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Using high-power ultrasounds in red winemaking: Effect of operating conditions on wine physico-chemical and chromatic characteristics

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ABSTRACT

This study presents the application of high-power ultrasounds (US) to crushed grapes using winery-scale equipment and studies, for the first time at this scale, how the frequency of the ultrasounds affects the extraction of phenolic compounds from grapes and the chromatic and physico-chemical characteristics of the finished wines, in an attempt to optimize the maceration process. The results showed how US modified the physical characteristics of the grape skin, facilitating phenolic extraction and improving the wine chromatic characteristics but only produced slight differences in the physico-chemical characteristics of the finished wines. The two different frequencies applied led to some differences in the wine phenolic composition, especially higher tannin extraction at 20 kHz whereas anthocyanin extraction was favoured at 28 kHz. Wines made with sonicated grapes and 72 h of skin maceration time were chromatically very similar to control wines with four more days of skin contact, indicating that this technological process may increase the working capacity of the winery by allowing a reduction of more than 50% in the maceration time.

1. Introduction

In recent years, the highest market value of wines has been seen to be related with their phenolic compound content (Mercurio, Dambergs, Cozzolino, Herderich, & Smith, 2010). To obtain wines with good chromatic quality, a good diffusion of the phenolic compounds from the grape to the must or must-wine is necessary. This extraction process is carried out during the maceration step and its development is based on the breakage of the cell walls of the grape skin and seeds, in order to achieve the release of the compounds of interest into the medium. Thus, the maceration time is a determining factor in the wine quality (Alencar et al., 2018).

However, in some cases, the technical resources found in wineries and the availability of maceration tanks may be limited, making the reduction of the maceration time necessary, which can be detrimental to the quality of the final product. On the other hand, if very long maceration times are needed to achieve a high chromatic content, the productivity of wineries decreases and, moreover, it may even lead to physico-chemical and microbiological alterations in wines, triggering loss of their organoleptic quality.

In order to address such problems, many studies have been carried out using different techniques during winemaking to increase phenolic extraction with shorter maceration times. Among these techniques, some have been used for many years, such as those involving the use of pectolytic enzymes, cold soaking or partial must bleeding (all of them reviewed by Sacchi, Bisson, & Adams, 2005), or the use of heat, with techniques such as thermovinification and flash release (Atanackovic et al., 2012) and the use of microwaves (Carew, Gill, Close, & Dambergs, 2014), although some authors suggest that heat may affect the quality and stability of phenolic compounds (Geffroy et al., 2018).

However, there are more innovative techniques, based on cell rupture by physical methods without the need of an increase in temperature, such as the use of pulse electric fields (PEF) or high-power ultrasound (US). The use of PEF allows, through electroporation, the permeabilization of cell tissues, facilitating the extraction of phenolic compounds (López, Puértolas, Condón, Álvarez, & Raso, 2008a, 2008b; Puértolas, López, Saldaña, Álvarez, & Raso, 2010). Maza et al. (2020) presented its possible application to reduce maceration time in Garnacha wines.

The use of high-power ultrasound (US) is another technique that has received great interest in recent years in food technology. The technique consists of the application of high-power and low-frequency mechanical

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waves (from 20 kHz to 100 kHz). These sound waves, formed in a transducer that transfers a vibration to devices called sonotrodes or sonoplates, generate, in liquid media, small bubbles that, when reaching a critical point, collapse and implode, releasing large amounts of energy (Chemat, Huma, & Khan, 2011). When these bubbles collapse near the surface of a solid material, the high pressure and temperature released generate microjets capable of causing very localized damage in nearby cellular structures, increasing the permeabilization of cell membranes to the intra-cellular substances, and reducing selectivity of the cell membranes significantly (Clodoveo, Dipalmo, Rizzello, Corbo, & Crupi, 2016), thus enhancing processes such as the exchange of compounds through the altered structures and/or the inactivation of microorganisms (Gallo, Ferrara, & Naviglio, 2018; Terefe, Sikes, & Juliano, 2016). The collapse of cavitation bubbles also generates extreme temperature conditions within the bubbles and highly reactive radicals are generated. If water is the sonication medium, H● and OH● radicals are generated from water, and so, the application of ultrasound has developed a relatively new area of food processing, food sonochemistry, based on the generation of chemical modifications in the media through the formation of OH free radicals (Krasulya et al., 2014), which may, for example, improve the aging process in wines (Zhang et al., 2015b).

It is clear that parameters intrinsically related to the ultrasonic devices (such as the frequency, wavelength and amplitude of the wave, the ultrasonic power, and consequently, intensity) will have an effect on the outcomes of the technique. The frequency of the US affects the acoustic cavitation and therefore, its two main effects: the mechanical damage resulting from the collapse of acoustic cavitation bubbles in the water near the surface and the formation of short-lived radicals due to the breakdown of water inside the bubble. Research has shown that the ultrasound mechanical effects are less pronounced at higher frequencies, because the negative pressure is insufficient to produce cavitation during the rarefaction period or the time is insufficient for the bubbles to collapse during compression (Ashokkumar et al., 2008). The other effects of US, the chemical effects due to cavitation, is also affected by frequency. Ashokkumar (2015) and Mason, Cobley, Graves, and Morgan (2011) showed how the formation of radicals increased at higher frequencies.

Previous studies on grapes and wines of different red varieties have provided very promising results on the extraction capacity of US and the consequent physico-chemical quality of the wines (Bautista-Ortín et al., 2017; Ferraretto, Cacciola, Batlló, & Celloti, 2013; Zhang, Shen, Fan, & García-Martín, 2015a), as well as the acceleration capacity of wine aging (Ferraretto & Celotti, 2016; García-Martín & Sun, 2013) or microbiological control (Gracin et al., 2016; Luo, Schmid, Grbin, & Jiranek, 2012). Furthermore, US has provided interesting results on the extraction of aromatic compounds in white and red wines (Bautista-Ortín et al., 2017; Roman et al., 2020) and on the recovery of bioactive compounds from pomace and lees (Bosiljkov et al., 2017; Drosou, Kyriakopoulou, Bimpilas, Tsimogiannis, & Krokida, 2015). Taken together, all the above suggests that the application of this technology could be very interesting for wineries, in fact, this technology has already been approved by the International Organization of Vine and Wine (OIV, 2019) in 2019 for its use in wineries.

However, as can be observed in the literature, most of the studies on the application of US in wine technology have been developed at laboratory scale, using laboratory ultrasonic baths (González-Centeno et al., 2014; Osete-Alcaraz, Bautista-Ortín, Ortega-Regules, & Gómez-Plaza, 2019) or probes (Celotti & Ferraretto, 2016; Lukić et al., 2019; Roman et al., 2020). We could only find two studies, besides our own publications, where a pilot or a winery scale system was used (Celotti & Ferraretto, 2016; Gambacorta et al., 2017), although all the studies were made at a fixed frequency.

Our research group is devoted to the optimization of the use of US at winery scale. The frequency of the applied US is one of the operational parameters that need to be optimized. This study presented here investigated, for the first time at winery scale, the effect of two different frequencies (20 kHz and 28 kHz) on facilitating the extraction of phenolic compounds from crushed grapes and, therefore, on optimizing the maceration process, the aim being to reduce the processing time while maintaining the colour quality and stability of the wines.

2. Materials and methods

2.1. Grape samples

Monastrell red grapes (approximately 1400 kg) were hand-harvested from a commercial vineyard in Jumilla (Murcia, Spain) when they reached 14° Baumé, and packed in 20 kg perforated plastic boxes before being rapidly transported to the winery for processing.

2.2. Winemaking process

A flow diagram of the process is shown in Fig. 1. The grapes were destemmed and crushed, and then the crushed grapes were treated with a pilot-scale power ultrasound system (MiniPerseo, Agrovin S.A., Alcazar de San Juan, Spain) using two different frequencies, 20 kHz (S20) and 28 kHz (S28). This system can treat 400 kg of crushed grapes per hour and operates at 2500 W with a power density of 8 W/cm^2. This equipment is composed inside of one sonoreactor, a hexagonal pipe (1 m length) in which the sonoplates are coupled and through which the crushed and destemmed grape must circulate, and is the place where the sonication occurs. The design of the equipment and the process limit the temperature increase to less than 2 °C. A batch of destemmed and crushed grapes were not treated and were used as control vinification (C). The musts were fermented in 50 kg stainless steel tanks, which were filled with the control or ultrasound-treated crushed grapes, maintaining the same solid/liquid ratio in each vessel. Total acidity was corrected to 5.5 g/L, and 20 g of selected dry yeast per 100 kg of grapes were added (Viniferm CT007, Agrovin, Alcazar de San Juan, Spain). Two different skin maceration times were tested for the sonicated grapes and for each frequency used: 48 h (S20-48h and S28-48h) and 72 h (S20-72h and S28-72h), and for the control wines (C48h and C72h), although for control wines a skin maceration time of 7 days was also tested (C7d). All vinifications were made in triplicate, and the tanks were maintained in a temperature-controlled room at 23 ± 2 °C. The cap was punched down twice a day during maceration, after which the wines were pressed in a 75 L pneumatic press. Free-run and pressed wines were combined and stored at room temperature until the end of alcoholic fermentation. When fermentation was finished, wines were cold-stabilised at 2 °C for one month, racked from lees and bottled. Wines were analysed at the end of skin maceration, at the end of alcoholic fermentation and at the time of bottling.

2.3. Analytical determinations

2.3.1. Skin characterization by optical microscopy

Grape skin samples were obtained just after crushing, in the case for control grapes, and after sonication at 20 or 28 kHz in the case of sonicated samples, to observe the effects of the sonication process on the cellular structure by optical microscopy. Small pieces of skin (1 mm^3) were cut from the vascular tissue and fixed in McDowell's reagent following the conditions described by Apolinar-Valiente, Romero-Cascales, Gómez-Plaza, and Ros-García (2016) with some modifications. The reagent consisting of 25% (v/v) glutaraldehyde and 40% (v/v) formaldehyde in 0.2 M cacodylate buffer (4 °C, 24 h), and then washed in the buffer. The samples were postfixed in an osmium tetroxide solution (4 °C, 2.5 h) and a staining was performed with veronal uranyl acetate (4 °C, 2 h). After dehydration through a series of ethanol gradients from 30% (v/v) to absolute (100% v/v), they were embedded in SPURR resin. The semi-thin sections were stained with toluidine blue and observed by light microscopy, and micro-photographs were taken.

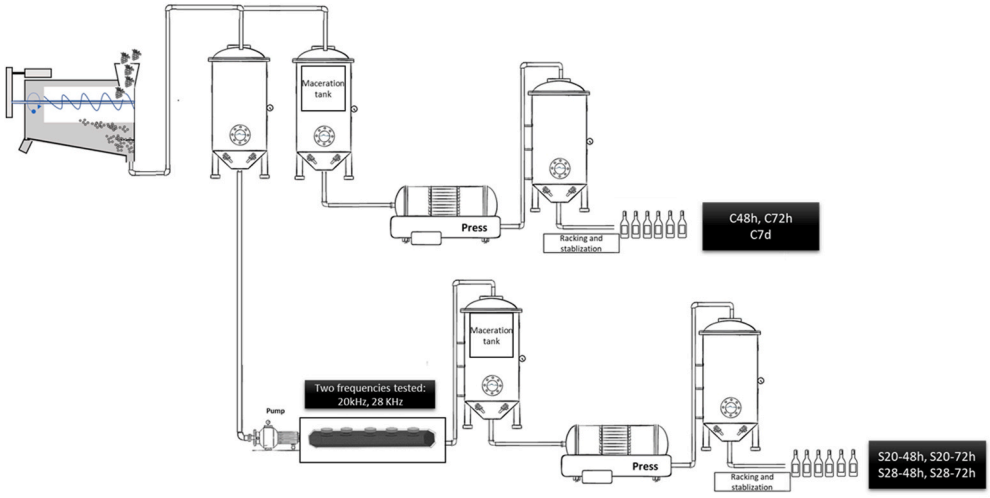

Fig. 1. Flow diagram of the sonication process.

2.3.2. Physico-chemical analysis

The wines were characterized by measuring the alcohol content, pH, and total and volatile acidity according to European Community methods (Commission Regulation ECC, 1990). Methanol, malic and tartaric acids and ethanal were determined by enzymatic methods carried out via an automated analyser (Miura One, TDI, Spain). The potassium (K) and calcium (Ca) contents were analysed by atomic absorption (Unicam 969 AA Spectrometer, Da Geleen, Netherlands).

2.3.3. Spectrophotometric parameters

Colour intensity (CI) was determined as the sum of absorbance at 620, 520 and 420 nm (Glories, 1984). Total and polymeric anthocyanins (TAnt, PolAnt) were measured using the method proposed by Ho, Da Silva, and Hogg (2001). The total phenol index (TPI) was calculated by measuring wine absorbance at 280 nm, according to Ribéreau-Gayon, Pontallier, and Glories (1983). Methyl cellulose precipitable tannins (MCPT) were determined by the methyl cellulose precipitation method (Smith, 2005).

2.3.4. Determination of tannins by HPLC

The tannin concentration and composition were measured using the phloroglucinolysis method. The preparation of wine samples and the elution conditions can be found in Busse-Valverde et al. (2010). Briefly, a Waters 2695 system (Waters, Milford, MA) equipped with a Waters 2996 photodiode array detector was used for this analysis. A volume of sample 10 μL was injected on an Atlantis dC18 column (250 × 4.6 mm, 5 μm packing) protected with a guard column of the same material (20 mm × 4.6 mm, 5 μm packing) (Waters, Milford, MA) and eluted with 0.8 mL/min of solvent A, water/formic acid (98:2, v/v) and solvent B, acetonitrile/solvent A (80:20 v/v), being 30 °C oven temperature. Elution began with 0% B for 5 min, linear gradient from 0 to 10%B in 30 min and gradient from 10 to 20% in 30 min, followed by washing and re-equilibration of the column.

These analyses allowed determination of the total tannin concentration (TTp), the apparent mean degree of polymerization (mDP), the percentage of galloylation (%Gal) and the percentage and total concentration of each constitutive unit (epicatechin gallate, ECG, and epigallocatechin, EGC). The mDP was calculated as the sum of all the subunits (flavan-3-ol monomer and phloroglucinol adducts, in moles) divided by the sum of all the flavan-3-ol monomers (in moles).

2.4. Statistical analysis

A one factor analysis of variance, a multivariate analysis of variance and principal component analysis were performed using the statistical package Statgraphics Centurion XVI.3 (Statpoint Technologies, Inc., The Plains, VA, USA).

3. Results and discussion

3.1. Optical microscopy of control and sonicated crushed grapes

To visualize the effects of US treatments on the cell structure of the grape berry skin, an optical microscopy study was carried out on the control berry skins obtained just after crushing and on the berry skins after crushing and sonication at 20 and 28 kHz.

All berry skins were characterized by one layer of epidermal cells and by five to six layers of hypodermal cells (Fig. 2). The distribution, size and shape of phenolic granulations in the vacuole allowed the skin cells to be categorized into five classes, as described by Cadot, Chevalier, and Barbeau (2011): cells without any colouration, cells with uniform colouration, cells with fine granulations homogeneously distributed in the vacuole, cells with small spherical inclusions, some of them stuck to the tonoplast, and cells with large round and distorted inclusions freely distributed inside the vacuole. The control grapes mainly had cells with small spherical inclusions, very homogeneously distributed. US-treated grapes, at both frequencies, showed slight epicarp compression, plasmolysis of subepidermal cells and a certain degree of mesocarp collapse. Sonicated grapes also showed more cells with uniform colouration and more cells without colouration. Cells with uniform colouration, as observed in sonicated samples, were not present in control grapes, as also observed by Cadot et al. (2011), but only in immature grapes.

A slight condensation of vacuolar material was observed in the epidermal layer, an effect that was already observed in grape skins with the use of pulsed electric fields (Cholet et al., 2014). Thus, the direct effect of US seems to be evacuation of the polyphenolic content, as well as the degradation and morphological modification of the external layers of skin.

3.2. Wine chromatic characteristics

After crushing and sonication, the musts fermented in stainless steel tanks. The fermentation process was controlled by monitoring the density and temperature throughout the period, until the sugar content fell below 2 g/L (Fig. 3). As can be observed in the corresponding graphs, the must from sonicated grapes fermented at a slightly lower speed than those of control grapes, especially when compared with the control vinifications with a 7-day maceration period. The fermentation of grapes

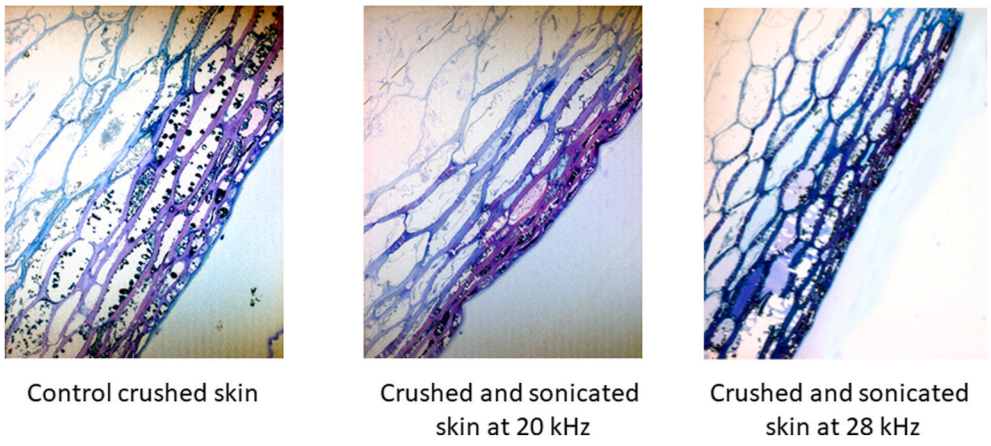

Fig. 2. Optical microscopy of grape skin just after crushing (A), after crushing and sonication at 20 kHz (B) and after crushing and sonication at 28 kHz.

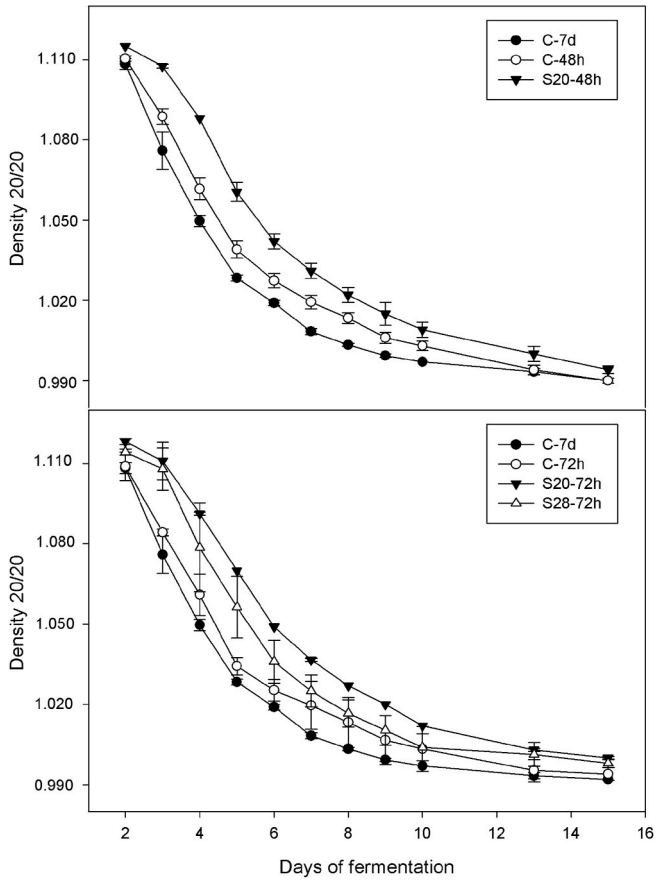

Fig. 3. Evolution of alcoholic fermentation on the different studied samples.

sonicated at 20 kHz had the lowest fermentation rate, especially during the first few days of fermentation. This could have been due to an effect of sonication on microbiological populations (Gracin et al., 2016; Luo et al., 2012), which led to a reduction in the endogenous microbial load of the grape prior to fermentation and a slight delay the initial fermentative rate. However, since a commercial yeast was added on the day after filling the tanks, fermentation ended around the same time in all the vinifications.

Our main interest in this study was the effect of sonication on the chromatic characteristics of the wines, and therefore, the evolution of these characteristics was followed during the winemaking process (Table 1). Two maceration times were assayed with the sonicated musts, 48 and 72 h, while the control must was also macerated for 7 days as is common practice in the commercial wineries using Monastrell variety.

At the moment of devatting, the control musts macerated for 48 h showed the lowest total phenol content of all the studied samples. As regards colour intensity, although the lowest values were observed for the control samples devatted at 48h differences were not significant compared with the other control wines. Interestingly, at this moment, the colour intensity of S20-48h and S28-48h wines did not significantly differ from that of the control wine with 7 days of skin contact time.

After 72 h of skin contact, the S28-72h wine presented the highest colour intensity of all the wines at the moment of devatting. Control wines after 48 and 72 h of maceration were the only wines that significantly differed from the control wine after 7 days of maceration in terms of the anthocyanin content, the sonication being highly effective for the extraction of anthocyanins. However, since the tannins are extracted more slowly than anthocyanins, a maceration time of 48 h, even with sonicated grapes, did not extract as many tannins as 7 days of maceration. Sonication of the crushed grapes and 72 h of maceration (S20-72h and S28-72h) did allow a similar extraction of tannins as when maceration lasted 7 days. The higher concentration of tannins in these wines also justifies the higher values of TPI. This confirms that sonication has a positive effect on the extraction of phenolic components, as reported in other studies (Gambacorta et al., 2017; Lukić et al., 2019), especially of those most tightly bound to cell walls, such as tannins. As regards the effect of the sonication frequency, almost no differences were observed at the end of skin maceration, except that S28-72h wines had a higher TPI.

These observations were mainly maintained at the end of the alcoholic fermentation and at the moment of bottling. However, it should be noted that there was a significant drop in the colour intensity and anthocyanin concentration in the 48- and 72-h macerated wines (both in the control and when sonicated grapes were used) from the moment of devatting to the end of the alcoholic fermentation, whereas the values of TPI and tannins were not similarly affected. These observations suggest that the precipitation of phenolic material was not the cause of this decrease in colour intensity and anthocyanin content. In young red wines, free anthocyanins are the principal source of their red colour, although monomeric anthocyanins are not particularly stable (He et al., 2012a). One of the most significant ways to stabilize them is through condensation with tannins to generate stable anthocyanin/tannin adducts. It is estimated that about 25% of anthocyanins will have polymerized with flavonoid compounds by the end of alcohol fermentation, the level rising to more than 40% after one year's aging (He et al., 2012b). However, if the concentration of tannins is not very high (as occurs in vinifications with short maceration times and as was observed in our wines macerated for 48 h), a fraction of free anthocyanins may be lost by degradation, oxidation, precipitation or formation of other

Table 1
Chromatic analysis of the different wines.

	Sample	CI	TPI	TAnt	PolAnt	MCPT
End of skin maceration	C-48h	13.24 ± 1.59**a**	35.25 ± 0.66**a**	539.79 ± 46.68**a**	5.42 ± 0.05**a**	822.72 ± 58.60**a**
	S20-48h	17.19 ± 0.66**bc**	44.55 ± 1.39**b**	639.18 ± 25.66 **ab**	10.14 ± 1.39**b**	995.89 ± 52.86**a**
	S28-48h	18.77 ± 0.14**bcd**	46.06 ± 1.51**b**	696.46 ± 14.48**bc**	10.44 ± 0.73**b**	1092.06 ± 62.51**a**
	C-72h	16.15 ± 1.05 **ab**	44.73 ± 0.43**b**	591.76 ± 39.93**a**	11.24 ± 0.28**b**	940.28 ± 34.99**a**
	S20-72h	20.28 ± 1.47**cd**	54.51 ± 1.94**c**	712.39 ± 27.10**bc**	16.63 ± 0.74**c**	1594.33 ± 142.41**b**
	S28-72h	21.66 ± 0.29**d**	68.25 ± 1.34**d**	776.69 ± 15.29**c**	18.85 ± 1.14**c**	1647.72 ± 143.33**b**
	C-7d	16.87 ± 1.63**abc**	67.85 ± 1.56**d**	709.26 ± 28.57**bc**	26.92 ± 2.14**d**	1707.46 ± 33.55**b**
End of alcoholic fermentation	C-48h	6.60 ± 0.62**a**	34.59 ± 0.70**a**	325.81 ± 19.11**a**	19.49 ± 1.52**a**	697.41 ± 27.82**a**
	S20-48h	10.02 ± 0.34**b**	44.75 ± 2.22**b**	415.86 ± 12.24**b**	33.03 ± 1.47**c**	1258.50 ± 37.89**bcd**
	S28-48h	10.20 ± 0.24**b**	43.73 ± 0.91**b**	433.98 ± 3.62**b**	31.60 ± 1.14**bc**	1068.86 ± 47.00**bc**
	C-72h	8.60 ± 0.67 **ab**	41.07 ± 2.77**b**	422.62 ± 27.24**b**	25.82 ± 0.52 **ab**	933.00 ± 87.31 **ab**
	S20-72h	13.54 ± 0.74**c**	54.38 ± 2.22**c**	501.48 ± 19.19**c**	43.89 ± 2.82**d**	1491.11 ± 141.84**de**
	S28-72h	15.21 ± 1.18**cd**	56.64 ± 1.29**c**	566.72 ± 35.41**d**	46.67 ± 4.01**d**	1411.88 ± 256.10**cd**
	C-7d	16.74 ± 0.97**d**	64.05 ± 0.98**d**	652.64 ± 4.02**e**	49.78 ± 3.07**d**	1768.80 ± 20.44**e**
Bottling	C-48h	7.00 ± 0.81**a**	32.93 ± 0.96**a**	321.03 ± 21.77**a**	23.30 ± 2.55**a**	667.04 ± 32.54**a**
	S20-48h	10.48 ± 0.39**b**	45.46 ± 1.31**b**	397.20 ± 6.51**b**	39.90 ± 1.57**b**	1153.65 ± 42.00**b**
	S28-48h	10.54 ± 0.33**b**	42.60 ± 0.86**b**	420.78 ± 6.20**bc**	37.64 ± 0.33**b**	937.57 ± 32.42 **ab**
	C-72h	9.21 ± 0.87 **ab**	44.01 ± 1.50**b**	406.19 ± 36.59**b**	31.43 ± 1.09 **ab**	1020.53 ± 133.44**b**
	S20-72h	14.66 ± 1.48**c**	54.04 ± 3.19**c**	482.28 ± 13.82**c**	54.25 ± 2.40**c**	1490.51 ± 93.17**c**
	S28-72h	14.80 ± 0.27**cd**	54.63 ± 0.71**c**	557.45 ± 17.30**d**	55.73 ± 2.94**cd**	1476.00 ± 145.33**c**
	C-7d	17.73 ± 1.48**d**	63.83 ± 2.63**d**	620.89 ± 28.16**d**	63.81 ± 6.75**d**	1878.43 ± 136.34**d**

CI: color intensity, TPI: total phenol index, TAnt: total anthocyanins (mg/L), PolAnt: polymeric anthocyanins (mg/L), MCPT: methyl cellulose precipitable tannins, (mg/L).
C: control wines, S20: wines made from sonicated grapes at 20 kHz, S28: wines made from grapes at 28 kHz, 48h: wines made with a skin maceration time of 48 h, 72h: wines made with a skin maceration time of 72 h, C-7d: wines made with a skin maceration time of 7 days.

colourless compounds. The fact that the values of TPI remained stable suggests that it was mainly the oxidation processes that led to the appearance of colourless phenolic compounds.

At the moment of bottling, there were significant differences between all the chromatic parameters of the wines made from sonicated grapes and the corresponding controls. Furthermore, the S28-72h wines reached similar values of colour intensity, total anthocyanins and polymeric anthocyanins to the wines that had been made after four more days of maceration time (C7d wines).

Thus, the results obtained showed that the use of ultrasound strongly favours the extraction of phenolic compounds. This was particularly noticeable in the wines elaborated with sonicated grapes and undergoing a 72-h maceration time, especially when a frequency of 28 kHz was used since the colour intensity of these wines presented values similar to those of conventional winemaking involving seven days of maceration.

3.3. Determination of the concentration and composition of wine tannins by phloroglucinolysis

The chemical characteristics of the wine tannins and how they were

Table 2
Concentration and characterization of the wine tannins using the phloroglucinolysis method.

	Sample	TTp	mDP	%EGC	%Gal	EGC	ECG
End of skin maceration	C-48h	540.29 ± 1.19**a**	7.45 ± 0.09**c**	19.56 ± 0.06**a**	1.99 ± 0.13**a**	356.86 ± 0.14**a**	36.35 ± 2.34**a**
	S20-48h	853.06 ± 28.82**b**	7.38 ± 0.06**c**	17.86 ± 0.21**a**	2.11 ± 0.07**a**	514.77 ± 23.65 **ab**	60.89 ± 0.13**a**
	S28-48h	764.29 ± 29.01 **ab**	6.75 ± 0.02 **ab**	20.23 ± 1.82**a**	2.39 ± 0.27 **ab**	521.27 ± 58.18 **ab**	61.52 ± 5.75**a**
	C-72h	747.01 ± 65.85 **ab**	6.61 ± 0.12**a**	19.78 ± 0.98**a**	2.21 ± 0.24**a**	499.24 ± 64.75 **ab**	55.73 ± 9.54**a**
	S20-72h	1112.04 ± 72.61**c**	7.11 ± 0.15**bc**	17.80 ± 0.26**a**	2.47 ± 0.00 **ab**	667.67 ± 53.21**bc**	92.72 ± 5.99**b**
	S28-72h	1092.27 ± 151.82**c**	6.95 ± 0.10 **ab**	18.46 ± 0.01**a**	2.59 ± 0.14 **ab**	679.05 ± 93.44**bc**	95.48 ± 18.15**b**
	C-7d	1183.74 ± 23.87**c**	6.93 ± 0.25 **ab**	19.00 ± 0.05**a**	3.04 ± 0.16**b**	755.72 ± 16.45**c**	121.09 ± 8.81**b**
End of alcoholic fermentation	C-48h	499.45 ± 13.28**a**	6.90 ± 0.24**a**	17.80 ± 0.44 **ab**	2.14 ± 0.11**a**	300.21 ± 12.36**a**	36.07 ± 1.26**a**
	S20-48h	853.87 ± 40.96**c**	7.46 ± 0.18**a**	17.33 ± 0.29**a**	2.20 ± 0.01**a**	499.89 ± 32.39**bc**	63.40 ± 2.82**bc**
	S28-48h	663.13 ± 9.15**b**	6.49 ± 0.23**a**	17.70 ± 0.16 **ab**	2.15 ± 0.05**a**	396.34 ± 5.15 **ab**	48.05 ± 0.74 **ab**
	C-72h	646.35 ± 43.99**b**	7.38 ± 0.26**a**	18.72 ± 0.84 **ab**	2.13 ± 0.09**a**	408.57 ± 33.79**abc**	46.50 ± 5.00 **ab**
	S20-72h	914.65 ± 139.29**c**	7.38 ± 0.47**a**	17.36 ± 0.76**a**	2.42 ± 0.11 **ab**	537.23 ± 104.40**cd**	74.92 ± 14.66**cd**
	S28-72h	1039.83 ± 70.26**d**	6.98 ± 0.11**a**	18.16 ± 0.22 **ab**	2.51 ± 0.20**b**	635.96 ± 34.58**de**	88.23 ± 12.90**de**
	C-7d	1099.43 ± 47.95**d**	7.10 ± 0.59**a**	19.14 ± 0.34**b**	2.89 ± 0.03**c**	707.75 ± 43.01**e**	106.80 ± 5.78**e**
Bottling	C-48h	482.67 ± 8.94**a**	6.21 ± 0.14**a**	17.26 ± 0.20**a**	1.77 ± 0.48**a**	282.03 ± 7.43**a**	28.97 ± 8.30**a**
	S20-48h	760.13 ± 27.97**b**	7.35 ± 0.40**c**	16.55 ± 0.49**a**	2.42 ± 0.02**cd**	424.81 ± 37.67**b**	62.11 ± 2.69**c**
	S28-48h	689.39 ± 13.53**b**	6.55 ± 0.24 **ab**	17.33 ± 0.30**a**	1.98 ± 0.25**abc**	403.72 ± 24.65**b**	46.16 ± 5.11**b**
	C-72h	665.07 ± 34.21**b**	6.81 ± 0.23**bc**	18.41 ± 0.87**bc**	1.85 ± 0.21 **ab**	413.74 ± 23.30**b**	41.74 ± 6.71**b**
	S20-72h	956.64 ± 113.72**c**	7.17 ± 0.44**c**	17.35 ± 0.34 **ab**	2.34 ± 0.06**bcd**	560.65 ± 139.24**c**	75.80 ± 10.82**c**
	S28-72h	987.81 ± 23.78**cd**	6.71 ± 0.01**abc**	17.41 ± 0.40 **ab**	2.03 ± 0.03**abc**	581.00 ± 22.02**c**	67.83 ± 0.52**c**
	C-7d	1097.38 ± 100.18**d**	7.15 ± 0.56**bc**	18.46 ± 0.51**c**	2.71 ± 0.02**d**	680.38 ± 111.95**d**	99.57 ± 8.22**d**

TTp: total tannins (mg/L), mDP: mean degree of polymerization, %EGC: percentage of epigallocatechin, %Gal: percentage of galloylation, ECG: concentration of epicatechin gallate (μM), EGC: concentration of epigallocatechin (μM).
C: control wines, S20: wines made from sonicated grapes at 20 kHz, S28: wines made from grapes at 28 kHz, 48h: wines made with a skin maceration time of 48 h, 72h: wines made with a skin maceration time of 72 h, C-7d: wines made with a skin maceration time of 7 days.

affected by the grape sonication were evaluated by the phloroglucinolysis method. As shown in Table 2, a higher total tannin extraction was observed in all the sonicated samples compared to their corresponding control samples, both for the 48-h and 72-h macerations. Sonicated samples after 48 h of maceration did not differ from the C72h wine, while the sonicated samples after 72 h of maceration did not differ from C7d wines in their tannin content. These results agree with previous studies that clearly showed a positive effect of sonication on total tannin extraction during winemaking (Ferraretto & Celotti, 2016; Bautista-Ortín et al., 2017). Moreover, Natolino and Da Porto (2020) reported that the effect of ultrasound on these compounds did not promote any degradation and increased their extraction from fresh and distilled grape marc.

Wine tannin composition is the result of the proportion of skin tannins and seed tannins extracted from grapes, the latter presenting lower mean degree of polymerization and higher percentage of galloylated units and absence of the trihydroxylated subunit epigallocatechin (Watrelot & Norton, 2020). The values of the tannin degree of polymerization at the moment of devatting were slightly higher in the samples obtained from grapes sonicated at 20 kHz, probably due to a higher extraction of high molecular weight skin tannins at this frequency. These differences observed at the moment of devatting were not observed at the end of alcoholic fermentation, when no differences were observed in tannin mDP in the studied wines, although, at the moment of bottling, samples sonicated at 20 kHz and control wines with a maceration of 7 days showed the highest values.

The galloylation (the quantity of galloylated tannin subunits) of the extracted tannins was very similar for all the wines macerated for 48 and 72 h at devatting, only the wine with a skin maceration time of 7 days showing a higher percentage of galloylation, since the longer maceration time facilitated seed tannin extraction.

At the moment of bottling, the highest values of epigallocatechin percentages were observed in two of the control wines (C72h and C7d) and the highest percentage of galloylation in the C7d and both wines made from grapes sonicated at 20 kHz, indicating that sonication, especially at the lower frequency, may also affected seed tannin extraction. In the same context, sonication has been described as a potential tool for releasing tannins from seeds (Da Porto, Porreto, & Decorti, 2013).

3.4. US effect on the physico-chemical composition of finished wines

Most of the research done in the application of US in winemaking have not put enough attention to the possible effect on other physico-chemical characteristics of the studied wines were analysed at the moment of bottling (Table 3) and, in general, as Zhang et al. (2015a) also found, any differences in the parameters due to grape sonication were slight.

No significant differences were observed regarding the alcohol content of the samples, except for the 7-day maceration control wine, in which it was slightly lower, probably because ethanol evaporation was more intense during the longer skin maceration time, when higher temperatures were recorded.

Since acidity was corrected at the beginning of the winemaking process, no differences in pH or total acidity were observed. The wines did not undergo a malolactic fermentation so malic acid was present, and no differences in this acid and tartaric acid were observed.

Volatile acidity was very similar in all the wines, and sonication of grapes did not lead to any problems related with the production of volatile acidity. The slightly higher values in the wines made from sonicated grapes at 20 kHz could have been due to the low fermentation rate observed in these vinifications, which could favour the development of acetic bacteria.

The process of ethanol oxidation generated ethanal, but no differences were observed in its concentration between any of the wines. This compound is used as a reference parameter of the oxidative processes that may take place in wine. Thus, the low amounts of ethanal found in the wines and the fact that wines made from sonicated wines did not show higher levels of this compound indicates that sonication did not induce oxidation during winemaking.

A higher concentration of methanol is observed in the C7d wines, as well as in the wines sonicated at 28 kHz and macerated for 72 h. The hydrolysis of pectins, which are found naturally in plant tissues, by pectolytic enzymes results in a production of methanol in wines (Rollero et al., 2018; Wei et al., 2020). The higher concentration in the C7d wines was expected since it is known that longer maceration times lead to a greater presence of solubilized plant material and therefore the formation of methanol. Sonication slightly increased the presence of methanol due to the higher extraction of pectic compounds from skin cell walls, although the differences were not significant when maceration lasted 48 h.

No differences in the calcium content and only small differences in the potassium content were found between the wines. However, it should be remembered that the wines underwent a cold stabilization process and, therefore, the effect of sonication on the content of these two cations would be more evident observing their content in the musts obtained after the treatment than in the finished wines. In these musts, the concentration of potassium and calcium was 1176 and 101 g/L, respectively, in the control must, 1323 and 124 g/L in the must after sonication at 28 kHz and 1281 and 110 g/L in the must sonicated at 20

Table 3
Physico-chemical analysis of the different wines at the time of bottling.

Sample	%Alc	pH	TAc	VAc	MeOH	Mal	Tart	Ethanal	K	Ca
C-48h	15.72 ± 0.23**b**	3.58 ± 0.10**a**	5.17 ± 0.15 **ab**	0.65 ± 0.06 **ab**	103.67 ± 7.64**a**	0.77 ± 0.06**a**	1.53 ± 0.32**a**	47.67 ± 6.11**a**	0.85 ± 0.02**a**	56.67 ± 5.51**a**
S20-48h	16.07 ± 0.15**b**	3.56 ± 0.01**a**	5.37 ± 0.06 **ab**	0.75 ± 0.04**b**	112.33 ± 12.50**a**	0.77 ± 0.06**a**	1.77 ± 0.06**a**	52.00 ± 1.00**a**	0.87 ± 0.01 **ab**	55.00 ± 1.73**a**
S28-48h	15.67 ± 0.01**b**	3.59 ± 0.01**a**	5.25 ± 0.05 **ab**	0.66 ± 0.02 **ab**	120.00 ± 14.00**a**	0.75 ± 0.05**a**	1.70 ± 0.00**a**	48.67 ± 2.52**a**	0.92 ± 0.03**bc**	66.67 ± 8.51**a**
C-72h	15.52 ± 0.59 **ab**	3.63 ± 0.07**a**	5.07 ± 0.15**a**	0.60 ± 0.06**a**	122.00 ± 3.00**a**	0.77 ± 0.06**a**	1.57 ± 0.15**a**	51.33 ± 5.51**a**	0.95 ± 0.01**c**	60.33 ± 3.06**a**
S20-72h	16.23 ± 0.07**b**	3.59 ± 0.03**a**	5.40 ± 0.00 **ab**	0.75 ± 0.01**b**	136.33 ± 15.04 **ab**	0.73 ± 0.06**a**	1.67 ± 0.06**a**	46.00 ± 6.25**a**	0.89 ± 0.04**abc**	56.33 ± 7.02**a**
S28-72h	15.70 ± 0.03**b**	3.59 ± 0.04**a**	5.43 ± 0.31 **ab**	0.64 ± 0.04 **ab**	173.00 ± 24.25**b**	0.70 ± 0.10**a**	1.63 ± 0.15**a**	54.33 ± 20.65**a**	0.93 ± 0.04**bc**	55.67 ± 6.43**a**
C-7d	14.92 ± 0.20**a**	3.57 ± 0.05**a**	5.57 ± 0.12**b**	0.60 ± 0.06**a**	265.33 ± 20.26**c**	0.80 ± 0.00**a**	1.40 ± 0.17**a**	40.67 ± 12.22**a**	0.96 ± 0.01**c**	65.00 ± 10.00**a**

%Alc: alcohol content, TAc: total acidity (g/L), VAc: volatile acidity (g/L), MeOH: methanol content (mg/L), Mal: malic acid (g/L), Tart: tartaric acid (g/L), K: potassium (mg/L), Ca: calcium (mg/L).
C: control wines, S20: wines made from sonicated grapes at 20 kHz, S28: wines made from grapes at 28 kHz, 48h: wines made with a skin maceration time of 48 h, 72h: wines made with a skin maceration time of 72 h, C-7d: wines made with a skin maceration time of 7 days.

Hz (data not shown). The sonication slightly increased the content of both ions, although the increase was small. 50% of grape potassium is present in the grape skin (Bigard, Romieu, Sire, & Torregrosa, 2020) and this increase seems to be linked to the intensification of extraction by the sonication process.

3.5. Evaluating the effect of the US frequency in the final wine composition

To separate the effect of maceration time (48 and 72 h) and sonication frequency (20 or 28 kHz) on polyphenolic extraction and other physicochemical parameters a multivariate analysis of variance (MANOVA) was also conducted with the data of the wines at the moment of bottling (Table 4). Regarding the maceration time, and as expected, wines obtained after 72 h of maceration presented greater colour intensity and a higher polyphenolic compound content than those with a 48-h maceration time. The maceration time did not affect the mDP or the percentage of galloylation of the tannins, but it favoured the percentage of EGC, the subunit arising from the grape skins. As regards other chemical parameters, only the methanol content and K content increased with increasing maceration time.

One of our main point of interest was the effect of frequency on the extraction. Some studies have been made at different frequencies but always at laboratory scale and with other matrixes not applicable to the winemaking process (Dranca & Oroian, 2019; Tchabo, Ma, Engmann, & Zhang, 2015).

As González-Centeno et al. (2014) reported, at lower frequency high disruptive forces can be expected due to violently collapsing cavitation bubbles with locally high temperatures, pressures and shear forces. At frequencies above 20 kHz, bubble collapse is less violent, and therefore local temperatures and pressures are much lower, which, in turn, may be gentler on the phenolic and antioxidant fraction. Although the two tested frequencies were close enough not to cause large variation in the wine characteristics, some differences due to the US frequency were observed. When grapes were sonicated at 20 kHz, the resulting wines presented a higher tannin content, with higher degree of polymerization and higher percentage of galloylation, higher alcohol content and volatile acidity, whereas those wines from grapes sonicated at 28 kHz presented higher anthocyanin and methanol content.

It is not easy to explain these differences since several effects may be occurring simultaneously. The more pronounced mechanical effects at 20 kHz than at 28 kHz may have led to a higher erosion of the skin and seed surfaces and a greater tannin extraction, favouring especially those tannins of greater molecular weight and, also, a higher extraction of seed tannins, as indicated by the high percentage of galloylation units. The higher final alcohol content observed in wines exposed to a frequency of 20 kHz may also have been due to a more intense rupture of the cell structures, which would lead to the greater availability of fermentable intracellular sugars. This is in agreement with the reports of Shirsath et al. (2017) which highlight that the application of low frequencies may promote the cell wall disruption, facilitating solvent access to the cell content and intensifying the mass transfer rate.

However, anthocyanins behaved in the opposite way, with higher concentrations observed in wines from grapes sonicated at 28 kHz. We hypothesized that, while 20 kHz could favour degradation of grape skin compared with sonication at 28 kHz, the differences were small. The slightly faster alcohol production rate during the first few days in the 28 kHz vinifications may have contributed to the higher concentration of easily extracted anthocyanins at the moment of devatting (Nell, van Rensburg, & Lambrechts, 2014), surpassing the effect of the lower frequencies.

Also, the methanol content was higher in the 28 kHz vinifications than in the control and after the application of 20 kHz. The formation of methanol is not only due to a higher presence of substrate but also to the enzyme (pectinase) activity. The studies of Ma et al. (2018) pointed to the stronger affinity between pectin and pectinase in sonicated samples and observed that ultrasound effectively decreased the degree of methoxylation (DM), which would have explained the higher methanol content. Wang, Cao, Sun, Wang, and Mo (2011) demonstrated that this enzymatic activity increased by 6.56, 14.79 and 17.85 at frequencies of 18, 20 and 24 kHz, respectively. They attributed this positive effect to the ability of ultrasound to increase the surface area of enzyme molecules, which would help to explain the higher methanol content observed at 28 kHz.

3.6. Can really help US to reduce skin maceration time during winemaking?

We have mentioned that one of our main objectives was to obtain wines similar to those obtained after seven days of maceration but using a shorter maceration time, and, in this respect, multivariate statistical analysis can help us to determine, using all the variables, the similarity

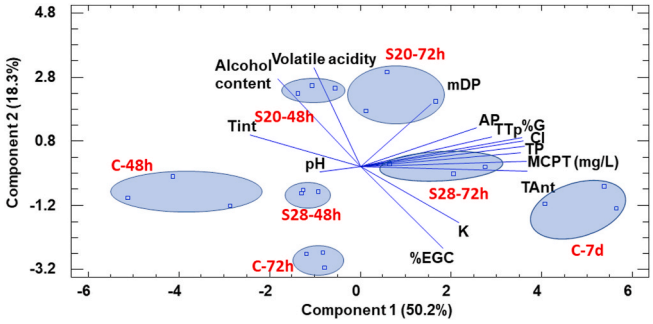

Fig. 4. Bidimensional plot of the different wines at the moment of bottling using the first two components resulting from a principal component analysis.

Table 4
MANOVA statistical analysis of the independent effect of maceration time (48 and 72 h) and sonication frequencies (20 and 28 kHz) at the time of bottling.

	CI	TPI	TAnt	PolAnt	MCPT	TTp	mDP	%EGC	%Gal	%Alc	TAc	VAc	MeOH	K	Ca
48 h	9.3**a**	40.3**a**	379.6**a**	33.6**a**	919.4**a**	638.1**a**	6.8**a**	17.2**a**	2.1**a**	15.8**a**	5.2**a**	0.6**a**	112.0**a**	883.3**a**	57.4**a**
72 h	12.9**b**	50.9**b**	481.9**b**	47.1**b**	1329.1**b**	818.8**b**	6.9**a**	17.8**b**	2.1**a**	15.9**a**	5.3**a**	0.7**a**	143.8**b**	926.5**b**	59.4**a**
Control	8.1**a**	38.5**a**	363.6**a**	27.4**a**	843.8**a**	573.8**a**	6.5**a**	17.8**b**	1.8**a**	15.6**a**	5.1**a**	0.6**a**	112.8**a**	903.2 **ab**	55.7**a**
S20	12.6**b**	48.6**b**	439.7**b**	46.7**b**	1322.1**c**	804.4**b**	7.3**b**	17.0**a**	2.4**b**	16.4**b**	5.4**b**	0.7**b**	124.3**a**	881.0**a**	57.4**a**
S28	12.6**b**	49.7**b**	489.1**c**	47.1**b**	1206.8**b**	806.9**b**	6.7**a**	17.6 **ab**	2.0**a**	15.7**a**	5.3**b**	0.6**a**	146.5**b**	930.7**b**	61.2**a**
Interactions	0.08	0.20	0.06	0.01	0.10	0.13	0.01	0.16	0.77	0.51	0.32	0.50	0.12	0.04	0.10

CI: color intensity, TPI: total phenol index, TAnt: total anthocyanins (mg/L), PolAnt: polymeric anthocyanins (mg/L), MCPT: methyl cellulose precipitable tannins (mg/L), TTp: total tannins (mg/L), mDP: mean degree of polymerization, %EGC: percentage of epigallocatechin, %Gal: percentage of galloylation, %Alc: alcohol content, TAc: total acidity (g/L), VAc: volatile acidity (g/L), MeOH: methanol content (mg/L), K: potassium (mg/L), Ca: calcium (mg/L).
C: control wines, S20: wines made from sonicated grapes at 20 kHz, S28: wines made from grapes at 28 kHz, 48h: wines made with a skin maceration time of 48 h, 72h: wines made with a skin maceration time of 72 h, C-7d: wines made with a skin maceration time of 7 days.

of wines. Fig. 4 presents the results of the principal component analysis (PCA), elaborated using all the data of the wines at the moment of bottling. As can be seen, the samples were distributed differently in the plane created by principal components 1 and 2. The control wines were located in the negative part of PC2 and were separated along PC1, moving towards the positive part of PC1 as maceration time increased, the 7-day maceration control wine located in the most positive part of PC1 due to its highest colour intensity and polyphenolic content (TPI, TT, TAnt, PolAnt) compared to wines macerated for 48 and 72 h. The frequency of the US also led to a separation of the wines along PC2, those wines obtained from crushed grapes sonicated at 20 kHz were located in the most positive part of PC2 due to their higher values of tannin content, tannin degree of polymerization and higher alcohol content. Looking for similarities with the 7-days skin macerated wines, the wines located closer to them where those made from 28 kHz sonicated grapes and 72 h of maceration time, indicating that these wines were those presenting the most similar chromatic and physico-chemical characteristics to C7d wines.

However, it is important to point out that the sonication of grapes at 20 kHz led to wines with higher tannin and alcohol content, and, according to Jackson (2017) and Aleixandre-Tudo and du Toit (2020) these initial high levels of alcohol content, tannins, together with high total phenols and polymeric pigments enhance the ageing potential of the wines.

4. Conclusions

Sonication of grapes has been proved as a very interesting technology easily applied in wineries that leads to wines with improved chromatic characteristics, compared with their control wines. If the same skin-maceration time is used, wines from sonicated grapes present higher colour and phenolic content than their control wines. Moreover, the wine obtained from sonicated grapes and skin-macerated for 72 h was very similar to the control wine resulting from 7 days of skin-maceration. Thus, sonication of grapes may allow a reduction of more than 50% in the maceration time without losing wine color or stability, thereby increasing the working capacity of the winery.

When optimizing operational parameters, the US frequency used led to different outcomes. Wines with higher tannin and alcohol content were obtained sonicating crushed grapes at 20 KHz, and this could ensure a long term wine aging stability. If wine anthocyanin content needs to be favoured, 28 kHz would be the most appropriate working frequency.

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CRediT authorship contribution statement

Paula Pérez-Porras: Conceptualization, Investigation, Formal analysis, Methodology, Writing - original draft, were in charge of the conceptualization as well as of the investigation, did the analysis of the data and were in charge of the methodology, oversaw the writing of the original draft. **Ana Belén Bautista-Ortín:** Conceptualization, Investigation, Formal analysis, Methodology, Writing - review & editing, were in charge of the conceptualization as well as of the investigation, did the analysis of the data and were in charge of the methodology, did the data curation and the writing, reviewing, and editing of the final manuscript. **Ricardo Jurado:** Conceptualization, Investigation, were in charge of the conceptualization as well as of the investigation. **Encarna Gómez-Plaza:** Conceptualization, Investigation, Writing - original draft, Writing - review & editing, were in charge of the conceptualization as well as of the investigation, oversaw the writing of the original draft, did the data curation and the writing, reviewing, and editing of the final manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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