.0 <sup>a</sup>	0.0 ± 0.0 <sup>b</sup>	0.0 ± 0.0 <sup>b</sup>	0.2 ± 0.0 <sup>a</sup>	0.2 ± 0.0 <sup>a</sup>
Total AG	178.9 ± 15.5 <sup>a</sup>	71.6 ± 8.2 <sup>b</sup>	76.9 ± 8.2 <sup>b</sup>	217.0 ± 10.9 <sup>a</sup>	208.0 ± 21.4 <sup>a</sup>
Mv3CG	22.3 ± 4.6 <sup>a</sup>	4.0 ± 0.7 <sup>b</sup>	4.8 ± 0.5 <sup>b</sup>	30.5 ± 1.7 <sup>a</sup>	28.2 ± 4.6 <sup>a</sup>
Pt3CG	0.8 ± 0.3 <sup>a</sup>	0.0 ± 0.0 <sup>b</sup>	0.1 ± 0.0 <sup>b</sup>	0.9 ± 0.1 <sup>a</sup>	0.9 ± 0.3 <sup>a</sup>
Dp3CG	0.1 ± 0.0 <sup>a</sup>	0.0 ± 0.0 <sup>b</sup>	0.0 ± 0.0 <sup>b</sup>	0.1 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>
Pn3CG	3.1 ± 0.7 <sup>a</sup>	1.4 ± 0.3 <sup>b</sup>	1.5 ± 0.2 <sup>b</sup>	3.1 ± 0.1 <sup>a</sup>	3.3 ± 0.7 <sup>a</sup>
Cy3CG	0.1 ± 0.0 <sup>a</sup>	0.0 ± 0.0 <sup>b</sup>	0.0 ± 0.0 <sup>b</sup>	0.1 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>
Total CG	26.4 ± 5.7 <sup>a</sup>	5.5 ± 1.0 <sup>b</sup>	6.3 ± 0.7 <sup>b</sup>	34.6 ± 1.9 <sup>a</sup>	32.5 ± 5.7 <sup>a</sup>

<sup>a</sup>Values with different letters denote a statistically significant difference ( $p < 0.05$ ). Results show the mean value and standard deviation. Dp3G (Delphinidin 3-O-glucoside); Cy3G (Cyanidin 3-O-glucoside); Pt3G (Petunidin 3-O-glucoside); Pn3G (Peonidin 3-O-glucoside); Mv3G (Malvidin 3-O-glucoside); Dp3AG (Delphinidin 3-O-acetylglucoside); Cy3AG (Cyanidin 3-O-acetylglucoside); Pt3AG (Petunidin 3-O-acetylglucoside); Pn3AG (Peonidin 3-O-acetylglucoside); Mv3AG (Malvidin 3-O-acetylglucoside); Dp3CG (Delphinidin 3-O-cumarilglucoside); Cy3CG (Cyanidin 3-O-cumarilglucoside); Pt3CG (Petunidin 3-O-cumarilglucoside); Pn3CG (Peonidin 3-O-cumarilglucoside); Mv3CG (Malvidin 3-O-cumarilglucoside).

**TABLE 9.** Procyanidin wine profile (M: monomers, D: dimers and T: trimers) for Tempranillo.

Analytes	C-2500 L	10 L	25 L	50 L	100 L
Gallic acid	41.0 ± 0.1 <sup>a</sup>	21.1 ± 0.9 <sup>c</sup>	19.5 ± 1.0 <sup>c</sup>	23.6 ± 2.9 <sup>bc</sup>	26.7 ± 2.0 <sup>b</sup>
Cat	40.7 ± 1.3 <sup>a</sup>	14.7 ± 1.0 <sup>bc</sup>	13.0 ± 0.7 <sup>c</sup>	17.7 ± 2.3 <sup>bc</sup>	17.3 ± 1.2 <sup>b</sup>
EC	15.0 ± 0.4 <sup>a</sup>	10.1 ± 0.8 <sup>b</sup>	9.5 ± 0.5 <sup>b</sup>	13.2 ± 1.7 <sup>a</sup>	12.8 ± 0.8 <sup>a</sup>
ECG	0.5 ± 0.0 <sup>c</sup>	0.8 ± 0.1 <sup>c</sup>	1.4 ± 0.1 <sup>a</sup>	1.5 ± 0.1 <sup>a</sup>	1.1 ± 0.1 <sup>b</sup>
Total M	97.3 ± 1.8 <sup>a</sup>	46.8 ± 2.8 <sup>c</sup>	43.2 ± 2.3 <sup>c</sup>	56.0 ± 7.0 <sup>b</sup>	57.9 ± 4.0 <sup>b</sup>
pdB1	20.5 ± 0.2 <sup>a</sup>	11.9 ± 0.7 <sup>c</sup>	10.8 ± 0.4 <sup>c</sup>	13.5 ± 1.7 <sup>bc</sup>	13.3 ± 0.8 <sup>b</sup>
pdB2	5.6 ± 0.3 <sup>d</sup>	14.1 ± 1.0 <sup>b</sup>	12.7 ± 1.0 <sup>c</sup>	15.9 ± 2.0 <sup>ab</sup>	16.8 ± 0.9 <sup>a</sup>
pdB3	7.1 ± 0.1 <sup>d</sup>	12.0 ± 0.7 <sup>b</sup>	10.8 ± 0.5 <sup>c</sup>	13.5 ± 1.7 <sup>ab</sup>	13.3 ± 0.7 <sup>a</sup>
pdB4	17.7 ± 0.5 <sup>a</sup>	13.7 ± 0.8 <sup>b</sup>	12.8 ± 0.9 <sup>c</sup>	15.6 ± 1.9 <sup>ab</sup>	16.2 ± 1.0 <sup>a</sup>
pdB2MG1	5.5 ± 4.1 <sup>a</sup>	1.2 ± 0.2 <sup>c</sup>	1.3 ± 0.3 <sup>c</sup>	1.9 ± 0.4 <sup>b</sup>	1.6 ± 0.2 <sup>b</sup>
pdB1G1	2.8 ± 0.1 <sup>b</sup>	4.4 ± 0.3 <sup>a</sup>	4.3 ± 0.5 <sup>a</sup>	4.5 ± 0.4 <sup>a</sup>	4.5 ± 0.3 <sup>a</sup>
DDG	0.5 ± 0.1 <sup>a</sup>	0.0 ± 0.0 <sup>b</sup>	0.0 ± 0.0 <sup>b</sup>	0.0 ± 0.0 <sup>b</sup>	0.0 ± 0.0 <sup>b</sup>
pdB1G2	5.4 ± 0.2 <sup>b</sup>	6.6 ± 0.8 <sup>b</sup>	10.4 ± 6.4 <sup>a</sup>	7.1 ± 0.5 <sup>b</sup>	6.9 ± 0.4 <sup>b</sup>
Total D	65.0 ± 5.6 <sup>a</sup>	63.9 ± 4.6 <sup>a</sup>	63.1 ± 10.2 <sup>a</sup>	72.0 ± 8.6 <sup>a</sup>	72.5 ± 4.3 <sup>a</sup>
ptC	5.0 ± 0.0 <sup>b</sup>	21.7 ± 0.9 <sup>a</sup>	21.8 ± 1.7 <sup>a</sup>	22.9 ± 2.0 <sup>a</sup>	22.6 ± 1.1 <sup>a</sup>
ptT2	20.1 ± 1.1 <sup>a</sup>	22.0 ± 1.0 <sup>a</sup>	21.8 ± 1.0 <sup>a</sup>	23.8 ± 2.0 <sup>a</sup>	22.2 ± 1.0 <sup>a</sup>
ptECG	15.7 ± 0.6 <sup>a</sup>	11.6 ± 1.1 <sup>b</sup>	9.7 ± 0.5 <sup>c</sup>	12.1 ± 1.4 <sup>b</sup>	12.4 ± 0.9 <sup>b</sup>
Total T	40.8 ± 1.7 <sup>b</sup>	55.3 ± 3.0 <sup>a</sup>	53.4 ± 3.2 <sup>a</sup>	58.8 ± 5.4 <sup>a</sup>	57.2 ± 3.0 <sup>a</sup>

<sup>a</sup>Values with different letters denote a statistically significant difference ( $p < 0.05$ ). Results show the mean value and standard deviation. ptC (Procyanidin trimer C); GA(Gallic acid); pdB3 (Procyanidin dimer B3); pdB1 (Procyanidin dimer B1); ptT2 (Procyanidin trimer T2); Cat ((+)-Catechin); pdB4 (Procyanidin dimer B4); pdB2 (Procyanidin dimer B2); PdB2MG1(Procyanidin dimer B2-3-O-gallate); PdB2MG2 (Procyanidin dimer B2-3'-O-gallate); EC ((-)-Epicatechin); ptECG(Procyanidin trimer C1 (-)-epicatechin-3-O-gallate); pdB1G1 (Procyanidin dimer B1-3-O-gallate); DDG (Dimer digallate); ECG ((-)-Epicatechin-O-gallate); pdB1G2 (Procyanidin dimer B1-3'-O-gallate).

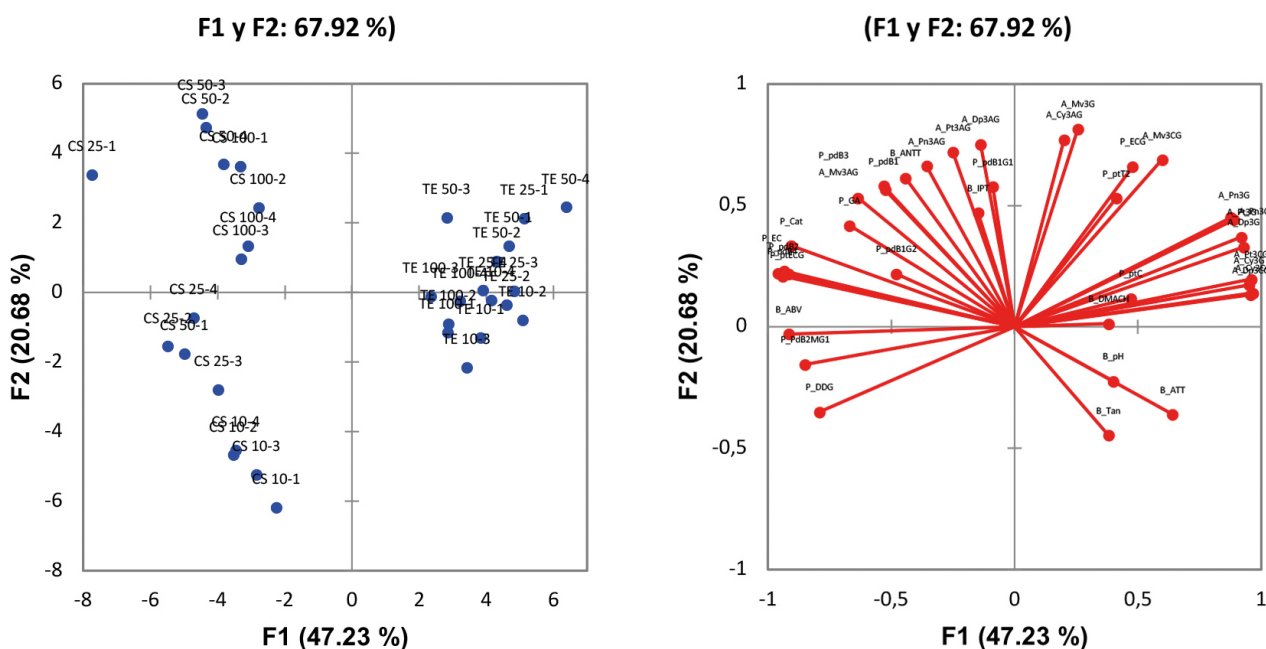
Variability in procyanidin content (Figure 2e and 2f) was relatively similar to that observed for anthocyanins; the median value was just 9 % except for CS-25 and TE-50 (12 and 22 %, respectively). The upper and lower CV values ranged between 10 % and 22 % in CS and between 5 % and 17 % in TE, with dimers showing a higher CV, which indicated that reproducibility was in general terms very satisfactory, particularly in TE, where it was almost always below 10 %. In both CS and TE, the lower CVs were associated with 10 L and 100 L volumes. In general, the effect of tank size on procyanidin content repeatability was less relevant in CS (table 10) than it was for anthocyanins.

#### 4. Comparison with commercial volume

PCA allowed the information provided by all the analysis variables included in the study to be condensed into a reduced number of components, with a minimum loss of information in both varieties (Figure 3). Thus, in CS, the first component accounted for 44.6 % of variability, the second

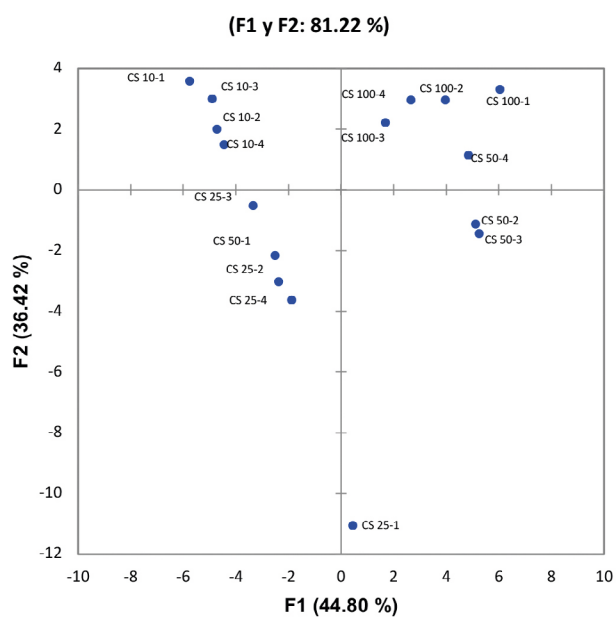
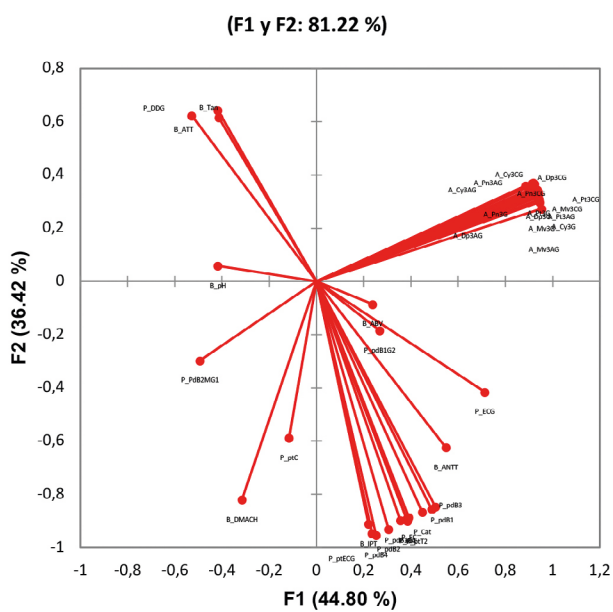
for 36.6 %, and the third for 5.9 % (Figure 4); whereas in TE the corresponding values were 44.0 %, 27.3 % and 7.2 %, respectively (Figure 5). In both varieties, the first component included mainly anthocyanin-content variables, the second included procyanidins-content variables, and the third component was linked predominantly to acidity (pH in CS, and TA in TE).

PCA scores for all small-scale tanks, average scores for each small-scale volume, and commercial-scale tank scores are shown in Figure 3a and 3b for CS and TE, respectively. For both varieties, the composition of the wine obtained in the 100 L tanks was clearly more similar to the commercial-scale wine for the main (first) component, related to anthocyanin content. For the second component, related to procyanidins, wines obtained in 10 L and 100 L volumes were the most similar to the commercial scale in CS, whereas, for TE, differences were smaller in this axis, with 10 L, 25 L and 100 L showing similar scores for this component compared with the commercial-scale wine (Figure 3c and 3d).

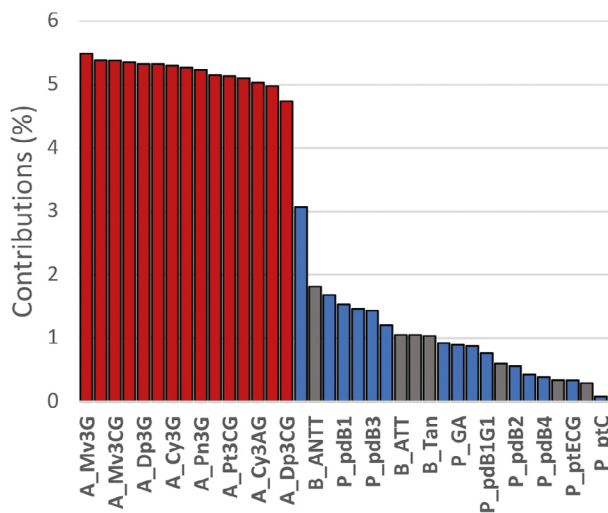


**FIGURE 3.** Contribution of wine composition variables to Principal Component Analysis dimensions 1 and 2 in all the small-scale fermentations.

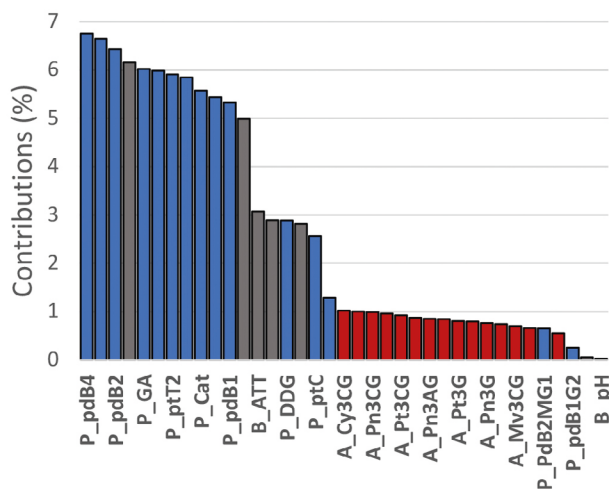
The resulting components from this transformation shows that the first two principal component have the highest variance and accounts for as most of the variability in the data. The first 2 components contribute to 67.92 % of the total variance. Choosing two components is good enough to show that the two grape and small-scale fermentations are well separated. This justifies that we do a separate analysis of the main components of each variety.



Contributions to Dim 1

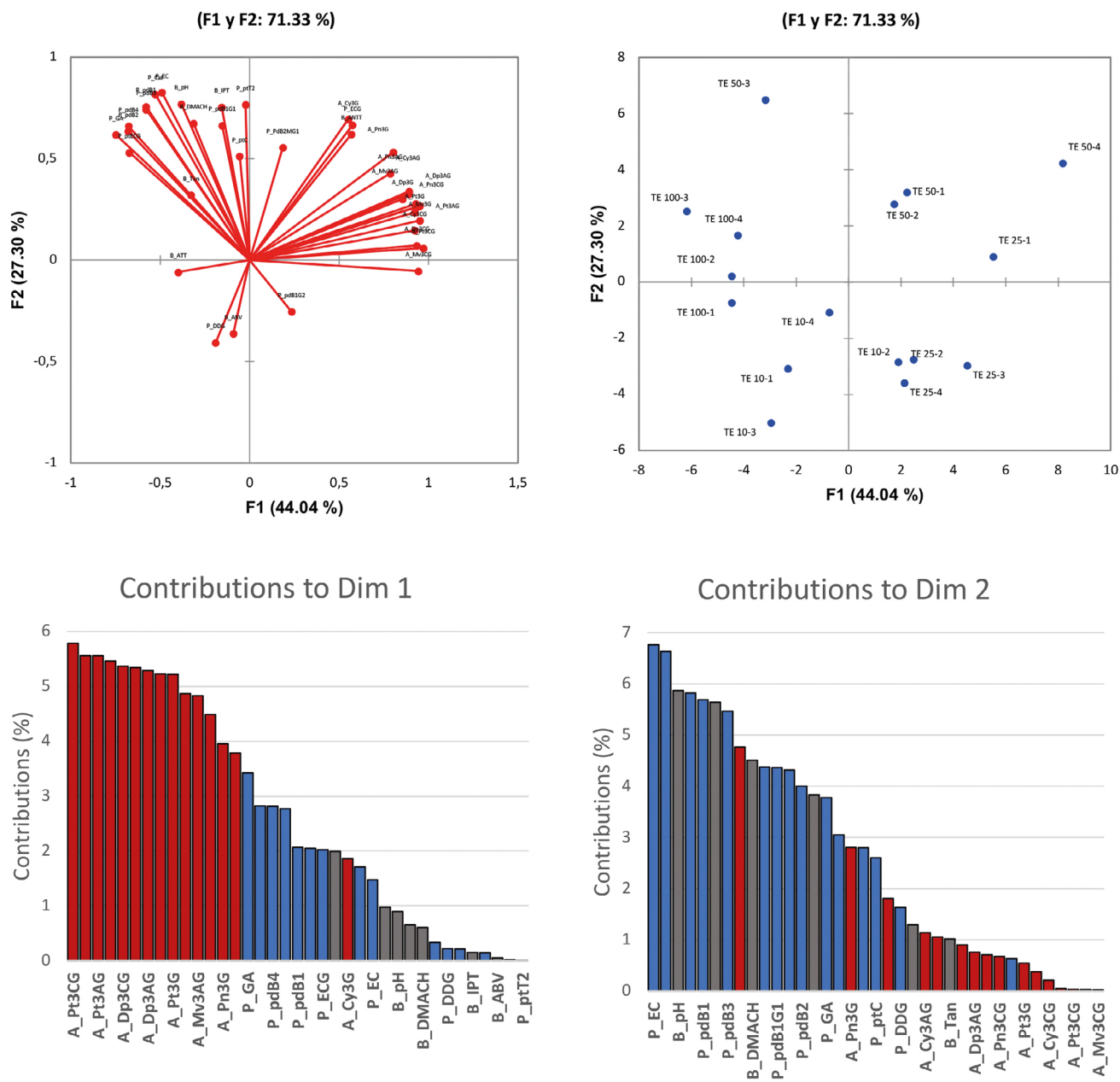


Contributions to Dim 2



**FIGURE 4.** Contribution of wine composition variables to Principal Component Analysis dimensions 1 and 2 in Cabernet-Sauvignon.

The first 2 components contribute to 81.22 % of the total variance. The commercial vessel (2500 L) was considered as a supplementary individual, i.e., not including it to calculate the principal components (PC) but evaluating its performance. Those variables contributing the most have been grouped according to their family as grey (basic wine parameters), red (anthocyanins) and blue (procyanidins). Variable codes as detailed in Table 1 and 2.



**FIGURE 5.** Contribution of wine composition variables to Principal Component Analysis dimensions 1 and 2 in Tempranillo.

The first 2 components contribute to 71.33 % of the total variance. The commercial vessel (2500 L) was considered as a supplementary individual, i.e., not including it to calculate the principal components (PC) but evaluating its performance. Those variables contributing the most have been grouped according to their family as grey (basic wine parameters), red (anthocyanins) and blue (procyanidins). Variable codes as detailed in Table 1 and 2. In both cases ABV and ATT do not contribute to distinguish the small volume vessels. Contrarily, Anthocyanin’s contribution is needed to explain variables in Dimension 1.

**TABLE 10.** Procyanidin wine profile (M: monomers, D: dimers and T: trimers) for Cabernet-Sauvignon.

Analytes	C-2500 L	10 L	25 L	50 L	100 L
Gallic acid	34.3 ± 0.1 <sup>a</sup>	21.6 ± 2.4 <sup>c</sup>	31.9 ± 5.6 <sup>a</sup>	29.6 ± 1.2 <sup>a</sup>	25.7 ± 0.9 <sup>b</sup>
Cat	64.1 ± 0.4 <sup>a</sup>	21.5 ± 2.0 <sup>d</sup>	37.0 ± 8.6 <sup>b</sup>	33.6 ± 3.0 <sup>bc</sup>	29.6 ± 1.3 <sup>c</sup>
EC	35.8 ± 0.3 <sup>a</sup>	22.3 ± 2.7 <sup>b</sup>	33.0 ± 6.4 <sup>a</sup>	31.2 ± 3.5 <sup>a</sup>	26.0 ± 1.1 <sup>b</sup>
ECG	0.1 ± 0.0 <sup>c</sup>	0.6 ± 0.1 <sup>b</sup>	0.9 ± 0.1 <sup>a</sup>	1.2 ± 0.2 <sup>a</sup>	0.8 ± 0.2 <sup>a</sup>
Total M	134.3 ± 0.9 <sup>a</sup>	65.9 ± 7.2 <sup>c</sup>	102.8 ± 20.6 <sup>b</sup>	95.7 ± 7.9 <sup>b</sup>	82.1 ± 3.5 <sup>b</sup>
pdB1	20.7 ± 0.6 <sup>a</sup>	11.0 ± 0.7 <sup>c</sup>	15.8 ± 3.4 <sup>b</sup>	14.9 ± 0.7 <sup>b</sup>	13.5 ± 0.6 <sup>b</sup>
pdB2	11.3 ± 0.7 <sup>c</sup>	22.5 ± 2.5 <sup>b</sup>	31.2 ± 7.2 <sup>a</sup>	29.3 ± 2.4 <sup>a</sup>	24.3 ± 0.8 <sup>b</sup>
pdB3	8.7 ± 0.6 <sup>c</sup>	11.1 ± 0.9 <sup>b</sup>	15.6 ± 3.3 <sup>a</sup>	14.9 ± 0.5 <sup>a</sup>	13.5 ± 0.5 <sup>b</sup>
pdB4	19.7 ± 1.9 <sup>b</sup>	22.2 ± 2.1 <sup>b</sup>	31.0 ± 7.3 <sup>a</sup>	28.3 ± 1.5 <sup>a</sup>	23.9 ± 0.7 <sup>b</sup>
pdB2MG1	1.1 ± 0.1 <sup>c</sup>	3.6 ± 0.3 <sup>a</sup>	3.1 ± 0.8 <sup>b</sup>	3.0 ± 0.2 <sup>b</sup>	2.6 ± 0.3 <sup>b</sup>
pdB1G1	2.4 ± 0.1 <sup>c</sup>	3.7 ± 0.4 <sup>b</sup>	4.8 ± 0.9 <sup>a</sup>	4.7 ± 0.3 <sup>a</sup>	4.1 ± 0.2 <sup>b</sup>
DDG	0.4 ± 0.1 <sup>a</sup>	0.3 ± 0.0 <sup>a</sup>	0.2 ± 0.1 <sup>ab</sup>	0.2 ± 0.1 <sup>ab</sup>	0.2 ± 0.0 <sup>b</sup>
pdB1G2	9.8 ± 0.3 <sup>b</sup>	11.0 ± 0.3 <sup>b</sup>	15.3 ± 8.0 <sup>a</sup>	10.8 ± 11.1 <sup>b</sup>	16.9 ± 5.8 <sup>a</sup>
Total D	74.0 ± 4.3 <sup>b</sup>	85.5 ± 7.2 <sup>b</sup>	116.9 ± 31.0 <sup>a</sup>	106.2 ± 16.8 <sup>a</sup>	98.8 ± 9.0 <sup>a</sup>
ptC	4.7 ± 0.1 <sup>c</sup>	15.8 ± 1.2 <sup>b</sup>	20.1 ± 3.5 <sup>ab</sup>	19.1 ± 1.0 <sup>a</sup>	14.6 ± 8.4 <sup>ab</sup>
ptT2	15.7 ± 2.1 <sup>b</sup>	15.3 ± 1.4 <sup>b</sup>	22.0 ± 4.7 <sup>ab</sup>	19.7 ± 1.2 <sup>a</sup>	18.3 ± 0.7 <sup>ab</sup>
ptECG	17.4 ± 1.6 <sup>b</sup>	15.9 ± 2.3 <sup>ab</sup>	22.1 ± 4.7 <sup>a</sup>	19.9 ± 1.4 <sup>a</sup>	16.8 ± 0.6 <sup>b</sup>
Total T	37.8 ± 3.8 <sup>b</sup>	47.0 ± 5.0 <sup>a</sup>	64.2 ± 12.9 <sup>a</sup>	58.8 ± 3.6 <sup>a</sup>	49.7 ± 9.8 <sup>a</sup>

<sup>a</sup>Values with different letters denote a statistically significant difference ( $p < 0.05$ ). Results show the mean value and standard deviation. ptC (Procyanidin trimer C); GA(Gallic acid); pdB3 (Procyanidin dimer B3); pdB1 (Procyanidin dimer B1); ptT2 (Procyanidin trimer T2); Cat ((+)-Catechin); pdB4 (Procyanidin dimer B4); pdB2 (Procyanidin dimer B2); PdB2MG1(Procyanidin dimer B2-3-O-gallate); PdB2MG2 (Procyanidin dimer B2-3'-O-gallate); EC ((-)-Epicatechin); ptECG (Procyanidin trimer C1 (-)-epicatechin-3-O-gallate); pdB1G1 (Procyanidin dimer B1-3-O-gallate); DDG (Dimer digallate); ECG ((-)-Epicatechin-O-gallate); pdB1G2 (Procyanidin dimer B1-3'-O-gallate).

## 5. Sensory evaluation

The tasting chart attributes (intensity, quality, acidity, astringency, mature tannins, body, unctuousness and balance) were chosen specifically to evaluate the different sizes for optimal small-scale fermentations. Although the two varieties are very different from each other in terms of profile, all the descriptors are relevant for both varieties.

The ANOVA results for Tempranillo (TE) showed differences in almost all the attributes evaluated. TE shows (Table 11, Figure 6) a trend of different values resulting in different sized vessels, scoring lower raw data intensity on attributes such as nose quality and body in the smallest volume (TE10). Most astringency is related to the increased volume (TE50, TE100) whose performance would be similar to that found for the body attribute.

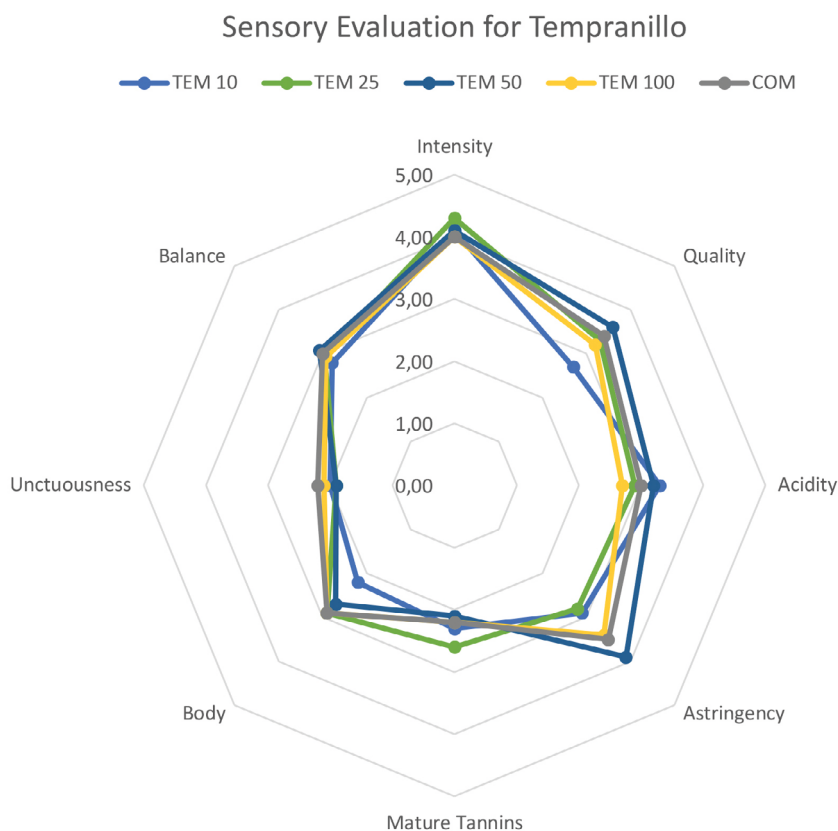
However, the most difficult element to assess was the mature tannin profile, showing little difference between treatments. Other attributes such as visual appearance, unctuousness and the overall perception showed insignificant differences.

CS data gathered from the expert panel showed fewer differences than TE between the attributes or aspects evaluated. As seen in Table 11 no differences were observed in the visual and nose attributes. Concerning body and overall balance, no appreciable differences were indicated. Only astringency, mature tannins and unctuousness seemed to vary between fermentation vessels. The highest astringency seems to be associated with 50 and 100 L, whilst mature tannins show less raw intensity with medium size fermentation vessels (25 and 50 L). There is also a trend away from unctuousness to score more in smaller volumes probably due to lower extraction.

**TABLE 11.** Sensory attributes. Raw data Intensity (1 to 5) for the Tempranillo attributes of each vessel.

Profile	Attribute	TE10	TE25	TE50	TE100
		Raw Data Intensity			
Visual	Intensity	4.10 ± 0.23	4.30 ± 0.21	4.10 ± 0.18	4.00 ± 0.23
Nose	Quality	2.70 ± 0.33 <sup>b</sup>	3.30 ± 0.40 <sup>ab</sup>	3.60 ± 0.31 <sup>a</sup>	3.20 ± 0.25 <sup>ab</sup>
	Acidity	3.30 ± 0.30 <sup>a</sup>	2.90 ± 0.23 <sup>b</sup>	3.20 ± 0.25 <sup>a</sup>	2.70 ± 0.26 <sup>b</sup>
Taste	Astringency	2.90 ± 0.31 <sup>b</sup>	2.80 ± 0.36 <sup>b</sup>	3.90 ± 0.18 <sup>a</sup>	3.40 ± 0.31 <sup>a</sup>
	Mature Tannins	2.30 ± 0.37 <sup>ab</sup>	2.60 ± 0.31 <sup>a</sup>	2.10 ± 0.18 <sup>ab</sup>	2.20 ± 0.33 <sup>ab</sup>
	Body	2.20 ± 0.20 <sup>b</sup>	2.90 ± 0.23 <sup>a</sup>	2.70 ± 0.26 <sup>a</sup>	2.90 ± 0.18 <sup>a</sup>
	Unctuousness	2.00 ± 0.15	1.90 ± 0.10	1.90 ± 0.23	2.10 ± 0.28
Overall Perception	Balance	2.79 ± 0.28	2.96 ± 0.28	3.07 ± 0.33	2.93 ± 0.25

Average of intensity judgments and standard errors of the intensity mean (SEM). Analysis of variance (ANOVA). Values with different letters in the same attribute are significantly different ( $p < 0.05$ ).



**FIGURE 6.** Sensory evaluation for Tempranillo wine assessing the main organoleptic descriptors from 5 different volume vessels (10 L, 25 L, 50 L, 100 L and 2500 L).

From these results (Table 12, Figure 7) it can be seen that as vessel volume increases up from 50 L, the wine seems to be more astringent (50-100 L). In Cabernet-Sauvignon, in particular, there were fewer differences between vessel volumes regarding previous chemical analysis

confirmed by the organoleptic analysis which reflects this trend as well. The volume of 10 L shows differences in chemical analysis from the other volumes corroborated in sensory analysis, and it also differs from the others because it is lighter in colour and body.

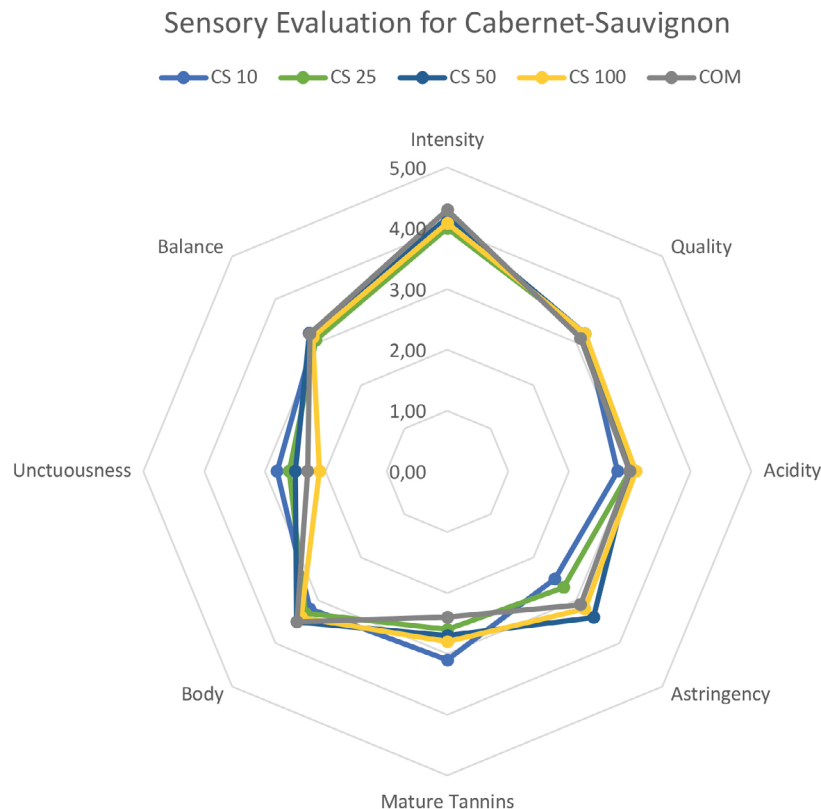
At this point of comparison between grape varieties, it is evident that the professional tasters did distinguish clear differences between the Tempranillo (TE) and the Cabernet-Sauvignon (CS) regarding how those varieties reacted to fermenting in different sized vessels. The professional panel found greater differences in the test results of TE than it did in the CS samples from the different sized test batches.

It is especially relevant and interesting that the tasting chart defines the score results regarding the perception of tannins on the palate. Because higher volumes extracted more quantity of tannins, the astringency attribute in CS is higher in 50 and 100 L. The smaller the vessel, the softer the tannins. Again TE showed a similar trend, where larger vessels also turned into more intense mouth filling.

**TABLE 12.** Raw data Intensity for the Cabernet attributes of each vessel.

		CS10	CS25	CS50	CS100
Profile	Attribute	Raw Data Intensity			
Visual	Intensity	4.08 ± 0.25	4.00 ± 0.23	4.17 ± 0.23	4.08 ± 0.21
Nose	Quality	3.20 ± 0.29	3.20 ± 0.20	3.20 ± 0.29	3.20 ± 0.25
Taste	Acidity	2.80 ± 0.29	3.00 ± 0.30	3.00 ± 0.30	3.10 ± 0.28
	Astringency	2.50 ± 0.17 <sup>b</sup>	2.70 ± 0.30 <sup>b</sup>	3.40 ± 0.27 <sup>a</sup>	3.20 ± 0.29 <sup>a</sup>
Taste	Mature tannins	2.10 ± 0.18 <sup>a</sup>	2.60 ± 0.22 <sup>b</sup>	2.70 ± 0.15 <sup>b</sup>	2.80 ± 0.25 <sup>ab</sup>
	Body	3.20 ± 0.25	3.30 ± 0.21	3.50 ± 0.17	3.40 ± 0.22
	Unctuousness	2.80 ± 0.25 <sup>a</sup>	2.60 ± 0.16 <sup>a</sup>	2.50 ± 0.17 <sup>ab</sup>	2.10 ± 0.23 <sup>b</sup>
Overall Perception	Balance	3.10 ± 0.19	3.06 ± 0.19	3.21 ± 0.21	3.13 ± 0.23

Average of intensity judgments and standard errors of the intensity mean (SEM). Analysis of variance (ANOVA). Values with different letters in the same attribute are significantly different ( $p < 0.05$ ).



**FIGURE 7.** Sensory evaluation for Tempranillo wine assessing the main organoleptic descriptors from 5 different volume vessels (10 L, 25 L, 50 L, 100 L and 2500 L).

## DISCUSSION

Although other studies investigating microscale fermentation have shown results using much smaller volumes (1 L), our contribution focuses on the relevance of volume fermentation size, even when larger volumes are considered. With regards to repeatability, all tank sizes proved to be adequate, since CV values were low in general. A certain trend of increased variability in 25 and 50 L tanks was observed, but the differences were small, and the CVs obtained were very satisfactory (usually below 15 % when determining phenolics and 5 % on grape and wine composition). This is an important result as one of the main concerns of researchers in viticulture and oenology is that reducing tank size in their experiments can increase variability during the fermentation stage, thus producing less reliable results. According to our data, decreasing the tank size from 100 L to 10 L does not cause an increase in variability and, therefore, the reliability of the results is very good.

However, having similar reliability in terms of variability does not mean that tank size did not affect wine typicality. For both varieties, we observed that the greatest volume was more representative of commercial-scale fermentation, particularly for anthocyanins (the first component in PCA). Thus, 10 L tanks achieved the lowest concentration of anthocyanin and phenol extraction into the wine, with the benefit of extraction of non-acylated anthocyanins. Non-acylated glycosides are more easily extracted, followed by acetyl glycosides, and *p*-coumaroyl. Alternately, procyanidins, included predominantly in the second component of PCA, were extracted in larger quantities in the commercial-sized tank, although 10 L, 25 L and 100 L showed similar scores for this component compared with commercial-scale wine. The pump-overs that takes place in commercial wine may have a different effect when compared to the gently hand-punched action used on the small scale. This may be due to the additional mechanical action of the pump, which does not apply to small volumes and leads to a much greater concentration of monomers moving into the wine. However, despite different extraction of monomers, dimers, and trimers, the total procyanidin content was more similar between tanks than that observed in the extraction of anthocyanins.

Tank size affected fermentation dynamics in both varieties, with the effects being clearer in CS tanks, where fermentation took place more slowly due to the smaller berry size. In both varieties,

the smallest tank (10 L) fermented the fastest (with no differences found between the remaining three sizes in TE) and gradually fermented more slowly as the tank size increased in CS.

Overall, according to our results, the smallest tank size used in this study could be sufficiently representative when the goal of winemaking is to compare different winemaking research (i.e., yeast performance and growth, microbiological assessment, wine fermentation kinetics), as variability was not affected by tank size. Nevertheless, when the objective of small-scale winemaking is to examine colour extraction and phenolic composition (i.e., validating red winemaking techniques or vineyards with variability due to orography) an increase in the tank volume (up to 50 L) is needed to obtain comparable results in colour and tannin extraction when comparing to commercial-scale wines.

In conclusion, small-scale winemaking is a valuable tool for viticulture and oenological research, although small-size tanks should only be used when the objective of the research is to compare different fields or winery treatments in relative terms. However, to approach the reality of wineries, the methodology used in this article helps to identify the true applicability between small-scale and large-scale fermentation to define the phenolic extraction of different grape styles for commercial wines. Larger volumes than 50 L must be used for evaluating the phenolic composition of red grapes, as small vessels would compromise research to estimate commercial phenolic extraction levels.

From the results of sensorial analyses it can be seen that as fermentation tank volume increases up from 50 L, the wine is more astringent (50–100 L). In Cabernet-Sauvignon, in particular, there were fewer differences between tank volumes regarding chemical analysis, and the organoleptic analysis reflects this trend as well. The volume of 10 L shows differences in chemical analysis from the other volumes corroborated in sensory analysis, and it also differs from the others because it is lighter in colour and body. At this point of comparison between grape varieties, it is evident that the professional tasters did distinguish clear differences between the Tempranillo (TE) and the Cabernet-Sauvignon (CS) wines regarding how those varieties reacted to fermenting in different sized vessels. The professional panel found greater differences in the test results of TE than it did in the CS samples from the different sized test batches.

The tasting notes also indicated the different mouth-feel of tannins in terms of green or ripe notes between the different volumes studied. The corroboration of the sensorial analysis with the HPLC results, and according to the objectives, lead us to suggest that the most appropriate small fermentation vessel is 50 L, to produce wines with properties and phenolic content comparable to results found in commercial winemaking.

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