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# Combining high-power ultrasound and enological enzymes during winemaking to improve the chromatic characteristics of red wine

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ABSTRACT

Several techniques have been used by the winemaking industry to facilitate the extraction of grape phenolic compounds during the maceration process. Interest in innovative techniques such as high-power ultrasound (US) has increased in recent years, replacing more traditional techniques such as the addition of pectolytic enzymes (E). This study evaluates the combined effect of ultrasound and enological enzymes, used at semi-industrial scale at the time of crushing, on the chromatic characteristics of red wine. Variables such as the timing of enzyme addition and the ripening state of the grapes were considered. The results showed that ultrasound had a greater effect than enological enzymes when used alone, especially when the ripest grapes were used. The results also indicate that, when added after sonication, the enzymes favored the US effect, enabling the contact time necessary to achieve a wine with chromatic characteristics similar to those resulting from a traditional maceration process to be reduced by 4 days. Carried out on a semi-industrial scale, the study demonstrates that the adequate combination of these two techniques can optimize the maceration process both in terms of time and the wine organoleptic characteristics, making the technique of special interest for industrial application.

## 1. Introduction

High-power ultrasound (>19kHz) is of great interest in the food industry (Rojas et al., 2021; Vilkhu et al., 2008), and its presence is increasing in the enological industry because of the potential it has shown to improve a wine's organoleptic characteristics (Oliver Simancas et al., 2021), for reducing the processing time (Bautista-Ortín et al., 2017) and for enabling greater microbiological control in wines (Cabredo-Pinillos et al., 2006; Carrera et al., 2015; Morata et al., 2017; Roman et al., 2020). The technique is especially useful in terms of wine color improvements (Ferraretto et al., 2013; Zhang et al., 2015; Bautista-Ortín et al., 2017). However, even though the International Organization of Vine and Wine (OIV) has approved its industrial use with crushed grapes to favor the extraction of phenolic and aroma compounds during winemaking (OIV, 2019), most published studies have tended to use small laboratory devices such as ultrasonic baths or probes, meaning that the results may not be applicable on an industrial scale (Celotti & Ferraretto, 2016; González-Centeno et al., 2014; Lukić et al., 2019; Osete-Alcaraz et al., 2019; Roman et al., 2020). Although studies of US used on a semi-industrial or industrial scale are scarce (Celotti & Ferraretto, 2016; Gambacorta et al., 2017; Martínez-Pérez et al., 2020; Pérez-Porras et al., 2021), most of those that do exist have shown very encouraging results and suggest the method could be useful in the wine industry.

Ultrasound is based on cavitation phenomena, the shock waves created being capable of breaking solid surfaces. For this reason, US has been proposed for use in enology to help degrade the cell walls of grape skins, thus facilitating the extraction of the compounds located inside the skin cells, mainly phenolics and aroma compounds. Previously, our group has worked on optimizing the maceration process in red wine making by using US on a semi-industrial scale (Pérez-Porras et al., 2021), finding that the maceration time can be reduced from 7 days to 3

*Abbreviations:* US, High-power ultrasound; E, Enzyme addition; C, Control wine; C + E, Control wine with enzyme addition; US + E, Sonication followed by enzyme addition; E + S, Enzyme addition followed by sonication; 3d, 3 days of maceration; 7d, 7 days of maceration; 12, Wine made from 12° Baumé grapes; 14, Wine made from 14° Baumé grapes; CI, Color intensity; TPI, Total polyphenol 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; ECG, Concentration of epicatechin gallate (μM); EGC, Concentration of epigallocatechin (μM).

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days without losing the wine chromatic or organoleptic quality. Indeed, other studies suggest that US not only improves the chromatic quality of the wine, but also its volatile profile (Oliver Simancas et al., 2021) and polysaccharide composition (Martínez-Lapuente et al., 2021).

When used in crushed grapes, the effect of US is similar to that observed when maceration enzymes are used. Such enzymes are a commercial mixture of different pectolytic activities, mainly endo-polygalacturonase, pectin-methyl esterase and pectin-lyase activities. The same activities have been found in some non-Saccharomyces yeasts (Belda et al., 2016). Their whose purpose is to disassemble the cell wall structure, thus facilitating extraction of the compounds located inside the grape cells (Pinelo et al., 2006). However, although these enzymes and yeasts have been shown to have a positive effect in several studies (Belda et al., 2016; Busse-Valverde et al., 2011; Gil-Muñoz et al., 2009; Kelebek et al., 2007; Revilla & González-San José, 2003; Romero-Cascales et al., 2012), and even though enzymes have not always produced significant effects on the total phenolic content of a wine (Clare et al., 2002) or anthocyanin content (Parley et al., 2001).

Among the reasons for these differing results when such enzymes are used may be the composition of the enzymatic cocktail itself (Bautista-Ortín et al., 2005; Romero-Cascales et al, 2008), the varietal effect on the composition of the cell walls that the enzymes have to degrade (Ortega-Regules et al., 2008) and the ripening stage of the treated grapes that may change the extractability of intracellular compounds (Nogales-Bueno et al., 2020; Zietsman et al., 2015). It is well known that during the ripening process important changes take place in the cell wall composition, including the cell wall interaction with phenolic compounds and hence their extractability (Peyrot des Gachons and Kennedy, 2003; Bindon & Kennedy, 2011; Zietsman et al., 2015; Bindon et al., 2014; Bautista-Ortín et al., 2012; Hernández-Hierro et al., 2014). Such changes may also affect the extent of the effect of enzymes or US on grape phenolic extraction.

Since both E and US seek the same target, it seemed possible that the combination of both techniques might have a synergistic effect and so improve the extraction of phenolic compounds. In recent years, interest has grown in ultrasound-assisted enzyme extractions in the food industry, as a way for optimizing the extraction of different compounds (e.g. proteins, antioxidant compounds or polysaccharides) from a variety of plants, such as wheat or sesame bran, rice or pumpkin (Görgüç et al., 2019; Wang et al., 2014; Wu et al., 2014; Yang et al., 2018). As regards its use in enology, Dalagnol et al. (2017) showed that the combined effect of both techniques was greater than that obtained when they were used individually to extract enzymes from Cabernet Sauvignon grapes, adding weight to the study by Lieu and Le (2010) concerning the optimization of conditions for combining both techniques. However, both studies were conducted using high temperatures (50–80 °C), short periods of time (5–15 min) and at laboratory scale. In the study carried out by Osete-Alcaraz et al. (2019), conducted at a controlled temperature of 18 ± 1 °C and also at laboratory scale, it was found that the combination of enzymes and US, when applied at the beginning of the maceration step, did not improve the results obtained when the treatments were applied separately; however, when the enzyme was allowed to act alone during the first few days of the maceration process before US was applied, a statistically significant synergistic effect was observed, and both the color intensity and total phenol content increased in the resulting wines.

Based on the above results, the objective of this study is to determine, at semi-industrial scale, whether the combination of US and E at the beginning of the maceration process enhances the effect of either technique applied on its own and whether the ripening state of the grapes affects the results. To date, no studies regarding the effect of grape maturation on the results obtained when using ultrasound, alone or in combination with enzymes, have been published.

## 2. Material and methods

### 2.1. Grape samples and vinifications

The wine was made with grapes of Monastrell variety at two stages of ripeness (12° Baumé, equivalent to 21.8° Brix, and 14° Baumé, equivalent to 25.4° Brix) harvested from a vineyard in Jumilla (Murcia, Spain). Grapes were destemmed and crushed and six different vinifications (in triplicate) were carried out for each type of grapes (see Fig. 1): two control vinifications with neither enzymatic nor ultrasound treatment with 3 or 7 days of skin maceration (C3d and C7d wines), two vinifications with sonicated crushed grapes and 3 or 7 days of skin maceration time (US3d and US7d wines) and two vinifications where a pectolytic enzyme was used, alone (C + E3d wine) or in combination with US (US + E3d wine). For the enzymatic treatments, a commercial pectolytic enzyme (EnozymLux®, Agrovin, S.A., Alcazar de San Juan, Spain) was added at the concentration recommended by the supplier (3 mL/hL). For the US treatment, the crushed grapes were treated with a pilot-scale power ultrasound system (Agrovin S.A., Alcazar de San Juan, Spain) using a frequency of 30 kHz, a power of 9000 W and a power density of 58.5 W $cm^{-2}$. The ultrasound system comprised two hexagonal sonoreactors with sonoplates arranged along the pipes. The ultrasound system worked with a low rate of 400 kg of grapes per hour. The temperature of the crushed grapes did not increase by more than 2 °C.

For the 12 Baumé grapes, another vinification was carried out adding the pectolytic enzyme before the crushed grapes were sonicated (E + US3d wine), to determine the effect of the moment of enzyme addition on the outcome of the final wine.

All vinifications were conducted in 50 L stainless steel tanks. Total acidity was corrected to 5.5 g/L and selected yeasts were added at a dose of 20 g per 100 kg of grapes (Viniferm CT007, Agrovin, Alcazar de San Juan, Spain). The tanks were kept in a controlled temperature room at 24 °C ± 2 °C and the cap was punched down twice a day. When the desire skin maceration time was achieved (3 or 7 days) the musts-wines were pressed in a 75 L pneumatic press. Free-run and pressed wines were combined and the liquid was returned to clean tanks at room temperature until the end of fermentation. The wines obtained were cold-stabilized, at a temperature of 2 °C for one month, and bottled. The wines were analyzed at this moment.

### 2.2. Analytical determinations

#### 2.2.1. Physico-chemical analysis

The alcohol content, pH, total acidity and volatile acidity were determined in accordance with European Commision Regulations (ECC, 1990).

#### 2.2.2. Spectrophotometric parameters

Different chromatic parameters were carried out in wines filtered through 0.45-μm nylon filters using a HEλIOS α spectrophotometer (ThermoSpectronic, USA). Color intensity (CI) was determined from the sum of absorbance at 420, 520 and 620 nm, as described by Glories (1984). The total polyphenol index (TPI) was determined by absorbance at 280 nm (Ribéreau-Gayon et al., 1983). Total and polymeric anthocyanins (TAnt and PolAnt) were measured following the method of Ho et al. (2001). For the determination of methylcellulose precipitable tannins (MCPT), the method proposed by Smith (2005) was used.

#### 2.2.3. Determination of tannins by HPLC

In order to determine the total tannin concentration and composition of the wines, the phloroglucinolysis method was followed, according to the conditions proposed by Busse-Valverde et al. (2010). The following equipment was used: a Waters 2695 HPLC system (Waters, Milford, MA) coupled to a Waters 2996 photodiode array detector, and 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). The

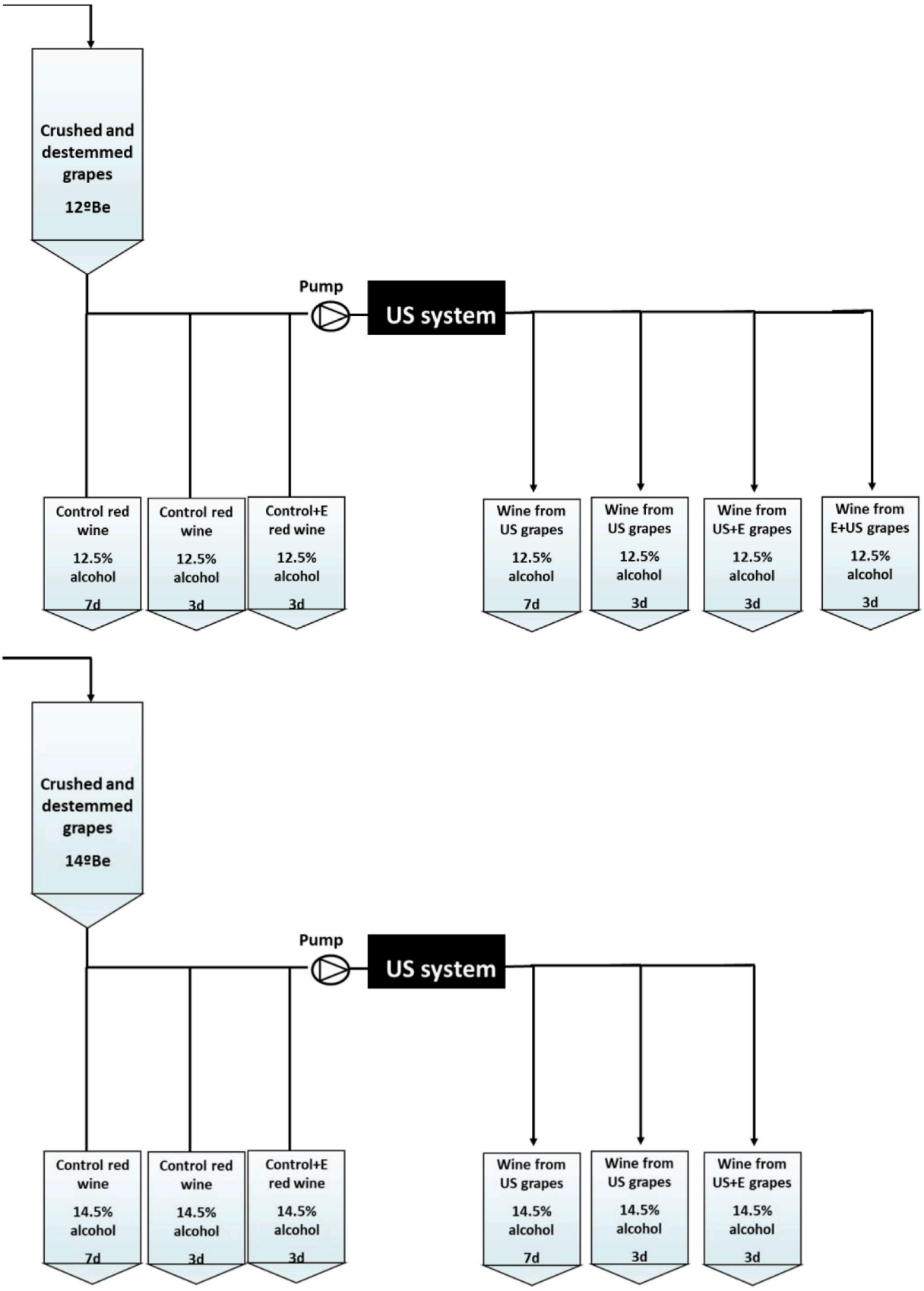


**Fig. 1.** Diagram of the experimental design.

injection volume was 10 μL. A water/formic acid mixture (98:2, v/v) was used as solvent A, and acetonitrile/solvent A (80:20 v/v) as solvent B, at a constant flow rate of 0.8 mL/min and an oven temperature of 30 °C. Elution conditions were as follows: 100% A for 5 min, linear gradient from 100 to 90% A in 30 min and gradient from 90 to 80% in 30 min, followed by washing and re-equilibration of the column.

This method provides information on the total tannin concentration (TTp), the mean apparent degree of polymerization (mDP) - calculated as the sum of the monomeric flavan-3-ol subunits and floroglucinol adducts divided by the sum of all flavan-3-ol monomers -, the percentage of galloylation (%Gal) and the percentage of epigallocatechin (%EGC) and the concentration of the epicatechin gallate (ECG) and epigallocatechin (EGC) subunits.

## 3. Statistical analysis

ANOVA, MANOVA and Principal Component Analyses were developed using the statistical package Statgraphics Centurion XVI.3 (Statpoint Technologies, Inc., The Plains, VA, USA).

## 4. Results and discussion

This study sought to investigate the combined effect of ultrasounds and pectolytic enzymes on a wine's chromatic characteristics (see Fig. 1) and how the ripening degree of the treated grapes affects the final wine characteristics. Moreover, with using the less ripe grapes, we also studied the effect of the moment of enzyme addition (before or after sonication), since contradictory results have been reported regarding the effect of ultrasounds on enzyme activity (Lieu & Le, 2010; Osete-Alcaraz et al., 2019; O'Donnell et al., 2010; Yachmenev et al., 2009).

### 4.1. Effect of ultrasound and enzyme treatments on the physico-chemical composition of finished wines

Table 1 shows the main physico-chemical parameters of the different wines. The results of the ANOVA analysis of the data pointed to almost no differences between them. As regards alcoholic degree, samples made from 12° Baumé grapes had an alcohol content that ranged from 11.7 (for the 12-C + E3d wine) to 12.6° (for the 12-C3d wine) while the wines made from 14° Baumé grapes provided values ranging from 14.55 (14-US7d wine) to 15.1° 14-C3d wine). There were no significant differences in pH or total acidity, regardless of the degree of maturation or the different winemaking methodologies since must acidity was corrected at the beginning of the elaboration process in the case of the more ripe grapes. Volatile acidity was very low, especially in the wines made from the less ripe grapes. As expected, the highest values in both wines were found for those made with seven days of skin maceration.

**Table 1**
Physico-chemical parameters analyzed at the *time of bottling*.

| *Sample* | %Alc | pH | TAc | VAc |
|---|---|---|---|---|
| 12-C3d | 12.62±0.46**b** | 3.79±0.03**a** | 5.89±0.20**a** | 0.17±0.04**a** |
| 12-C+E3d | 11.67±0.47**a** | 3.74±0.13**a** | 5.74±0.48**a** | 0.18±0.06**a** |
| 12-US3d | 12.46±0.04**ab** | 3.80±0.01**a** | 5.57±0.23**a** | 0.23±0.02**ab** |
| 12-US+E3d | 12.27±0.32**ab** | 3.75±0.01**a** | 5.79±0.10**a** | 0.20±0.02**a** |
| 12-E+US3d | 12.47±0.00**ab** | 3.73±0.01**a** | 5.94±0.31**a** | 0.17±0.01**a** |
| 12-C7d | 12.12±0.42**ab** | 3.86±0.02**a** | 5.34±0.04**a** | 0.25±0.02**ab** |
| 12-US7d | 12.42±0.06**ab** | 3.81±0.02**a** | 5.60±0.19**a** | 0.29±0.03**b** |
| 14-C3d | 15.15±0.20**c** | 3.67±0.01**bc** | 5.75±0.04**a** | 0.26±0.03**a** |
| 14-C+E3d | 15.04±0.28**bc** | 3.69±0.03**c** | 5.63±0.29**a** | 0.32±0.06**a** |
| 14-US3d | 14.86±0.04**abc** | 3.59±0.04**a** | 5.45±0.25**a** | 0.28±0.03**a** |
| 14-US+E3d | 14.60±0.10**ab** | 3.61±0.02**ab** | 5.43±0.21**a** | 0.31±0.06**a** |
| 14-C7d | 14.89±0.18**abc** | 3.73±0.03**c** | 6.04±0.86**a** | 0.45±0.02**b** |
| 14-US7d | 14.51±0.11**a** | 3.69±0.02**c** | 5.47±0.17**a** | 0.44±0.02**b** |

### 4.2. Effect of US and E treatments on the chromatic profile and phenolic composition of finished wines

Our main interest in this work was to study the chromatic characteristics of the wines obtained when sonication and enzymes were used with the crushed grapes. The results of the ANOVA analysis for the chromatic characteristics and phenolic composition of the different wines are shown in Tables 2 and 3. The combination of US + E was applied to grapes with two different levels of ripening, and for each ripening level the results were compared with those obtained when US and E were applied separately.

Using the less ripe grapes, we first tested whether the moment of enzyme addition was relevant for the results. In the first case, no positive effect was observed, and the wines did not differ chromatically from the control wine. In this respect, contradictory results on the effect of US on enzymes activity are to be found in literature. Some authors have pointed to the ability of US to improve the action of some enzymes (Ma et al., 2016; Yachmenev et al., 2009), while others showed that US may inactivate enzymes (Mawson et al., 2010; Rojas et al., 2016; Zhang et al., 2017), especially when used together with other technologies such as high pressure or high temperature (O'Donnell et al., 2010). Our results also pointed to enzyme inactivation by US. In a study developed by Osete-Alcaraz et al. (2019), the application of a combination of both techniques only had a favorable effect, compared with the use of one or the other technique, when US were applied after three days of skin maceration with enzyme, and no synergistic effect was observed when US was applied just after the application of the enzyme. Given these results, the same experiment (E + S) was not repeated with the more mature grapes, when and only the combination S + E was tested.

The results showed that when the pectolytic enzyme alone was added at the beginning of the vinification, the color intensity of the resulting wine was slightly higher (but not significantly so) than that of the corresponding control wine with no added enzyme. On the other hand, statistical differences were observed in the anthocyanin content of the

**Table 2**
Chromatic parameters analyzed at the time of bottling.

| *Sample* | CI | TPI | TAnt | PolAnt | MCPT |
|---|---|---|---|---|---|
| 12-C3d | 6.53 ± 0.19**a** | 31.50 ± 0.71**ab** | 292.69 ± 5.58**a** | 13.23 ± 0.19**a** | 555.38 ± 55.45**a** |
| 12-C + E3d | 7.07 ± 0.16**ab** | 34.02 ± 0.62**bc** | 323.87 ± 6.03**b** | 16.11 ± 1.56**bc** | 669.74 ± 51.38**a** |
| 12-US3d | 6.81 ± 0.37**ab** | 32.46 ± 1.13**abc** | 307.60 ± 17.83**ab** | 15.02 ± 0.71**ab** | 639.26 ± 41.96**a** |
| 12-US + E3d | 7.39 ± 0.23**b** | 34.05 ± 0.83**bc** | 323.82 ± 7.34**b** | 16.86 ± 0.71**bc** | 693.68 ± 94.64**ab** |
| 12-E + US3d | 6.29 ± 0.32**a** | 30.21 ± 1.32**a** | 285.13 ± 8.25**a** | 13.37 ± 0.90**a** | 649.38 ± 28.67**a** |
| 12-C7d | 7.52 ± 0.29**b** | 34.52 ± 1.04**c** | 322.17 ± 10.24**b** | 17.57 ± 0.47**c** | 843.12 ± 68.80**b** |
| 12-US7d | 8.61 ± 0.44**c** | 43.12 ± 0.46**d** | 323.31 ± 11.38**b** | 21.77 ± 0.90**d** | 1303.85 ± 18.26**c** |
| 14-C3d | 7.91 ± 0.23**a** | 36.68 ± 0.27**a** | 320.69 ± 8.65**ab** | 19.03 ± 1.65**a** | 1224.39 ± 37.56**a** |
| 14-C + E3d | 8.72 ± 0.20**ab** | 41.54 ± 1.91**b** | 335.77 ± 10.53**abc** | 22.81 ± 0.90**a** | 1239.41 ± 34.22**a** |
| 14-US3d | 10.69 ± 0.57**cd** | 47.55 ± 2.18**cd** | 401.32 ± 12.35**d** | 24.89 ± 2.03**ab** | 1441.40 ± 152.71**ab** |
| 14-US + E3d | 11.20 ± 0.24**d** | 49.10 ± 1.31**d** | 374.12 ± 35.90**cd** | 31.27 ± 2.83**bc** | 1728.71 ± 131.92**bc** |
| 14-C7d | 9.62 ± 0.26**bc** | 43.25 ± 1.19**bc** | 299.26 ± 16.14**a** | 31.55 ± 3.11**bc** | 1319.42 ± 103.55**a** |
| 14-US7d | 11.80 ± 0.73**d** | 57.85 ± 2.22**e** | 354.60 ± 12.97**bcd** | 37.50 ± 3.31**c** | 1981.95 ± 128.75**c** |

CI: color intensity, TPI: total polyphenol index, TAnt: total anthocyanins (mg/L), PolAnt: polymeric anthocyanins (mg/L), MCPT: methyl cellulose precipitable tannins (mg/L), 12: wine made from 12° Baumé grapes; 14: wine made from 14° Baumé grapes, C: control wine; US: ultrasound application; E: enzyme addition, 3d: 3 days of maceration; 7d: 7 days of maceration. Different letters in the same column mean statistically significant differences ($P < 0.05$) ($n = 3$).

**Table 3**
Concentration and characterization of the wine tannins using the phloroglucinolysis method.

| *Sample* | TTp | mDP | %EGC | %Gal | EGC | ECG |
|---|---|---|---|---|---|---|
| 12-C3d | 305.03 ± 43.83**a** | 4.68 ± 0.31**a** | 15.12 ± 0.51**b** | 2.39 ± 0.18**a** | 180.85 ± 51.20**ab** | 28.11 ± 5.60**a** |
| 12-C + E3d | 369.29 ± 14.78**ab** | 4.90 ± 0.12**abc** | 14.92 ± 1.16**b** | 2.40 ± 0.62**a** | 186.08 ± 16.43**ab** | 30.05 ± 8.43**a** |
| 12-US3d | 362.05 ± 28.04**ab** | 4.81 ± 0.05**ab** | 15.06 ± 0.50**b** | 2.52 ± 0.08**ab** | 184.45 ± 13.78**ab** | 30.93 ± 2.98**a** |
| 12-US + E3d | 439.28 ± 20.37**bc** | 5.03 ± 0.20**abc** | 14.57 ± 0.87**b** | 2.55 ± 0.16**ab** | 216.60 ± 22.67**ab** | 37.92 ± 4.04**a** |
| 12-E + US3d | 363.83 ± 37.07**ab** | 4.59 ± 0.15**a** | 14.02 ± 0.37**ab** | 2.62 ± 0.17**ab** | 172.12 ± 17.74**a** | 32.09 ± 2.45**a** |
| 12-C7d | 508.13 ± 1.70**c** | 5.38 ± 0.15**c** | 14.57 ± 0.65**b** | 3.23 ± 0.06**bc** | 249.11 ± 10.31**b** | 55.14 ± 1.19**b** |
| 12-US7d | 794.23 ± 34.69**d** | 5.26 ± 0.08**bc** | 12.20 ± 0.80**a** | 3.93 ± 0.07**c** | 325.58 ± 29.82**c** | 104.71 ± 6.24**c** |
| 14-C3d | 633.07 ± 62.45**a** | 6.80 ± 0.15**cd** | 18.44 ± 0.12**c** | 2.38 ± 0.04**a** | 393.73 ± 41.22**a** | 50.82 ± 5.78**a** |
| 14-C + E3d | 758.63 ± 33.40**ab** | 7.06 ± 0.19**d** | 18.34 ± 0.38**c** | 2.29 ± 0.13**a** | 469.25 ± 21.13**abc** | 58.80 ± 5.60**a** |
| 14-US3d | 910.65 ± 50.77**bc** | 6.30 ± 0.11**b** | 16.31 ± 0.09**b** | 2.39 ± 0.08**ab** | 501.48 ± 29.54**bcd** | 73.48 ± 4.92**b** |
| 14-US + E3d | 1017.13 ± 76.90**c** | 6.18 ± 0.18**ab** | 16.31 ± 0.38**b** | 2.41 ± 0.31**ab** | 560.75 ± 56.16**cd** | 82.19 ± 4.90**b** |
| 14-C7d | 797.88 ± 34.05**b** | 6.52 ± 0.14**bc** | 16.93 ± 0.13**b** | 2.78 ± 0.01**b** | 454.85 ± 16.53**ab** | 74.82 ± 3.18**b** |
| 14-US7d | 1293.72 ± 61.88**d** | 5.83 ± 0.11**a** | 13.57 ± 0.41**a** | 3.74 ± 0.08**c** | 590.07 ± 45.62**d** | 162.20 ± 5.20**c** |

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), 12: wine made from 12° Baumé grapes; 14: wine made from 14° Baumé grapes, C: control wine; US: ultrasound application; E: enzyme addition, 3d: 3 days of maceration; 7d: 7 days of maceration. Different letters in the same column mean statistically significant differences ($P < 0.05$) ($n = 3$).

wines (12-C versus 12-C + E3d wines), both total and polymeric anthocyanins, that increased significantly. Neither the tannin content nor tannin profile was not modified by the use of the enzyme. As regards the effect of US alone, the chromatic characteristics of the wines obtained after three days of maceration (12-US3d) did not differ from the wines obtained using pectolytic enzymes or from the control wine. Seven days of maceration was needed before any clear effect of sonication was observed on wine chromatic and phenolic characteristics (12-C7d *vs* 12-US7d), the wine made from sonicated grapes and following seven days of maceration displayed a higher color intensity, greater total phenol and tannin content, and their tannins presented a higher percentage of galloylation, probably due to the degradation of the seed cellular structures by the effect of sonication and the increasingly presence of ethanol during fermentative maceration (Hernández-Jiménez et al., 2012). In agreement with our results, Busse-Valverde et al. (2011) observed an increase in the percentage of galloylation as the alcohol content of the medium increased.

When the grapes were sonicated and then the maceration enzyme was added, the combination of both processes increased the color intensity and the anthocyanin and tannin concentration compared with control wines, while the composition of tannins was similar to that of the control wines. Moreover, the characteristics of the 12-US + E3d wines did not differ from those observed for the control wine made with seven days of maceration (12-C7d), and neither did the tannin composition, indicating that the combination of both techniques could help reduce maceration time in wineries.

As regards the more mature grapes, the wines, in general and as expected, had a higher color intensity, TPI, total and polymeric anthocyanin content, and tannin content than the corresponding wines made from the less ripe grapes. These differences were statistically significant, as shown by the multifactorial analysis of variance (MANOVA, Table 4). The technological maturity of the grape is usually accompanied by an increase in phenolic maturity and this, together with the higher alcohol content of these wines, might have facilitated the extractability of the phenolic compounds from grapes.

The wine tannins from the riper grapes also had a higher mDP and % EGC (Tables 3 and 4). Tannin mDP is mainly linked to the presence of skin tannins, since they present more than 20 subunits compared to the 7–8 subunits found in seed tannins. Skin tannins are rich in epigallocatechin, a tannin subunit absent from seed tannins (Watrelot & Norton, 2020), and the percentage of this subunit can be used as a distinguishing factor for evaluating the quantity of skin or seed tannins extracted during vinification. The higher percentage of this tannin subunit in the wines made from the more mature grapes pointed to a higher degree of degradation of the skin structures in the riper grapes, which facilitated the extraction of these high molecular mass tannins.

The use of enzymes in the elaboration of wines from the more mature grapes (14-C + E3d wines) barely changed the resulting wine chromatic and phenolic characteristics, and only the total phenol content was significantly higher than in the control wine (14-C3d). Bautista-Ortín et al. (2007) reported the low effect of maceration enzymes when used during the vinification of grapes with an advanced degree of ripening, possibly due to the naturally occurring greater degradation of grape structures with ripening. However, the results shown in Table 4 show that the effect of enzymes was hardly affected by the ripening status, the interactions between these two effects only being significant in the case of total and polymeric anthocyanins, which were positively affected when more mature grapes were used.

**Table 4**
MANOVA statistical analysis of the independent effect of ripening, sonication and enzyme addition at the time of bottling for wines made with 3 days of maceration.

| | CI | TPI | TAnt | PolAnt | MCPT | TTp | mDP | %EGC | %Gal |
|---|---|---|---|---|---|---|---|---|---|
| 12° Baumé | 6.95**a** | 33.01**a** | 312.00**a** | 15.30**a** | 639.52**a** | 369.16**a** | 4.86**a** | 14.92**a** | 2.47**a** |
| 14° Baumé | 9.63**b** | 43.72**b** | 357.97**b** | 24.50**b** | 1408.48**b** | 829.87**b** | 6.58**b** | 17.35**b** | 2.37**a** |
| Control (US) | 7.56**a** | 35.93**a** | 318.26**a** | 17.79**a** | 922.23**a** | 516.51**a** | 5.86**b** | 16.70**b** | 2.37**a** |
| US | 9.02**b** | 40.79**b** | 351.71**b** | 22.01**b** | 1125.76**b** | 682.53**b** | 5.58**a** | 15.56**a** | 2.47**a** |
| Control (E) | 7.98**a** | 37.05**a** | 330.58**a** | 18.04**a** | 965.11**a** | 552.95**a** | 5.65**a** | 16.23**a** | 2.42**a** |
| Enzyme | 8.59**b** | 39.68**b** | 339.39**a** | 21.76**b** | 1082.89**b** | 646.08**b** | 5.79**a** | 16.03**a** | 2.41**a** |
| Interactions | | | | | | | | | |
| RxUS | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.73 |
| RxE | 0.69 | 0.29 | 0.04 | 0.05 | 0.36 | 0.24 | 0.33 | 0.55 | 0.81 |
| USxE | 0.59 | 0.06 | 0.04 | 0.55 | 0.15 | 0.93 | 0.21 | 0.86 | 0.79 |

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, 12: wine made from 12° Baumé grapes; 14: wine made from 14° Baumé grapes, C: control wine; US: ultrasound application; E: enzyme addition; RD: ripening degree; 3d: 3 days of maceration; 7d: 7 days of maceration. Statistically significant interactions show a P-value lower than 0.05.

Turning our attention to the effect of US, the effect of sonication on the wine chromatic characteristics was much more significant when the more mature grapes were sonicated. The wine color intensity, total phenol content, total anthocyanins and tannins (determined by phloroglucinolysis) were significantly improved when the more mature grapes were sonicated compared with the corresponding control wines (14-US3d wines *vs.* 14-C3d wines). In particular, the CI increased by 35.15%. The interaction between ripening and sonication was significant for all the studied parameters except the percentage of galloylation (Table 4). Comparison of the different effects of sonication when using the two type of grapes led us to hypothesize that the weaker effect of US on the less ripe grapes might be related to a greater rigidity of their cell wall structures.

The improvement in chromatic characteristics was greater than when enzymes were used (the 14-C + E3d wine only showed a 10.24% increase in CI compared with the 14-C3d wine). Although Osete-Alcaraz et al. (2019) found a similar effect of both techniques, our results agree with those of Lieu and Le (2010), whose study pointed to a higher concentration of phenolic compounds after US treatment compared with those observed after enzymatic treatment. However, the different outcomes of these two studies may have been be due to differences in the ripening stage of the grapes used in the assays. Moreover, both studies were developed at laboratory scale, so their results may not be totally comparable with ours, since our experiment was carried out on a semi-industrial scale using apparatus very similar to that found in large wineries.

The wine tannins from sonicated ripen grapes also differed from those of the control wine, since they presented a lower mDP and lower % of EGC, which may have been due to a more intense extraction of tannins from the seed helped by the effects of sonication, as reported by Da Porto et al. (2013).

When both ultrasound and enzyme were applied using the riper grapes, the differences between these wines and the control wines were even more pronounced, the 14-US + E3d wines showing greater color intensity and higher total phenol, anthocyanin and tannin content than the control wine made with four more days of skin maceration time (14-US + E3d wine *vs.* 14-C7d wine), as also observed when the less ripe grapes were used. These results agree with those of Lieu and Le (2010), in which the combined use of ultrasound and enzyme (added after sonication) produced more favorable results than other ways of combination or the exclusive application of enzyme, with an increase of 7.3% in the extraction of compounds of interest. Similarly, the study developed by Dalagnol et al. (2017) found a 35% increase in total anthocyanin concentrations over control assay levels when US and E were combined, being as high as 41% when mechanical stirring was also used after the ultrasound treatment.

### 4.3. Principal component analysis of the wine samples using all the studied variables

To graphically compare the results obtained taking into account all the studied factors (state of maturation, application of ultrasound and/or addition of pectolytic enzymes), a principal component analysis was carried out (Fig. 2). As can be seen, the different wines were distributed along two main components (PC1 on the x-axis and PC2 on the y-axis). As expected, and coinciding with previously reported results, wines made from less ripe grapes were separated from those of the more mature grapes, the former being located in the negative part of PC1, while the wines from the more mature grapes being located in the positive part of PCI, due to higher values of almost all the chromatic parameters.

In the wines made from 12° Baumé grapes, the application of enzymes, sonication and the increase in maceration time separated the wines along PC1, due to increases in color intensity, total phenols and tannin levels, although the separation between wines was slight. The only wine clearly separated from all the other samples was 12-US7d, which had higher PC1 values and lower PC2 values, mainly due its higher color intensity and phenolic content and higher percentage of tannin galloylation. In fact, this wine was located close to 14-C7d wines, with similar values in the PC1 axis, only differing in the values of PC2 since both wines presented similar total phenol index, total anthocyanins and total tannins. However, it is of note that the alcohol content of 12-US7d was 16.6% lower than that of 14-C7d (12.42% *vs.* 14.89%). In recent years, the mismatch between technological and phenolic maturity has become even more accentuated, especially in warm areas, where technologically mature grapes do not necessarily reflect their optimal phenolic maturity, leading to poorly colored wines. However, if viticulturists delay harvest until optimum phenolic maturity is reached, the resulting wines will have an excessive alcohol content. This is why techniques that increase the extraction of phenolic compounds from grapes with a low phenolic maturity index are of particular interest, ultrasound being a potentially appropriate technique in this respect. Previous results from Martínez-Perez et al. (2020) indicated that US could be applied to grapes to favor the extraction of phenolic compounds even when full phenolic maturity has not been reached, allowing the production of quality wines with a reduced alcohol content.

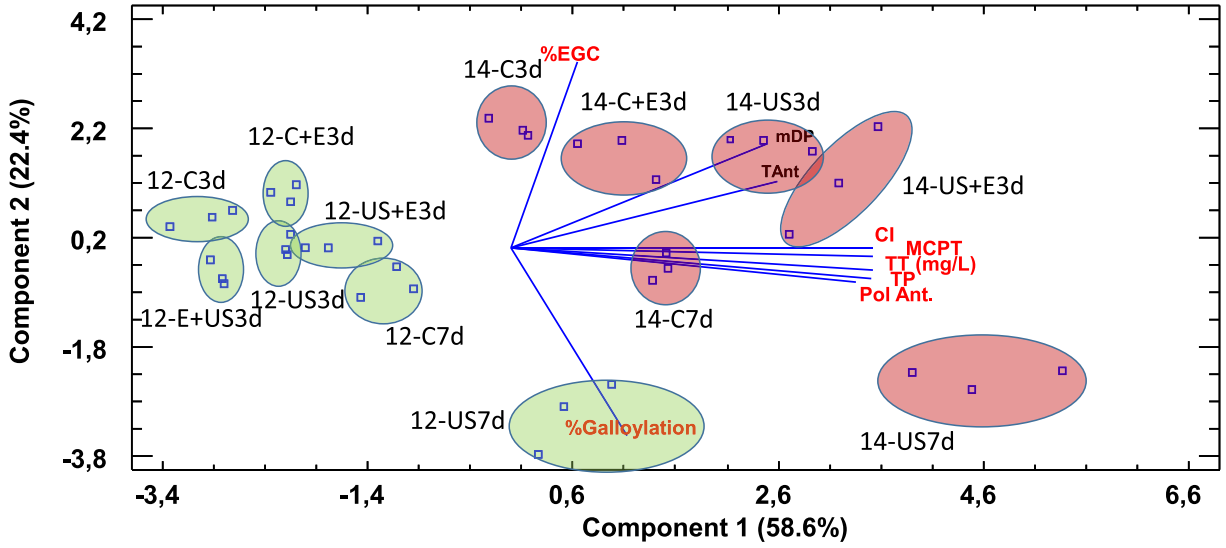


**Fig. 2.** Bidimensional plot of the Principal component analysis for the different wines at the moment of bottling.

Separation was much more evident in the wines made from the more mature grapes; indeed, the 14-C3d wines were quite different from 14-US + E3d wines, which presented intermediate characteristics between the two wines made after seven days of skin maceration, the control wine and the one made from sonicated grapes (14-C7d and 14S7d) since the 14-US + E3d wine showed chromatic characteristics over the 14-C7d wine, as it presented a higher concentration of phenolic compounds, a higher percentage of skin tannin and greater degree of polymerization. This effect, which was also evident in the wines made with the less ripe grapes, confirmed that the combination of sonication and enzyme intensified the chromatic characteristics of the wines; similar (or even higher) values for most chromatic compounds were observed between US + E3d and C7 wines. In fact, in the wines obtained from the less ripe grapes, only PolAnt, TT and tannin mDP were higher in 12-C7d than in 12-US + E3d, as reflected in Table 2, and when the same comparison was made with the wines from the more mature grapes, all the chromatic values were statistically similar, total anthocyanis being even higher in the 14-US + E3d wine.

## 5. Conclusions

The results show that the sonication of crushed grapes led to an improvement in the wine chromatic characteristics. Ultrasounds was more effective than pectolytic enzymes in terms of increasing wine color and the phenolic content, and when the enzymes were added to sonicated crushed grapes, a synergistic effect was evident. Indeed, the chromatic characteristics of the 12-US + E3d wines were very similar to those of a control wine with 4 more days of maceration, or even better in the case of the more ripe grapes (14-US + E3d). Taking into account that the higher temperatures we are experienced during the maturation period have led to an advanced and compressed vintage period, the availability of maceration tanks in the wineries may be limited, making the reduction of the maceration time necessary, these results indicate that the combination of US and E could be considered a very interesting practice for reducing maceration times, thus increasing the productivity of wineries, since a reduction of more than 50% in the maceration time is possible. However, no such improvement was observed when the enzyme was added to crushed grapes before the sonication process.

Another important fact is that the combination of sonication and long maceration times may facilitate the extraction of phenolic compounds, allowing highly colored wines to be obtained from less ripe grapes. This would also serve to reduce the alcohol content of the wines without reducing the quantity of phenols extracted, an aspect of great interest in the face of probable climate change.

## Industrial relevance

This work describes different procedures for optimizing the process of winemaking at an industrial scale through the combined use of enological enzymes and high-power ultrasound.

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## CRediT authorship contribution statement

**Paula Pérez-Porras:** Formal analysis, Methodology, Writing – original draft. **Ana Belén Bautista-Ortín:** Conceptualization, Investigation, Writing – original draft, Data curation, Writing – review & editing, and Dr. **Ricardo Jurado:** Conceptualization, Investigation, Formal analysis, Methodology, Data curation, Writing – review & editing, were in charge of the conceptualization as well as in the investigation, helped by Mr. **Encarna Gómez-Plaza:** Conceptualization, Investigation, Writing – original draft, Data curation, Writing – review & editing.

## Declaration of competing interest

None.

## References

Bautista-Ortín, A. B., Fernández-Fernández, J. I., López-Roca, J. M., & Gómez-Plaza, E. (2007). The effects of enological practices in anthocyanins, phenolic compounds and wine colour and their dependence on grape characteristics. *Journal of Food Composition and Analysis, 20*(7), 546–552. https://doi.org/10.1016/j.jfca.2007.04.008

Bautista-Ortín, A. B., Jiménez-Martínez, M. D., Jurado, R., Iniesta, J. A., Terrades, S., Andrés, A., & Gómez-Plaza, E. (2017). Application of high-power ultrasounds during red wine vinification. *International Journal of Food Science and Technology, 52*(6), 1314–1323. https://doi.org/10.1111/ijfs.13411

Bautista-Ortín, A. B., Martínez-Cutillas, A., Ros-García, J. M., López-Roca, J. M., & Gómez-Plaza, E. (2005). Improving color extraction and stability in red wines: The use of maceration enzymes and enological tannins. *International Journal of Food Science and Technology, 40*(8), 867–878. https://doi.org/10.1111/j.1365-2621.2005.01014.x

Bautista-Ortín, A. B., Rodríguez-Rodríguez, P., Gil-Muñoz, R., Jiménez-Pascual, E., Busse-Valverde, N., Martínez-Cutillas, A., López-Roca, J. M., & Gómez-Plaza, E. (2012). Influence of berry ripeness on concentration, qualitative composition and extractability of grape seed tannins. *Australian Journal of Grape and Wine Research, 18*(2), 123–130. https://doi.org/10.1111/j.1755-0238.2012.00178.x

Belda, I., Conchillo, L., Ruiz, J., Navascués, E., Marquina, D., & Santos, A. (2016). Selection and use of pectinolytic yeasts for improving clarification and phenolic extraction in winemaking. *International Journal of Food Microbiology, 223*, 1–8. https://doi.org/10.1016/j.ijfoodmicro.2016.02.003

Bindon, K. A., & Kennedy, J. A. (2011). Ripening-induced changes in grape skin proanthocyanidins modify their interaction with cell walls. *Journal of Agricultural and Food Chemistry, 59*, 2696–2707. https://doi.org/10.1021/jf1047207

Bindon, K. A., Madani, S. H., Pendleton, P., Smith, P. A., & Kennedy, J. A. (2014). Factors affecting skin tannin extractability in ripening grapes. *Journal of Agricultural and Food Chemistry, 62*(5), 1130–1141. https://doi.org/10.1021/jf4050606

Busse-Valverde, N., Gómez-Plaza, E., López-Roca, J. M., Gil-Muñoz, R., & Bautista-Ortín, A. B. (2011). The extraction of anthocyanins and proanthocyanidins from grapes to wine during fermentative maceration is affected by the enological technique. *Journal of Agricultural and Food Chemistry, 59*(10), 5450–5455. https://doi.org/10.1021/jf2002188

Busse-Valverde, N., Gómez-Plaza, E., López-Roca, J. M., Gil-Muñoz, R., Fernández-Fernández, J. I., & Bautista-Ortín, A. B. (2010). Effect of different enological practice on skin and seed proanthocyanidins in three varietal wines. *Journal of Agricultural and Food Chemistry, 58*(21), 11333–11339. https://doi.org/10.1021/jf102265c

Cabredo-Pinillos, S., Cedrón-Fernández, T., González-Briongos, M., Puente-Pascual, L., & Sáenz-Barrio, C. (2006). Ultrasound-assisted extraction of volatile compounds from wine samples: Optimisation of the method. *Talanta, 69*(5), 1123–1129. https://doi.org/10.1016/j.talanta.2005.12.011

Carrera, C., Ruiz-Rodríguez, A., Palma, M., & Barroso, C. G. (2015). Ultrasound assisted extraction of amino acids from grapes. *Ultrasonics Sonochemistry, 22*, 499–505. https://doi.org/10.1016/j.ultsonch.2014.05.021

Celotti, E., & Ferraretto, P. (2016). Studies for the ultrasound application in winemaking for a low impact enology. *Application of Ultrasound in the Beverage Industry*, 115–143.

Clare, S. S., Theaud, L., & Skurray, G. (2002). Effect of a pectolytic enzyme on the colour of red wine. *Australian and New Zealand grapegrower and winemaker, 456*, 29–35.

Da Porto, C., Porretto, E., & Decorti, E. (2013). Comparison of ultrasound-assisted extraction with conventional extraction methods of oil and polyphenols from grape (*Vitis vinifera* L.) seeds. *Ultrasonics Sonochemistry, 20*(4), 1076–1080. https://doi.org/10.1016/j.ultsonch.2012.12.002

Dalagnol, L. M. G., Dal Magro, L., Silveira, V. C. C., Rodrigues, E., Manfroi, V., & Rodrigues, R. C. (2017). Combination of ultrasound, enzymes and mechanical stirring: A new method to improve Vitis vinifera Cabernet Sauvignon must yield, quality and bioactive compounds. *Food and Bioproducts Processing, 105*, 197–204. https://doi.org/10.1016/j.fbp.2017.07.009

European Commission Regulation, E. E. C. (1990). No. 2676/90 Determining Community methods for the analysis of wines. *Official Journal of the European Communities*, 1, 192. L272 http://data.europa.eu/eli/reg/1990/2676/oj/.

Ferraretto, P., Cacciola, V., Batlló, I. F., & Celotti, E. (2013). Ultrasounds application in winemaking: Grape maceration and yeast lysis. *Italian Journal of Food Science, 25*(2), 160.

Gambacorta, G., Trani, A., Punzi, R., Fasciano, C., Leo, R., Fracchiolla, G., & Faccia, M. (2017). Impact of ultrasounds on the extraction of polyphenols during winemaking of red grapes cultivars from southern Italy. *Innovative Food Science & Emerging Technologies, 43*, 54–59. https://doi.org/10.1016/j.ifset.2017.07.029

Gil-Muñoz, R., Moreno-Pérez, A., Vila-López, R., Fernández-Fernández, J. I., Martínez-Cutillas, A., & Gómez-Plaza, E. (2009). Influence of low temperature prefermentative techniques on chromatic and phenolic characteristics of Syrah and Cabernet Sauvignon wines. *European Food Research and Technology, 228*(5), 777–788. https://doi.org/10.1007/s00217-008-0989-5

González-Centeno, M. R., Knoerzer, K., Sabarez, H., Simal, S., Rosselló, C., & Femenia, A. (2014). Effect of acoustic frequency and power density on the aqueous ultrasonic-assisted extraction of grape pomace (*Vitis vinifera* L.) – a response surface approach. *Ultrasonics Sonochemistry, 21*(6), 2176–2184. https://doi.org/10.1016/j.ultsonch.2014.01.021

Görgüç, A., Bircan, C., & Yılmaz, F. M. (2019). Sesame bran as an unexploited by-product: Effect of enzyme and ultrasound-assisted extraction on the recovery of protein and antioxidant compounds. *Food Chemistry, 83*, 637–645. https://doi.org/10.1016/j.foodchem.2019.01.077

Hernández-Hierro, J. M., Quijada-Morín, N., Martínez-Lapuente, L., Guadalupe, Z., Ayestarán, B., Rivas-Gonzalo, J. C., & Escribano-Bailón, M. T. (2014). Relationship between skin cell wall composition and anthocyanin extractability of *Vitis vinifera* L. cv. Tempranillo at different grape ripeness degree. *Food Chemistry, 146*, 41–47. https://doi.org/10.1016/j.foodchem.2013.09.037

Hernández-Jiménez, A., Kennedy, J. A., Bautista-Ortín, A. B., & Gómez-Plaza, E. (2012). Effect of ethanol on grape seed proanthocyanidin extraction. *American Journal of Enology and Viticulture, 63*(1), 57–61. https://doi.org/10.5344/ajev.2011.11053

Ho, P., Da Silva, M., & Hogg, T. A. (2001). Changes in colour and phenolic composition during the early stages of maturation of port in wood, stainless steel and glass. *Journal of the Science of Food and Agriculture, 81*(13), 1269–1280. https://doi.org/10.1002/jsfa.938

Kelebek, H., Canbas, A., Cabaroglu, T., & Selli, S. (2007). Improvement of anthocyanin content in the cv. Okuzgozu wines by using pectolytic enzymes. *Food Chemistry, 105*(1), 334–339. https://doi.org/10.1016/j.jfoodeng.2005.08.032

Lieu, L. N., & Le, V. V. M. (2010). Application of ultrasound in grape mash treatment in juice processing. *Ultrasonics Sonochemistry, 17*(1), 273–279. https://doi.org/10.1016/j.ultsonch.2009.05.002

Lukić, K., Brnčić, M., Ćurko, N., Tomašević, M., Valinger, D., Denoya, G. I., Barba, F. J., & Ganić, K. K. (2019). Effects of high power ultrasound treatments on the phenolic, chromatic and aroma composition of young and aged red wine. *Ultrasonics Sonochemistry, 59*, 104725. https://doi.org/10.1016/j.ultsonch.2019.104725

Martínez-Lapuente, L., Guadalupe, Z., Ayestarán, B., Pérez-Porras, P., Bautista-Ortín, A. B., & Gómez-Plaza, E. (2021). Ultrasound treatment of crushed grapes: Effect on the must and red wine polysaccharide composition. *Food Chemistry, 356*, 129669. https://doi.org/10.1016/j.foodchem.2021.129669

Martínez-Pérez, M. P., Bautista-Ortín, A. B., Pérez-Porras, P., Jurado, R., & Gómez-Plaza, E. (2020). A new approach to the reduction of alcohol content in red wines: The use of high-power ultrasounds. *Foods, 9*(6), 726. https://doi.org/10.3390/foods9060726

Mawson, R., Gamage, M., Terefe, N. S., & Knoerzer, K. (2010). *Ultrasound in enzyme activation and inactivation. Ultrasound technologies for food and bioprocessing, 369–404*. New York, NY: Springer. https://doi.org/10.1007/978-1-4419-7472-3_14

Ma, X., Zhang, L., Wang, W., Zou, M., Ding, T., Ye, X., & Liu, D. (2016). Synergistic effect and mechanisms of combining ultrasound and pectinase on pectin hydrolysis. *Food and Bioprocess Technology, 9*(7), 1249–1257. https://doi.org/10.1007/s11947-016-1689-y

Morata, A., Loira, I., Vejarano, R., González, C., Callejo, M. J., & Suárez-Lepe, J. A. (2017). Emerging preservation technologies in grapes for winemaking. *Trends in Food Science & Technology, 67*, 36–43. https://doi.org/10.1016/j.tifs.2017.06.014

Nogales-Bueno, J., Baca-Bocanegra, B., Heredia, F. J., & Hernández-Hierro, J. M. (2020). Phenolic compounds extraction in enzymatic macerations of grape skins identified as low-level extractable total anthocyanin content. *Journal of Food Science, 85*(2), 324–331. https://doi.org/10.1111/1750-3841.15006

O'Donnell, C. P., Tiwari, B. K., Bourke, P., & Cullen, P. J. (2010). Effect of ultrasonic processing on food enzymes of industrial importance. *Trends in Food Science & Technology, 21*(7), 358–367. https://doi.org/10.1016/j.tifs.2010.04.007

OIV. (2019). *Treatment of crushed grapes with ultrasound to promote the extraction of their compounds. Resolution OIV-OENO 616-2019*. Paris, France: OIV.

Oliver Simancas, R., Díaz-Maroto, M. C., Alañón Pardo, M. E., Pérez Porras, P., Bautista-Ortín, A. B., Gómez-Plaza, E., & Pérez-Coello, M. S. (2021). Effect of power ultrasound treatment on free and glycosidically-bound volatile compounds and the sensorial profile of red wines. *Molecules, 26*(4), 1193. https://doi.org/10.3390/molecules26041193

Ortega-Regules, A., Ros-García, J. M., Bautista-Ortín, A. B., López-Roca, J. M., & Gómez-Plaza, E. (2008). Changes in skin cell wall composition during the maturation of four premium wine grape varieties. *Journal of the Science of Food and Agriculture, 88*(3), 420–428. https://doi.org/10.1002/jsfa.3102

Osete-Alcaraz, A., Bautista-Ortín, A. B., Ortega-Regules, A. E., & Gómez-Plaza, E. (2019). Combined use of pectolytic enzymes and ultrasounds for improving the extraction of phenolic compounds during vinification. *Food and Bioprocess Technology, 12*(8), 1330–1339. https://doi.org/10.1007/s11947-019-02303-0

Parley, A., Vanhanen, L., & Heatherbell, D. (2001). Effects of pre-fermentation enzyme maceration on extraction and colour stability in Pinot Noir wine. *Australian Journal of Grape and Wine Research, 7*(3), 146–152. https://doi.org/10.1111/j.1755-0238.2001.tb00203.x

Pérez-Porras, P., Bautista-Ortín, A. B., Jurado, R., & Gómez-Plaza, E. (2021). Using high-power ultrasounds in red winemaking: Effect of operating conditions on wine physicochemical and chromatic characteristics. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 138*, 110645. https://doi.org/10.1016/j.lwt.2020.110645

Peyrot des Gachons, C., & Kennedy, J. A. (2003). Direct method determining seed and skin proanthocyanidin extraction into red wine. *Journal of Agricultural and Food Chemistry, 51*, 5877–5881. https://doi.org/10.1021/jf034178r

Pinelo, M., Arnous, A., & Meyer, A. S. (2006). Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. *Trends in Food Science & Technology, 17*(11), 579–590. https://doi.org/10.1016/j.tifs.2006.05.003

Revilla, I., & González-San José, M. L. (2003). Compositional changes during the storage of red wines treated with pectolytic enzymes: Low molecular-weight phenols and flavan-3-ol derivative levels. *Food Chemistry, 80*(2), 205–214. https://doi.org/10.1016/S0308-8146(02)00255-8

Ribéreau-Gayon, P., Pontallier, P., & Glories, Y. (1983). Some interpretations of colour changes in young red wines during their conservation. *Journal of the Science of Food and Agriculture, 34*(5), 505–516. https://doi.org/10.1002/jsfa.2740340512

Rojas, M. L., Hellmeister Trevilin, J., & Duarte Augusto, P. E. (2016). The ultrasound technology for modifying enzyme activity. *Scientia Agropecuaria, 7*(2), 145–150. https://doi.org/10.17268/sci.agropecu.2016.02.07

Rojas, M. L., Kubo, M. T., Caetano-Silva, M. E., & Augusto, P. E. (2021). Ultrasound processing of fruits and vegetables, structural modification and impact on nutrient and bioactive compounds: A review. *International Journal of Food Science and Technology*. https://doi.org/10.1111/ijfs.15113

Roman, T., Tonidandel, L., Nicolini, G., Bellantuono, E., Barp, L., Larcher, R., & Celotti, E. (2020). Evidence of the possible interaction between ultrasound and thiol precursors. *Foods, 9*(1), 104. https://doi.org/10.3390/foods9010104

Romero-Cascales, I., Fernández-Fernández, J. I., Ros-García, J. M., López-Roca, J. M., & Gómez-Plaza, E. (2008). Characterization of the main enzymatic activities present in six commercial macerating enzymes and their effects on extracting colour during winemaking of Monastrell grapes. *International Journal of Food Science and Technology, 43*(7), 1295–1305. https://doi.org/10.1111/j.1365-2621.2007.01608.x

Romero-Cascales, I., Ros-García, J. M., López-Roca, J. M., & Gómez-Plaza, E. (2012). The effect of a commercial pectolytic enzyme on grape skin cell wall degradation and colour evolution during the maceration process. *Food Chemistry, 130*(3), 626–631. https://doi.org/10.1016/j.foodchem.2011.07.091

Smith, P. A. (2005). Precipitation of tannin with methyl cellulose allows tannin quantification in grape and wine samples. *Technical Review, 158*, 3–7 (Adelaide, Australia: Australian Wine Research I(nstitute).

Vilkhu, K., Mawson, R., Simons, L., & Bates, D. (2008). Applications and opportunities for ultrasound assisted extraction in the food industry — a review. *Innovative Food Science & Emerging Technologies, 9*(2), 161–169. https://doi.org/10.1016/j.ifset.2007.04.014

Wang, J., Sun, B., Liu, Y., & Zhang, H. (2014). Optimisation of ultrasound-assisted enzymatic extraction of arabinoxylan from wheat bran. *Food Chemistry, 150*, 482–488. https://doi.org/10.1016/j.foodchem.2013.10.121

Watrelot, A. A., & Norton, E. L. (2020). Chemistry and reactivity of tannins in vitis spp.: A review. *Molecules, 25*(9), 2110. https://doi.org/10.3390/molecules25092110

Wu, H., Zhu, J., Diao, W., & Wang, C. (2014). Ultrasound-assisted enzymatic extraction and antioxidant activity of polysaccharides from pumpkin (*Cucurbita moschata*). *Carbohydrate Polymers, 113*, 314–324. https://doi.org/10.1016/j.carbpol.2014.07.025

Yachmenev, V., Condon, B., Klasson, T., & Lambert, A. (2009). Acceleration of the enzymatic hydrolysis of corn stover and sugar cane bagasse celluloses by low intensity uniform ultrasound. *Journal of Biobased Materials and Bioenergy, 3*(1), 25–31.

Yang, X., Li, Y., Li, S., Oladejo, A. O., Wang, Y., Huang, S., Zhou, C., Ye, X., Ma, H., & Duan, Y. (2018). Effects of ultrasound-assisted α-amylase degradation treatment with multiple modes on the extraction of rice protein. *Ultrasonics Sonochemistry, 40*, 890–899.

Zhang, Z., Niu, L., Li, D., Liu, C., Ma, R., Song, J., & Zhao, J. (2017). Low intensity ultrasound as a pretreatment to drying of daylilies: Impact on enzyme inactivation, color changes and nutrition quality parameters. *Ultrasonics Sonochemistry, 36*, 50–58. https://doi.org/10.1016/j.ultsonch.2016.11.007

Zhang, Q.-A., Shen, Y., Fan, X.-H., & García-Martín, J. F. (2015). Preliminary study of the effect of ultrasound on physicochemical properties of red wine. *CyTA - Journal of Food, 14*(1), 55–64. https://doi.org/10.1080/19476337.2015.1045036

Zietsman, A. J. J., Moore, J. P., Fangel, J. U., Willats, W. G. T., Trygg, J., & Vivier, M. A. (2015). Following the compositional changes of fresh grape skin cell walls during the fermentation process in the presence and absence of maceration enzymes. *Journal of Agricultural and Food Chemistry, 63*(10), 2798–2810. https://doi.org/10.1021/jf505200m