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Application of high power ultrasounds during red wine vinification

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Summary

Wine color is one of the main organoleptic characteristics influencing its quality. It is of special interest in red vinifications due to the economic resources that wineries have to invest for the extraction of the phenolic compounds responsible for wine color, compounds that are mainly located inside the skin cell vacuoles, where the volatile compounds are also found. The transfer of phenolic compounds from grapes to must during vinification is closely related with the type of grapes and the winemaking technique. During traditional winemaking, grapes are crushed and skin macerated for several days, with pumps overs to facilitate the color extraction. To increase this extraction, some chemical (maceration enzymes) or physical technologies (thermovinification, cryomaceration, flash-expansion) can be applied. In this work, a new methodology has been tested. This methodology consists in the application of high power ultrasounds to crushed grapes to increase the extraction of phenolic compounds. Crushed grapes were treated with this non-thermal technology and vinified, with 3, 6 and 8 days of skin maceration time, and the results were compared

with a control vinification, where crushed grapes were not subjected to any treatment and were skin macerated during 8 days. The wine chromatic characteristics (determined spectrophotometrically) and the individual phenolic compounds (anthocyanins and tannins, determined by HPLC) were followed during the maceration period, at the end of alcoholic fermentation and after two months in bottle. Also, the wine volatile compounds were determined by GC-MS. The wines made with ultrasound treated grapes showed differences with the control wine, especially regarding total phenol content and tannin content. The wines elaborated with sonicated grapes and with only three days of skin maceration time presented similar concentration of anthocyanins and twice the concentration of tannins than control wines elaborated with 8 days of skin maceration.

Keywords: Ultrasound, phenolic compounds, proanthocyanidins, anthocyanidins, volatile compounds

Introduction

Red wine vinification implies the maceration of grape skins with the must, this step being one of the most important processes of this type of vinification. Red wine quality and its stability are mainly associated with the type and concentration of phenolic compounds in the grapes and with the extraction to must and wine that occurs during the skin fermentation period.

The optimum skin contact time needed to achieve the adequate level and composition of wine phenols depends on the desired wine style. It is commonly assumed that maximum anthocyanin and colour is achieved within 4 to 5 days of the start of the fermentation, but tannins and other flavonoids usually continue to be extracted from the pomace up to the end of fermentation (Sacchi et al., 2005).

For wineries, the maceration process is a very important technological part of the winemaking process due to the influence in the resulting wine and the economic

54 inputs that have to be used to extract the desirable substances from grape skins, not
55 only phenolic but also volatile compounds.

56 Therefore, to achieve a good and stable wine color and a desirable varietal
57 aroma a certain length of skin maceration is needed, in order to promote the extraction
58 of anthocyanins (responsible of the wine red color and located inside the cells in the
59 skin), tannins (located in skin and seeds, their presence is necessary for stabilizing the
60 unstable anthocyanins) and aroma compounds (also mainly located in the skin cells).
61 The maceration process, and therefore, the extraction of phenolics and aroma
62 compounds, starts when the grapes are crushed and it is facilitated when the ethanol
63 from the fermentation is present (Sacchi et al., 2005), so the necessary presence of
64 some ethanol determine the minimun length of the maceration. To facilitate the contact
65 of skin and must, pumping-overs are done frequently. Bautista-Ortín et al. (2004)
66 stated that the best chromatic characteristics of young Monastrell wines (as regard
67 color intensity and stability) were obtained with 10 days of skin maceration, shorter
68 times led to poor phenolic extraction and unstable colour.

69 However, sometimes problems appear for large wineries, especially in the
70 middle of the harvest time, when the capacity of the winery, especially regarding
71 maceration tanks, is exceeded due to the high quantities of grapes being transported to
72 the winery. In this case, the winery can be forced to reduce the maceration time and,
73 as a consequence, the quality of the wine and its potential for aging can be
74 compromised.

75 To face this problem some strategies have been used to shorten the maceration
76 time but maintaining color and wine quality. These techniques are mainly focused on
77 facilitating the disgregation of the cell walls of the skins (for an easier extraction of the
78 compounds located inside the cells) or facilitating the diffusion. Among these
79 techniques we can find the use of enzymes (Bautista-Ortín et al., 2005; Romero-
80 Cascales et al., 2008; Romero-Cascales et al., 2012) and the use of physical
81 methodologies as the thermovinification (de Andrade Neves et al., 2014; Jackson,

2000; Ribéreau-Gayon et al., 1998) or the flash release systems (Morel-Salmi et al., 2006).

Among the physical methodologies, thermovinification has been developed to enhance the extraction of phenolic components. This technique consists in warming the crushed grapes with a heat exchange column (70-85ºC). Heating leads to a better solubilization and diffusivity of cell components; however, it may cause a significant reduction in the levels of anthocyanins, flavonoids and total phenolic compounds after aging in the bottle (de Andrade Neves et al., 2014) and the apparition of cooked flavors and losses of volatile compounds (Geffroy et al., 2015) and it is also energy costly. The flash release systems consist in rapidly heating the grapes and then applying strong vacuum. This technique has also been proposed for increasing the polyphenol content of red wines. Its impact on polyphenol extraction kinetics and on the polyphenol composition of red juice and wines was studied by Morel-Salmi et al. (2006) and they stated that the flash release process allowed an initial fast extraction of phenolic compounds although the concentration of all polyphenols dramatically decreased throughout fermentation when pressing was achieved immediately after the flash release. If a pomace maceration time followed the application of the flash release, the wines were enriched in polyphenols compared to the corresponding control wines. However, in comparison to a standard vinification, a two-hour heat treatment at 70º C induced a significant loss in several grape-derived aroma compounds. Moreover, the process consumes relatively high quantities of energy.

Together with these well-known techniques, other different alternative must pretreatments, such as ultrasound, pulsed electric fields, and high voltage electrical discharges had been tested to enhance the extraction of phenolic compounds (El Darra et al., 2013). Among these last three techniques, ultrasound is the technology that is closer to be found in the market as an industrial commercial technology for optimizing the maceration process.

The ultrasound technology is based on mechanical waves at a frequency higher than the upper limit of human hearing (> 16kHz) that are transmitted through any substance which possesses elastic properties (Ferraretto et al., 2013). In the food industry, ultrasound can be divided into two frequency ranges: high frequency ultrasound (100 kHz-1 MHz) and power ultrasound (16-100 kHz). High frequency ultrasound is commonly applied as an analytical technique to provide information on the physicochemical properties of food such as ripeness, sugar content, acidity, etc (Demirdoven and Baysal, 2008) and power ultrasound can be used to generate emulsions, disrupt cells and disperse aggregated materials (Knorr et al., 2004; Tiwari et al., 2010).

In enology, power ultrasound may enhance the extraction of intracellular compounds from skin cells during vinification, in fact, some works can be found indicating that ultrasound application at 20–35 kHz enhances the extraction of polyphenols from red-grape residues (Tao, Zhang, & Sun, 2014) and from grape seeds (Da Porto et al., 2013). An extraction method using a sonication was developed to recover total phenolic compounds and anthocyanins from grape skins (Ghafoor and Choi, 2009). Furthermore, ultrasound-assisted extraction allowed the extraction of anthocyanins, condensed tannins and other phenolics present in grape in a very short time, compared with a classical solvent extraction (Carrera et al., 2012).

Other authors are exploring the possibilities of using power ultrasounds for accelerating the wine aging process (Zhang et al., 2016; Zhang et al., 2015) since the high temperatures and pressures generated by the collapse of cavitation bubbles can induce chemical reactions, and accelerate some reactions that usually occur during wine aging.

In this paper, we focused our attention on the application of a small scale power ultrasound system to crushed grapes, looking for a reduction of the maceration time needed for the extraction of phenolic and volatile compounds.

Materials and Methods

Grapes

Monastrell red grapes were harvested from vineyards in the province of Murcia (Spain) and they were transported the same day to the winery for their processing.

Winemaking (micro-vinification)

The grapes (200 kg) were destemmed and crushed. The crushed grapes were treated with a pilot scale power ultrasound system (MiniPerseo, Agrovin S.A., Alcazar de San Juan, Spain) that could treat 400 kg of crushed grapes per hour. The system operated at 2500 W and 28 kHz frequency, with a power density of 8 W/cm2. A batch of crushed grapes was not treated (control vinification). 10 kg stainless-steel small tanks were filled with the control and ultrasound treated crushed grapes. Must homogeneity in each tank was achieved weighting separately the solid parts and the liquid and filling each 10 L vessels with the same quantity and proportion to assure the same solid:liquid ratio in each vessel. Total acidity was corrected to 5.5 g/L and selected yeasts were added (Viniferm CT007, acidity Agrovin, Spain, 20 g of dry yeast/100 kg of grapes). Three different skin maceration times were tested for the sonicated must: 3 (SW3d), 6 (SW6d) and 8 (SW8d) days, whereas the control vinification had a skin maceration time of 8 days (CW). All vinifications were done in duplicate. Throughout the fermentation pomace contact period, the cap was punched down twice a day. At the end of this period, the wines were pressed. Free-run and press wines were combined and stored at room temperature. After alcoholic fermentation was finished, wines were cold stabilized at 2ºC for one month and bottled. Must and wines were

analysed after the ultrasound treatment, at the end of skin maceration, at the end of alcoholic fermentation and after two months in the bottle.

Analytical determinations

Determination of wine anthocyanins: This analysis was performed by direct injection of wine samples on a Waters 2695 liquid chromatograph (Waters, Milford, PA, USA), equipped with a Waters 2996 diode array detector and a Licrochart RP-18 column (Merck, Darmstadt, Germany), 25 x 0.4 cm, 5 µm particle size, using as solvents water plus 4.5% formic acid (solvent A) and HPLC grade acetonitrile (solvent B) at a flow rate of 0.8 mL/min. The chromatographic conditions were those described by Busse-Valverde et al. (2011). The anthocyanins were quantified at 520 nm as malvidin-3-glucoside, using malvidin-3-glucoside chloride as an external standard (Extrasynthese, Genay, France).

Determination of proanthocyanidins: Wine samples were prepared by an optimization of the method described by Pastor del Rio and Kennedy (2006) and the detailed methodology can be found in Busse-Valverde et al. (2010). Briefly, five mL of wine were evaporated in a centrivap concentrator (Labconco, USA), redissolved in 3 mL of water and then passed through a C18-SPE column (1 g, Waters, Milford, MA). The cartridge was washed with 20 mL of water, and compounds of interest were eluted with 10 mL of methanol, evaporated, and then dissolved in 1 mL of methanol. The analyses of proanthocyanidins were done by depolymerizing the molecule using the phloroglucinol reagent. The depolymerized samples (10 µL injection volume) were analyzed by HPLC. The elution conditions can be found in Busse-Valverde et al. (2010). Proanthocyanidin cleavage products were estimated using their response factors relative to (+)-catechin, which was used as the quantitative standard. These analyses allowed determination of the total proanthocyanidin content, the apparent

mean degree of polymerization (mDP) and the percentage of each constitutive unit. The mDP was calculated as the sum of all subunits (flavan-3-ol monomer and phloroglucinol adducts, in moles) divided by the sum of all flavan-3-ol monomers (in moles). Wine tannin mass conversion yield was also calculated at the end of alcoholic fermentation and after bottle storage, reporting a conversion yield of 37.98%±5.64 and 38.16%±5.70 respectively.

Spectrophotometric parameters: Colour intensity (CI) was calculated as the sum of absorbance at 620, 520 and 420 nm, and tint as the ratio between absorbance at 420 nm and absorbance at 520 nm. Total and polymeric anthocyanins were determined spectrophotometrically (Boulton, 2001). Total phenols (TP) were calculated by measuring wine absorbance at 280 nm, according to Ribereau-Gayon et al. (1998).

Isolation of wine and grape volatile compounds by SPME: For the isolation of volatile compounds by SPME, a divinylbenzene-carboxen-polydimethylsiloxane 50/30 micras (DVB/CAR/ PDMS) fiber was used. It was conditioned before the first use by insertion into the GC injector, as recommended by the manufacturer.

For the analysis of wine volatile compounds, 10 mL of wine were added to a 20 mL headspace vial. 4 g of sodium chloride and 50 µL of the internal standard (125 µL/L of 2-octanol in absolute ethanol) were added to the same vial. The vial was tightly sealed and loaded onto a Gerstel autosampling device (Gerstel GmbH & Co.KG, Mellinghofen, Germany). The program of the autosampling device consisted on swirling the vial at 500 rpm for 15 min at 40ºC, then inserting the fiber into the headspace for 30 min at 40ºC as the solution was swirled again, then transferring the fiber to the injector for desorption at 240ºC for 5 min. The conditions of the gas chromatograph and the mass spectra can be found in Gómez-Plaza et al. (2012). Injections were done in the splitless mode for 0.75 min, using a 2 mm I.D. non-deactivated direct liner for the SPME.

Peak identification was carried out by comparing mass spectra with those of the mass library (Wiley 6.0) and comparing the calculated retention indices with those published in the literature. Semiquantitative data were obtained by calculating the relative peak area (or TIC signal) in relation to that of the internal standard (2-octanol).

Statistical analysis

The Analysis of Variance and the Principal component analysis were carried out using the statistical package Statgraphics Centurion XVI.

Results and Discussion

The results of the chromatic parameters are shown in Table 1. The initial must already had large differences in total phenols, total anthocyanins and color intensity, the sonicated must presenting the highest values for these parameters. The control grapes were put in stainless steel tanks and the skins were separated from the must/wine after 8 days of contact time; the same was done for sonicated crushed grapes and the skins were separated from the must/wine after 3, 6 or 8 days of contact time. After that, alcoholic fermentation was completed without the skins. Comparing the chromatic characteristics of the different samples at the moment the skins were pressed-off, sonicated samples with only 3 days of skin maceration (SW3d) showed the highest values of the chromatic parameters, even when the control wine (CW8d) had 5 more days of skin contact time. The content of total anthocyanins for SW3d was also maximum and no differences were observed between the wines in the case of polymeric anthocyanins. The highest color intensity was also found in SW3d, and the lowest was observed in SW8d. It is clear that the sonication of the crushed grapes led to a disruption of the cell structures that facilitated the extraction of phenolic

248 compounds during the maceration process, but the length of the skin maceration
249 modified the final chromatic characteristics of the fermenting must.

250 At the end of alcoholic fermentation, we found a slight decrease in phenolic
251 content in the wines elaborated from sonicated crushed grapes (especially those with 3
252 and 6 days of skin contact time) although for SW3d and SW8d values were still higher
253 than those of control wine. At this moment, SW3d also presented higher total and
254 polymeric anthocyanins and color intensity whereas the other wines presented similar
255 color intensity. After two months in the bottle, the phenolic content did not significantly
256 decrease for any of the wines, although there was a decrease in anthocyanins and
257 color intensity. At that moment, SW3d still maintained its chromatic differences with all
258 the other wines.

259 Anthocyanins were also analysed by HPLC (Table 2). The results were quite
260 coincident with those of the spectrophotometric values. It was quite curious that, in the
261 must, just after sonication, the anthocyanins found at maximum concentration were
262 cyanidin-3-glucoside and peonidin-3-glucoside, both being the dihydroxylated
263 anthocyanins. Romero-Cascales et al. (2005) observed a similar behaviour during the
264 initial steps of the Monastrell maceration process. At the end of skin contact time and
265 alcoholic fermentation, the profile was similar to that described for the Monastrell wine
266 variety (Romero-Cascales et al., 2005), malvidin-3-glucoside being the monomeric
267 anthocyanin present at the highest quantity, the acylation percentage being around
268 10% and the sonication process not affecting the qualitative composition of the wine
269 anthocyanin profile. The maximum content of free anthocyanins was measured right
270 after the separation of skins, the quantities presented at the end of alcoholic
271 fermentation being slightly lower (around 22 and 30% lower), the lowest decrease
272 being observed in SW3d. After two months in the bottle, the measured content
273 decreased again, probably due to factors such as polymerization with other phenolic
274 compounds and oxidation reactions (Cano-López et al., 2008).

Since one of the main differences between control and sonicated musts and wines were related to total phenol content (as observed in Table 1), we were interested in the study of the concentration and type of tannins extracted from sonicated crushed grapes (Table 3). The analysis of tannins by phloroglucinolysis and HPLC gave us information on the total tannin content, the mean degree of polymerization of these tannins, the percentage of galloylation and the concentration of epigallocatechin, a subunit that only appears in the tannins extracted from grape skin since it is absent in seed tannins (Labarbe et al., 1999; Souquet et al., 1996) and can inform us of the proportion of skin tannins in the wines.

The results showed that the sonication of the crushed grape samples doubled the tannin concentration in must (in fact, tannins could not be detected in the first control must samples) and wines, these compounds being the most favoured by the ultrasound application, with no difference between the wines from sonicated grapes at the end of skin contact time, even when different maceration times were used. Differences with control wine were maintained at the end of alcoholic fermentation and no decreases in tannin content could be observed after two months in bottle. The percentage of galloylation was not affected by the sonication process and decreased after 2 months in the bottle, however, the content of epigallocatechin was higher in wines from sonicated grapes during all the studied period, indicating that the extraction of skin tannins was favoured by the ultrasound treatment. This could positively affect wine quality since skin tannins have frequently been described as “soft” or “ripe,” contrary to seed tannins, which have been associated with more aggressive and less desirable sensory descriptors like “green” or “hard.” No differences in mDP were observed between the different wines and its values slightly decreased at the end of alcoholic fermentation for all the wines.

Ferrareto et al. (2013) also studied, at laboratory scale, the effect of the application of ultrasound to crushed grapes on wine color. The maceration time for the ultrasound treated samples was 2, 3 and 4 days, while the time for the control sample

303 was 5 days. Coincident with our results, the sonicated crushed grapes led to musts
304 with higher polyphenol content in comparison with the reference at the beginning of
305 maceration. The same was found at the end of the process and the influence of the
306 sonication process on total polyphenols index was higher than on anthocyanins
307 content. These results are also similar to our findings, corroborating that tannin
308 extraction seems to be more favoured by the application of ultrasounds than
309 anthocyanin extraction. This could be related to the localization of tannins in skin cells,
310 ultrasound facilitating the liberation of those integrated in skin cell walls (Gagne et al.,
311 2006), and to the fact that ultrasound may also facilitate the extraction of seed tannins,
312 although probably to a lesser extent than favored skin tannins. Some authors have
313 already studied the positive effect of ultrasound in extracting tannins from grape seeds
314 (Da Porto et al., 2013).

315 Ferrareto et al. (2013) also reported that the treatment caused an enrichment of
316 the medium in colloidal fractions. The same phenomenon was observed in our
317 experiment, the fraction presumably being formed of polysaccharides and other
318 fractions from the cell walls. The high presence of suspended cell wall material in the
319 sonicated musts could explain why longer maceration techniques did not lead to higher
320 tannin content since the affinity of the suspended cell walls for tannins is a adsorption
321 mechanism clearly established and proved (Bautista-Ortin et al., 2014; Bindon et al.,
322 2010; Castro-Lopez et al., 2016). Therefore, in wines from sonicated grapes, longer
323 maceration time might have led to higher adsorption of tannins in the suspended cell
324 walls. But not only tannins might be adsorbed in cell wall but also anthocyanins
325 (Bautista-Ortin et al., 2016) and this fact could also explain the lowest anthocyanin
326 content of SW6d and SW8d compared with SW3d and CW8d.

327 El Darra et al. (2013) also compared the effect of different physical treatments
328 on Cabernet Franc red grapes. Crushed grapes were sampled and then subjected to a
329 laboratory ultrasound bath that enhanced the polyphenols yield, much more than a
330 thermovinification process at 50ºC. The effect of ultrasound is usually attributed to the

331 acoustic cavitations causing mechanical rupture of solid particles and cell structure,
332 increasing the contact surface area between the solid and liquid phase and, as a result,
333 permitting better diffusivity of solute from the solid phase to the media.

334 Even less studied than the effect of ultrasound on phenolic compounds is the
335 effect on the volatile composition. The results of the semiquantitative analysis of
336 volatile compounds of control and sonicated wines at 3 different maceration times (3, 6,
337 and 8 d) after two months in the bottle are presented in Table 4. The identified
338 compounds included alcohols, monoterpenes and norisoprenoids, acetates and fatty
339 acid ethyl esters, three ketones and fatty acids. No significant differences were
340 observed in the total concentration of volatile compounds, neither due to the sonication
341 nor to the maceration length.

342 Acetates of higher alcohols and fatty acids ethyl esters contribute to aroma of
343 young wines, exhibiting floral and fruity odors. Although no significant differences were
344 observed in the sum of these compounds, the quantities of esters were slightly higher
345 in control than in the sonicated wines, where their quantities seemed to be slightly
346 reduced as maceration time increased. It has been reported that long maceration time
347 could generate a decrease of esters, probably as a result of nonenzymatic hydrolysis
348 (Rapp, 1988). Furthermore, during the maceration and alcoholic fermentation, fatty
349 acids could be used by the yeast as a carbon source, causing a decline of their
350 amounts.

351 Fatty acid production is associated with the initial must composition and the
352 fermentation conditions and was higher in SW3d (although not differing statistically
353 from control wine). The concentration of acids decreased in SW6d and SW8d.

354 The alcohol fraction was mainly composed of four compounds present in
355 highest amount: 3-methyl-1-butanol, 2-methyl-1-propanol, phenylethyl alcohol and 1-
356 hexanol, which is in agreement with literature data (Petropulos et al., 2014). It was
357 observed that the concentration of higher alcohols slightly increased with sonication
358 and with maceration time although, again, the differences were not significant. Contrary

359 to these results, it has been reported that the production of higher alcohols declines
360 with maceration time, because of blockage of the Ehrlich mechanism, the main
361 pathway for the formation of these compounds (Rapp and Versini, 1996).

362 Terpenes and norisoprenoids are considered to be closely related to the variety
363 and are important for the expression of varietal characteristics in wine. Both terpenes
364 and norisoprenoids have a low olfactory threshold and are generally associated with
365 floral and citric aromas. In the studied wines, several terpenes were detected, the
366 largest quantity being found in the sesquiterpene nerolidol and the monoterpenes
367 linalool and citronellol. Contrary to what was expected, wines from sonicated grapes
368 did not show higher concentration of these compounds than control wines, however,
369 the levels of terpens and norisoprenoids in SW3d did not significantly differ from those
370 of control wines made with 8 days of skin maceration.

371 A Principal component analysis was conducted, using all the measured
372 chromatic parameters, the total concentration of anthocyanins determined by HPLC
373 and, given the large number of identified compounds, the sum of the different families
374 of volatile compounds as variables. The objective was to find out how the wine
375 samples, after two months in the bottle, were grouped and which variables were
376 responsible for the grouping. This analysis reduced the information provided by all the
377 measured variables to two principal components, which explained 78% of the variability
378 of the data (Figure 1). The analysis clearly showed how different the wines were.
379 Control wines and those elaborated from sonicated grapes were separated along PC1,
380 the control wines presenting lower values of the chromatic parameters but, in general,
381 slightly higher content of volatile compounds. Wines from sonicated grapes were
382 separated along PC2, SW3d located in the positive part of PC2 and being
383 characterized by the highest values in total anthocyanins and color intensity. SW6d and
384 SW8d were located in the negative part of PC2, being characterized by their higher
385 content of total tannins and phenols.

386 The results of the application of this small scale industrial ultrasound system to
387 crushed grapes indicate that this technology facilitates the extraction of phenolic
388 compounds from grape to must. It could be applied as a continuous pre-treatment of
389 crushed red grapes, before loading the maceration-vinification tanks, representing a
390 possibility to optimize winery capacity by reducing the skin maceration time without
391 losing the quality characteristics of the obtained wines.
392
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396
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Table 1. Chromatic characteristics of the control and sonicated musts and wines

Sample	TP	TA (mg/L)	PA (mg/L)	CI	Tint
Initial must					
Control must	9.9 ± 0.5^{a}	4.6 ± 0.0^{b}	2.3 ± 0.0^{b}	1.1 ± 0.0^{a}	2. 01 ± 0.0^{b}
Sonicated must	23.2 ± 0.8^{b}	124.0 ± 0.0^{b}	4.1 ± 0.1^{b}	3.2 ± 0.0^{b}	0.83 ± 0.0^{a}
End of skin maceration					
Control wine (8 days)	33.1 ± 1.3^{a}	265.7 ± 5.6^{a}	10.5 ± 0.7^{a}	6.2 ± 0.3^{ab}	0.51 ± 0.0^{b}
Sonicated wine (3 days)	50.3 ± 2.6^{b}	363.6 ± 24.9^{b}	10.8 ± 0.6^{a}	8.9 ± 0.4^{c}	0.46 ± 0.0^{a}
Sonicated wine (6 days)	47.5 ± 3.3^{b}	283.4 ± 1.6^{a}	11.1 ± 0.3^{a}	6.6 ± 0.2^{b}	0.52 ± 0.0^{b}
Sonicated wine (8 days)	45.2 ± 1.3^{b}	247.5 ± 8.9^{a}	10.8 ± 0.1^{a}	5.7 ± 0.0^{a}	0.56 ± 0.0^{c}
End of alcoholic fermentation					
Control wine (8 days)	34.7 ± 3.2^{a}	233.3 ± 1.6^{ab}	13.8 ± 1.1^{a}	6.3 ± 0.1^{b}	0.57 ± 0.0^{a}
Sonicated wine (3 days)	43.6 ± 1.6^{b}	266.9 ± 20.1^{b}	20.4 ± 2.1^{a}	7.9 ± 0.5^{c}	0.54 ± 0.0^{a}
Sonicated wine (6 days)	39.8 ± 6.5^{b}	224.8 ± 2.4^{ab}	17.4 ± 3.2^{a}	6.3 ± 0.9^{b}	0.62 ± 0.0^{b}
Sonicated wine (8 days)	44.5 ± 1.3^{b}	213.9 ± 16.1^{a}	14.1 ± 1.6^{a}	5.4 ± 0.3^{a}	0.65 ± 0.0^{b}
Two months in bottle					
Control wine (8 days)	31.8 ± 2.4^{a}	180.9 ± 4.8^{a}	4.0 ± 0.8^{a}	4.8 ± 0.7^{a}	0.66 ± 0.0^{a}
Sonicated wine (3 days)	41.9 ± 1.6^{b}	207.7 ± 8.9^{b}	6.4 ± 1.1^{b}	6.3 ± 0.5^{b}	0.67 ± 0.0^{a}
Sonicated wine (6 days)	38.1 ± 1.4^{b}	173.5 ± 4.0^{a}	5.6 ± 0.3^{ab}	5.5 ± 0.1^{ab}	0.74 ± 0.0^{b}
Sonicated wine (8 days)	42.6 ± 0.8^{b}	165.6 ± 18.5^{a}	4.7 ± 0.7^{ab}	4.8 ± 0.0^{a}	0.77 ± 0.0^{b}

TP: total phenols, TA: total anthocyanins, PA: polymeric anthocyanin, CI: color intensity

Different letters within same column and for each of the different moments of sampling indicate significant differences ($p<0.05$) according to a LSD test

Table 2. Anthocyanin content (mg/L) of the different control and sonicated musts and wine samples

Sample	Del-Glu	Cyan-Glu	Pet-Glu	Peon-Glu	Malv-Glu	AAT	AT
Initial must							
Control must	nd	2.40±1.84^{a}	0.00±0.00^{a}	6.70±1.29^{a}	20.86±5.55^{a}	0.00±0.0^{a}	31.27±8.69^{a}
Sonicated must	1.99±0.84^{b}	21.49±1.44^{b}	8.71±2.03^{b}	24.66±1.08^{b}	97.92±4.70^{b}	6.14±0.05^{b}	168.55±14.33^{b}
End of skin maceration							
Control wine (8 days)	19.97±3.73^{ab}	8.22±0.35^{ab}	39.09±3.81^{ab}	16.22±0.02^{ab}	183.43±8.67^{b}	25.97±1.99^{b}	295.53±18.53^{b}
Sonicated wine (3 days)	24.15±1.47^{b}	14.54±0.83^{c}	39.71±3.26^{b}	25.03±1.77^{c}	204.08±9.83^{c}	33.88±1.12^{c}	340.35±18.27^{c}
Sonicated wine (6 days)	14.18±0.19^{a}	9.11±0.41^{b}	29.39±1.49^{a}	18.96±0.43^{b}	166.22±0.43^{ab}	23.95±0.42^{ab}	261.93±2.53^{ab}
Sonicated wine (8 days)	15.24±2.25^{a}	7.31±0.55^{a}	30.57±2.78^{a}	14.94±0.58^{a}	156.16±8.74^{a}	21.54±2.07^{a}	247.35±16.98^{a}
End of alcoholic fermentation							
Control wine (8 days)	10.97±1.80^{a}	5.28±0.32^{ab}	26.13±2.09^{ab}	13.01±0.55^{a}	151.34±0.72^{b}	21.07±0.15^{b}	229.08±3.89^{b}
Sonicated wine (3 days)	17.70±3.50^{b}	5.84±0.79^{b}	28.41±2.45^{b}	12.38±1.16^{a}	142.27±5.27^{b}	21.15±3.86^{b}	225.27±9.30^{b}
Sonicated wine (6 days)	9.59±1.23^{a}	5.46±0.00^{ab}	21.71±2.09^{a}	12.41±0.34^{a}	121.58±2.54^{a}	10.52±1.57^{a}	182.14±0.45^{a}
Sonicated wine (8 days)	8.19±1.97^{a}	5.29±0.18^{a}	17.43±2.20^{a}	12.63±0.65^{a}	127.12±9.88^{a}	11.75±1.52^{a}	181.04±8.11^{a}
Two months in bottle							
Control wine (8 days)	9.59±0.70^{ab}	3.49±0.22^{a}	16.95±1.94^{ab}	7.71±1.05^{a}	87.75±13.85^{a}	8.68±1.51^{a}	134.17±19.27^{b}
Sonicated wine (3 days)	12.11±0.41^{b}	4.16±0.45^{a}	20.42±0.83^{b}	9.44±0.65^{a}	96.36±7.20^{a}	12.60±1.42^{a}	155.08±9.24^{b}
Sonicated wine (6 days)	7.18±0.50^{a}	3.31±0.02^{a}	12.89±0.81^{a}	7.53±0.31^{a}	66.66±5.51^{a}	7.62±0.56^{a}	105.20±6.63^{a}
Sonicated wine (8 days)	8.92±2.13^{a}	3.85±0.76^{a}	16.61±3.32^{ab}	8.81±2.55^{a}	88.06±18.54^{a}	9.59±2.25^{a}	135.83±29.56^{b}

Del-Glu: delphinidin-3-glucoside, Cyan-Glu: cyanidin-3-glucoside, Pet-Glu: petunidin-3-glucoside, Peon-Glu: peonidin-3-glucoside, Malv-Glu: malvidin-3-glucoside, AAT: total acylated anthocyanins, AT: sum of anthocyanins

Different letters within same column and for each of the different moments of sampling indicate significant differences ($p<0.05$) according to a LSD test

Table 3. Tannin content (mg/L) and composition of the control and sonicated musts and wine samples

Sample	TT	mDP	%Galoyllation	EGC (mM)
Initial must				
Control must	nd	--	--	--
Sonicated must	297.8 ± 13.8	3.8 ± 0.0	6.1 ± 0.1	105.2 ± 0.5
End of skin maceration				
Control wine (8 days)	448.4 ± 96.0^{a}	4.1 ± 0.1^{a}	4.9 ± 0.4^{a}	221.7 ± 52.1^{a}
Sonicated wine (3 days)	861.7 ± 32.5^{b}	4.3 ± 0.1^{b}	5.0 ± 0.0^{a}	350.2 ± 17.0^{b}
Sonicated wine (6 days)	948.9 ± 70.4^{b}	4.1 ± 0.1^{a}	5.4 ± 0.1^{a}	331.9 ± 12.9^{b}
Sonicated wine (8 days)	927.9 ± 76.2^{b}	4.0 ± 0.1^{a}	5.0 ± 0.2^{a}	331.3 ± 37.4^{b}
End of alcoholic fermentation				
Control wine (8 days)	397.5 ± 46.8^{a}	3.4 ± 0.2^{a}	5.3 ± 0.6^{a}	182.1 ± 28.7^{a}
Sonicated wine (3 days)	774.7 ± 25.5^{b}	3.9 ± 0.1^{b}	4.8 ± 0.0^{a}	329.2 ± 29.3^{b}
Sonicated wine (6 days)	926.1 ± 107.4^{bc}	3.8 ± 0.0^{b}	5.3 ± 0.2^{a}	306.6 ± 16.8^{b}
Sonicated wine (8 days)	941.4 ± 57.9^{c}	3.8 ± 0.0^{b}	5.3 ± 0.1^{a}	310.0 ± 27.0^{b}
Two months in bottle				
Control wine (8 days)	379.1 ± 46.6^{a}	3.7 ± 0.0^{a}	4.1 ± 0.4^{b}	195.1 ± 25.6^{a}
Sonicated wine (3 days)	821.3 ± 87.4^{b}	4.3 ± 0.1^{b}	3.3 ± 0.1^{a}	352.1 ± 52.8^{b}
Sonicated wine (6 days)	902.5 ± 28.5^{b}	4.1 ± 0.0^{b}	3.6 ± 0.2^{ab}	313.6 ± 36.9^{b}
Sonicated wine (8 days)	905.1 ± 106.8^{b}	4.1 ± 0.0^{b}	3.6 ± 0.1^{ab}	312.7 ± 50.7

TT: total tannins, mDP: mean degree of polymerization, EGC (mM): concentration of epigallocatechin

Different letters within same column and for each of the different moments of sampling indicate significant differences ($p<0.05$) according to a LSD test

Table 4. Semiquantitative analysis of the volatile compounds in the different control and sonicated samples

	Control wine	Sonicated wine (3d)	Sonicated wine (6d)	Sonicated wine (9d)
Esters				
2-Methyl propyl acetate	33.84[b]*	9.39[a]	7.54[a]	6.60[a]
Ethyl butanoate	14.33[a]	17.80[c]	13.56[a]	16.23[b]
Ethyl 2-methylbutanoate	6.02[a]	4.92[a]	4.39[a]	6.09[a]
Ethyl 3-methylbutanoate	10.64[b]	4.40[a]	4.53[a]	5.32[a]
3-Methyl butanol acetate	505.42[b]	164.42[a]	142.05[a]	123.63[a]
2-Methyl butanol acetate	14.58[b]	4.14[a]	2.87[a]	2.65[a]
Pentil acetate	3.99[a]	4.68[a]	3.30[a]	3.43[a]
3-Methyl butanol butanoate	1.19[a]	1.24[a]	1.04[a]	0.96[a]
Ethyl hexanoate	429.00[a]	528.00[a]	488.75[a]	512.50[a]
Ethyl heptanoate	21.92[b]	14.42[a]	15.62[a]	15.16[a]
Ethyl lactate	42.65[a]	32.02[a]	40.00[a]	44.79[a]
Methyl octanoate	25.21[a]	19.89[a]	20.28[a]	21.40[a]
Ethyl octanoate	2260.99[a]	2482.74[a]	2247.37[a]	2158.24[a]
Isopentyl hexanoato	39.86[b]	10.72[a]	35.48[b]	38.38[b]
Ethyl nonanoate	56.19[a]	72.75[ab]	67.94[ab]	82.38[b]
Methyl decanoate	27.11[a]	25.37[a]	25.64[a]	28.38[a]
Ethyl decanoate	1275.68[a]	1620.80[b]	1408.97[ab]	1437.06[ab]
3-Methylbutanol octanoate	35.02[a]	84.71[b]	88.51[b]	84.36[b]
Diethyl succinate	491.56[b]	179.00[a]	441.38[b]	528.13[b]
Ethyl 9-decenoate	64.38[a]	104.61[b]	60.83[a]	57.02[a]
Ethyl undecanoate	53.41[a]	43.16[a]	41.65[a]	48.96[a]
Ethyl benzene acetate	18.32[a]	89.23[c]	nd	35.74[b]
2-Phenyl ethyl acetate	197.42[b]	nd	41.74[a]	nd
Ethyl dodecanoate	227.47[a]	239.01	217.17[a]	257.56[a]
Ethyl benzenepropanoate	36.25[a]	39.55[a]	35.45[a]	nd
3-Methyl butyl 2-hydroxybenzoate	15.32[a]	13.33[a]	12.80[a]	17.05[a]

Ethyl 3-hydroxydodecanoate	11.18[a]	14.53[a]	12.77[a]	15.66[a]
Ethyl cinnamaate	16.59[b]	6.90[a]	5.78[a]	7.67[a]
3-Methylbutyl benzoate	10.72[a]	13.30[a]	nd	nd
2-Propenyl benzeneacetate	7.96[b]	7.97[b]	4.73[a]	8.82[b]
Ethyl hexadecanoate	12.69[a]	20.48[b]	nd	11.86[a]
Ethyl hydrogen succinate	53.94[b]	23.28[a]	nd	nd
Sum esters	6020.87[b]	5896.76[b]	5492.14[a]	5581.67[a]
Alcohols				
Propanol	2.41[a]	2.47[a]	2.05[a]	3.30[a]
2-Methyl propanol	189.00[a]	199.67[ab]	209.50[bc]	223.50[c]
3-Methyl butanol	2224.80[a]	2197.15[a]	2223.63[a]	2324.04[a]
4-Methyl pentanol	1.26[a]	1.23[a]	1.41[a]	1.39[a]
Hexanol	109.98[a]	143.84[b]	154.79[c]	163.49[c]
3-Ethoxy-2-propanol	0.56[a]	1.86[a]	1.12[a]	2.01[a]
3-Hexen-1-ol	1.67[a]	1.84[a]	2.42[a]	2.30[a]
2-Ethyl hexanol	12.11[a]	9.40[a]	11.79[a]	10.79[a]
2-Nonanol	12.05[a]	23.00[b]	22.45[b]	24.37[b]
2.3-Butanodiol	40.35[a]	36.58[a]	37.54[a]	37.06[a]
Octanol	52.22[a]	92.03[b]	105.82[c]	107.18[c]
2.3 Butanodiol	31.14[a]	33.65[a]	36.76[a]	39.97[a]
4-Methyl guaiacol	28.46[a]	20.22[a]	19.30[a]	23.78[a]
Nonanol	38.20	nd	nd	nd
Methyl thiopropanol	45.42[b]	nd	34.57[a]	37.81[a]
2-Phenylethanol	1799.07[a]	1797.73[a]	1769.88[a]	1714.33[a]
4-Ethylguaiacol	31.79[a]	74.39[b]	157.13[c]	109.29[c]
Benzene propanol	12.38[b]	6.09[a]	8.03[a]	7.40[a]
4-Ethyl phenol	32.25[a]	186.95[b]	243.08[b]	176.28[b]
Sum alcohols	4679.55[a]	4849.11[a]	5058.06[a]	5025.60[a]
Ketones				
3-Hydroxy-2-butanone	32.65[b]	14.24[a]	11.86[a]	11.59[a]
2-Octanone	18.65[a]	21.92[a]	19.65[a]	20.00[a]

furanone	10.36[a]	6.11[a]	9.58[a]	9.97[a]
Sum ketones	61.66[b]	42.27[ab]	41.09[a]	41.56[a]
Acids				
Acetic acid	51.64[b]	77.67[c]	108.75d	39.34[a]
Methoxy benzofuran. 2 carboxilic acid	84.28[a]	71.67[a]	66.77[a]	74.13[a]
Hexanoic acid	66.33[a]	72.96[a]	71.55[a]	72.77[a]
Heptanoic acid	19.73[a]	14.60[a]	12.06[a]	17.10[a]
Octanoic acid	494.22[ab]	534.28[b]	476.18[a]	465.17[a]
Nonanoic acid	25.93[a]	26.65[a]	nd	27.16[a]
Decanoic acid	264.69[ab]	294.50[b]	245.09[a]	254.78[a]
Dodecanoic acid	9.98[a]	13.21[a]	nd	12.38[a]
Sum acids	1016.79[ab]	1105.53[b]	980.39[a]	962.83[a]
Terpens and norisoprenoids				
Carene	19.40	15.47	14.80	14.78
Limonene	25.90	23.70	22.96	24.72
Ionone	124.20[b]	94.47[a]	75.33[a]	93.83[a]
Linalool	51.14	46.67	44.66	46.29
Ddihydro-α-ionona	33.47	28.87	27.16	30.15
Citronellol	89.54[a]	109.74[b]	105.00[b]	109.25[b]
Damascenone	168.00[c]	137.78[b]	127.24[a]	116.79[a]
Nerolidol	130.25[b]	135.62[b]	86.92[a]	109.53[a]
Farnesol	12.38[ab]	15.12[b]	9.86[a]	nd
Sum	654.28[c]	607.43[bc]	513.94[a]	545.34[a]
Totals	12433.15[a]	12501.09[a]	12085.62[a]	12156.99[a]

***** different letters within the same row indicates significant differences ($p<0.05$)

FIGURE CAPTION

Figure 1. Biplot representation of the Principal component analysis showing the distribution of the wine samples along component 1 and 2.