



Original article

Application of high-power ultrasounds during red wine vinification

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Summary Wine colour 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 colour, 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 to 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 colour 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 Anthocyanidins, phenolic compounds, proanthocyanidins, ultrasound, 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 4–5 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 inputs that have to be used to extract the desirable substances from grape skins, not only phenolic but also volatile compounds.

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

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

To face this problem some strategies have been used to shorten the maceration time but maintaining colour and wine quality. These techniques are mainly focused on facilitating the disaggregation of the cell walls of the skins (for an easier extraction of the compounds located inside the cells) or facilitating the diffusion. Among these techniques, we can find the use of enzymes (Bautista-Ortín *et al.*, 2005; Romero-Cascales *et al.*, 2008, 2012) and the use of physical methodologies as the thermovinification (Ribéreau-Gayon *et al.*, 1998; Jackson, 2000; de Andrade Neves *et al.*, 2014) 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 (70–85 °C). Heating leads to a better solubilisation and diffusivity of cell components; however, it may cause a significant reduction in the levels of anthocyanins, flavonoids and total phenolic compounds after ageing in the bottle (de Andrade Neves *et al.*, 2014) and the apparition of cooked flavours 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 with a standard vinification, a two-hour heat treatment at 70 °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 optimising the maceration process.

The ultrasound technology is based on mechanical waves at a frequency higher than the upper limit of human hearing (>16 kHz) 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 (Demirdoven & 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 *et al.*, 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 & 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 ageing process (Zhang *et al.*, 2015, 2016) as 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 ageing.

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 (microvinification)

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 cm^{-2} . 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^{-1} and selected yeasts were added (Viniferm CT007, acidity Agrovin, Spain, 20 g of dry yeast per 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-stabilised at $2 \text{ }^{\circ}\text{C}$ 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), $25 \times 0.4 \text{ cm}$, $5 \mu\text{m}$ 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^{-1} . 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 optimisation of the method described by Pastor del Rio & Kennedy (2006) and the detailed methodology can be found in Busse-Valverde *et al.* (2010). Briefly, 5 mL of wine was evaporated in a centrivap concentrator (Labconco, Kansas City, MO, USA), redissolved in 3 mL of water and then passed through a C18-SPE column (1 g, Waters). 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 depolymerising the molecule using the phloroglucinol reagent. The depolymerised samples ($10 \mu\text{L}$ injection volume) were analysed 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 polymerisation (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 mean value for the conversion yield of 39.5% and 38.2%, 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 Ribéreau-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

microns (DVB/CAR/PDMS) fibre 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 was added to a 20-mL headspace vial. 4 g of sodium chloride and 50 μ L of the internal standard (125 μ L L⁻¹ 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, Mellminghofen, Germany). The program of the autosampling device consisted on swirling the vial at 500 r.p.m. for 15 min at 40 °C, then inserting the fibre into the headspace for 30 min at 40 °C as the solution was swirled again, then transferring the fibre to the injector for desorption at 240 °C 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 colour 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 five 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 colour 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 compounds during the maceration process, but the length of the skin maceration modified the final chromatic characteristics of the fermenting must.

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

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

As 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 polymerisation of these tannins, the percentage of galloylation and the concentration of epigallocatechin, a subunit that only

Table 1 Chromatic characteristics of the control and sonicated musts and wines

Sample	TP	TA (mg L ⁻¹)	PA (mg L ⁻¹)	CI	Tint
Initial must					
Control must	9.9 ± 0.5 ^a	4.6 ± 0.0 ^a	2.3 ± 0.0 ^a	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, colour 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⁻¹) 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.

appears in the tannins extracted from grape skin as it is absent in seed tannins (Souquet *et al.*, 1996; Labarbe *et al.*, 1999) 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.

Table 3 Tannin content (mg L⁻¹) and composition of the control and sonicated musts and wine samples

Sample	TT	mDP	% Galoyllation	EGC (µM)	% Conversion
Initial must					
Control must	nd	–	–	–	
Sonicated must	297.8 ± 13.8	3.8 ± 0.0	6.1 ± 0.1	105.2 ± 0.5	n.a.
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	n.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	n.a.
Sonicated wine (6 days)	948.9 ± 70.4 ^b	4.1 ± 0.1 ^a	5.4 ± 0.1 ^a	331.9 ± 12.9 ^b	n.a.
Sonicated wine (8 days)	927.9 ± 76.2 ^b	4.0 ± 0.1 ^a	5.0 ± 0.2 ^a	331.3 ± 37.4 ^b	n.a.
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	29.4
Sonicated wine (3 days)	774.7 ± 25.5 ^b	3.9 ± 0.1 ^b	4.8 ± 0.0 ^a	329.2 ± 29.3 ^b	37.5
Sonicated wine (6 days)	926.1 ± 107.4 ^{bc}	3.8 ± 0.0 ^b	5.3 ± 0.2 ^a	306.6 ± 16.8 ^b	48.5
Sonicated wine (8 days)	941.4 ± 57.9 ^c	3.8 ± 0.0 ^b	5.3 ± 0.1 ^a	310.0 ± 27.0 ^b	41.8
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	28.5
Sonicated wine (3 days)	821.3 ± 87.4 ^b	4.3 ± 0.1 ^b	3.3 ± 0.1 ^a	352.1 ± 52.8 ^b	40.1
Sonicated wine (6 days)	902.5 ± 28.5 ^b	4.1 ± 0.0 ^b	3.6 ± 0.2 ^{ab}	313.6 ± 36.9 ^b	40.9
Sonicated wine (8 days)	905.1 ± 106.8 ^b	4.1 ± 0.0 ^b	3.6 ± 0.1 ^{ab}	312.7 ± 50.7	43.2

TT, total tannins; mDP, mean degree of polymerisation; EGC (µM), concentration of epigallocatechin; n.a., not available.

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.

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 as 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.

Ferraretto *et al.* (2013) also studied, at laboratory scale, the effect of the application of ultrasound to crushed grapes on wine colour. The maceration time for the ultrasound-treated samples was 2, 3 and 4 days, while the time for the control sample was 5 days. Coincident with our results, the sonicated crushed grapes led to musts with higher polyphenol content in comparison with the reference at the beginning of maceration. The same was found at the end of the process and the influence of the sonication process on total polyphenols index was higher than on anthocyanins content. These results are also similar to our findings, corroborating that tannin extraction seems to be more favoured by the application of ultrasounds

than anthocyanin extraction. This could be related to the localisation of tannins in skin cells, ultrasound facilitating the liberation of those integrated in skin cell walls (Gagne *et al.*, 2006), and to the fact that ultrasound may also facilitate the extraction of seed tannins, although probably to a lesser extent than favoured skin tannins. Some authors have already studied the positive effect of ultrasound in extracting tannins from grape seeds (Da Porto *et al.*, 2013).

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

El Darra *et al.* (2013) also compared the effect of different physical treatments on Cabernet Franc red grapes. Crushed grapes were sampled and then

Table 4 Semiquantitative analysis of the volatile compounds in the different control and sonicated samples (µg/L)

	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 hexanoate	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 cinnamate	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-Butanediol	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 Butanediol	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 ^a	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

Table 4 (Continued)

	Control wine	Sonicated wine (3d)	Sonicated wine (6d)	Sonicated wine (9d)
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	108.75 ^d	39.34 ^a
Methoxy benzofuran-2 carboxylic 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
Terpenes 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
Dihydro- α -ionone	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
Total	12433.15 ^a	12501.09 ^a	12085.62 ^a	12156.99 ^a

*Different letters within the same row indicate significant differences ($P < 0.05$).

subjected to a laboratory ultrasound bath that enhanced the polyphenols yield, much more than a thermovinification process at 50 °C. The effect of ultrasound is usually attributed to the acoustic cavitations causing mechanical rupture of solid particles and cell structure, increasing the contact surface area between the solid and liquid phase and, as a result, permitting better diffusivity of solute from the solid phase to the media.

Even less studied than the effect of ultrasound on phenolic compounds is the effect on the volatile

composition. The results of the semiquantitative analysis of volatile compounds of control and sonicated wines at three different maceration times (3, 6, and 8 days) after two months in the bottle are presented in Table 4. The identified compounds included alcohols, monoterpenes and norisoprenoids, acetates and fatty acid ethyl esters, three ketones and fatty acids. No significant differences were observed in the total concentration of volatile compounds, neither due to the sonication nor due to the maceration length.

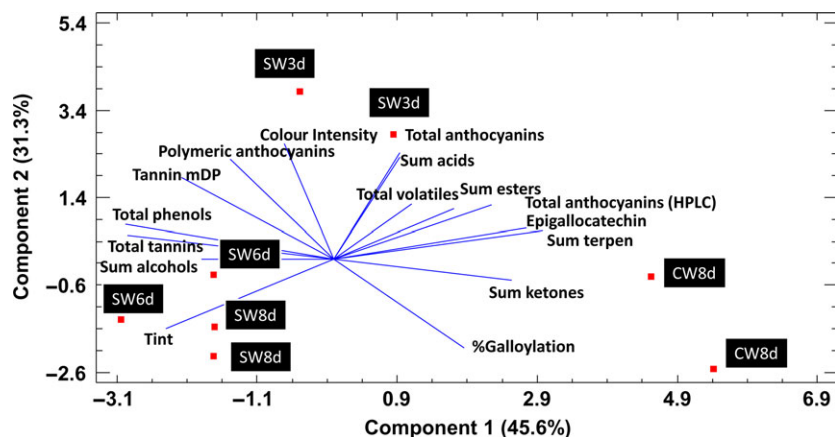


Figure 1 Biplot representation of the principal component analysis showing the distribution of the wine samples along components 1 and 2.

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

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

The alcohol fraction was mainly composed of four compounds present in highest amount: 3-methyl-1-butanol, 2-methyl-1-propanol, phenylethyl alcohol and 1-hexanol, which is in agreement with literature data (Petropoulos *et al.*, 2014). It was observed that the concentration of higher alcohols slightly increased with sonication and with maceration time although, again, the differences were not significant. Contrary to these results, it has been reported that the production of higher alcohols declines with maceration time, because of blockage of the Ehrlich mechanism, the main pathway for the formation of these compounds (Rapp & Versini, 1996).

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

A principal component analysis was conducted, using all the measured chromatic parameters, the total concentration of anthocyanins determined by HPLC and, given the large number of identified compounds, the sum of the different families of volatile compounds as variables. The objective was to find out how the wine samples, after two months in the bottle, were grouped and which variables were responsible for the grouping. This analysis reduced the information provided by all the measured variables to two principal components, which explained 78% of the variability of

the data (Fig. 1). The analysis clearly showed how different the wines were. Control wines and those elaborated from sonicated grapes were separated along PC1, the control wines presenting lower values of the chromatic parameters but, in general, slightly higher content of volatile compounds. Wines from sonicated grapes were separated along PC2, SW3d located in the positive part of PC2 and being characterised by the highest values in total anthocyanins and colour intensity. SW6d and SW8d were located in the negative part of PC2, being characterised by their higher content of total tannins and phenols.

The results of the application of this small-scale industrial ultrasound system to crushed grapes indicate that this technology facilitates the extraction of phenolic compounds from grape to must. It could be applied as a continuous pretreatment of crushed red grapes, before loading the maceration-fermentation tanks, representing a possibility to optimise winery capacity by reducing the skin maceration time without losing the quality characteristics of the obtained wines.

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