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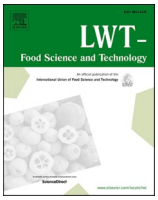

Ultrasound treatment of red wine: Effect on polyphenols, mathematical modeling, and scale-up considerations

Andrea Natolino *, Emilio Celotti

Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, via Sondrio 2/A, 33100, Udine, Italy

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ABSTRACT

High-power ultrasound has recently been approved for the treatment of crushed grapes; it can be considered as a highly promising technology that can be adopted for several purposes in the winemaking process. The effect of ultrasound used at different amplitudes (30, 60, and 90%) and over different periods of time (2, 6, and 10 min) on the main polyphenols and other analytical indices relating to the sensory properties of red wines was studied. An increase in the amplitude and sonication time did not affect the initial polyphenol profile, and no degradative phenomena were revealed. A significant increase in the HCl index and a decrease in the astringency index and particle size were highlighted in all sonicated samples. The significant effects of ultrasounds were described well by the power law function, logistic, and Peleg's model, with good fitting results obtained ($R^2 > 0.94$). Some scale-up considerations were reported with regard to the acoustic energy density (AED), as an intensive parameter of ultrasound treatments.

1. Introduction

Polyphenols play a fundamental role in enology, especially in the color and flavor of red wines. This class of compounds can be found in different part of grape; they have different chemical structures and, consequently, different chemical properties and reactivities. Anthocyanins and tannins are two of the most important groups of polyphenols responsible for many of the sensory properties of red wines.

Anthocyanins are the main compounds responsible for the color of young red wines. These molecules are unstable and the concentration of monomeric forms decreases constantly during wine maturation and aging. Several mechanisms might be involved, such as degradation; oxidation; precipitation with proteins, polysaccharides, or condensed tannins; and the progressive and irreversible formation of more complex and stable anthocyanin-derived pigments (He et al., 2012a, 2012b; Oliveira et al., 2014).

Tannins can be subdivided in two classes: hydrolysable and condensed tannins. Condensed tannins, also known as proanthocyanidins, are more or less complex polymers of flavan-3-ols or catechins. These are the main compounds affecting the perception of the astringency of wine and its evolution throughout wine aging (Wei et al., 2021). The initial aggressive astringency of young red wines is gradually softened throughout the aging process. This lessening of the perception of astringency is not solely due to a reduction in tannin concentration but may also be associated with condensation reactions with anthocyanins and changes in the mean degree of polymerization (Garcia-Estevez et al., 2017). During wine aging, proanthocyanidin compounds tend to polymerize, condense with anthocyanins, and combine with other polymers, such as proteins and polysaccharides. Condensation with anthocyanins is the main chemical reaction involved in the deepening and stabilization of the color of red wine, which changes from a bright red to reddish-brown tonality (Avizcuri et al., 2016).

Wine aging is a long-term process that is sometimes incompatible with the consumers' requirements and creates high costs for wineries (Carpena et al., 2020; GarcíaMartín et al., 2016). Several actions should be considered to accelerate the vinification processes and ensure the quality of the wine at the same time. The intentional and controlled addition of a small amount of oxygen into wine, known as micro-oxygenation, is a cheap and widespread technique used to improve the taste, aroma, and structural characteristics of red wine and reduce its aging time (Catania et al., 2021; Ćurko et al., 2021). Some other approaches are used by wineries, but these are strictly limited to specific specialty wines, such as Madeira wine, which undergoes a slow heating for several months during the vinification process (Tredoux & Silva Ferreira, 2012).

Other different innovative technologies, such as ultrasound (Lukić

* Corresponding author.
E-mail address: andrea.natolino@uniud.it (A. Natolino).

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et al., 2019; Zhang & Wang, 2017), gamma irradiation (Harder et al., 2013; KalkanYildiri & Dündar, 2017), and high pressure (Tao et al., 2016), have been applied to accelerate the aging process and shorten the production cycle of wine.

Ultrasound is regarded as the most promising technique for accelerating the wine aging process (García Martín & Sun, 2013), specifically considering its effectiveness in changing the chromatic characteristics and phenolic properties of red wines (Tiwari et al., 2010). Ultrasound can promote certain chemical reactions and encourage chemical and structural changes in wine that resemble those that occur after long periods of natural aging to take place (García Martín & Sun, 2013).

Sonication at low frequency (20–100 kHz) has shown several beneficial effects on numerous liquid and solid foods, especially fermented ones (Yu et al., 2021). Various investigations have suggested that ultrasound can increase the polysaccharide content of wine and reduce its ageing time (del Fresno et al., 2018). Generally, ultrasound can promote many chemical reactions involved in the maturation and aging of fermented foods, such as oxidation, esterification, and proteolysis, as well as improving their quality by promoting a favorable texture, color, flavor, and taste (Gao et al., 2020, 2021; Gavahian et al., 2021).

The high localized temperature and pressure created by acoustic cavitation induce the formation of reactive radical species, which enhance the reaction rates of existing processes or start new reaction mechanisms (McKenzie et al., 2019).

Ultrasound is a relative low-cost and eco-friendly technology that can be applied for several purposes in food technology (Bhargava et al., 2021). Despite the several studies on this topic, only a few experiments have been carried out on an industrial scale of operation due to the lack of unified design and scale-up strategies (Gogate et al., 2011). One extremely important aspect of the development of ultrasound technology that must be taken into account is that laboratory-scale experiments must be able to be up-scaled for industry; this is an aspect of sonochemistry that has been of interest for many years. Energy is an intensive parameter and independent of scale; thus, any ultrasonic process can be made scalable using this parameter (Patist & Bates, 2011).

In 2019, the International Organization of Wine (OIV) officially approved the use of ultrasound technology for the treatment of crushed grapes in order to increase the level of extraction of chemical compounds. Several authors have reported that the use of ultrasound makes it possible to prevent the degradation of polyphenol compounds and can positively affect some stability and evolution mechanisms involved in wine aging processes, if it is appropriately modulated (Celotti et al., 2020; Ferraretto & Celotti, 2016). It has also been highlighted that sonication can have positive effects on the protein stability of white wines (Celotti et al., 2021) and thiol precursors (Roman et al., 2020). Nevertheless, more detailed investigations on the effect of ultrasound on the main chemical compounds of wine are needed in order to define the right operating conditions for wine with different characteristics (grape variety, anthocyanin/tannin ratio, etc.). The aim of the present study was to evaluate the effect of ultrasounds on the polyphenolic compounds, especially tannins, in red wine. The effects of different levels of amplitude and sonication time on the total polyphenol, anthocyanin, flavan-3-ols, and catechins contents were studied. Other analytical indices relating to the chemical and sensory properties of tannins were considered in our evaluation of the effect of cavitation phenomena.

Moreover, some scale-up considerations were reported using the mathematical modelling of significant trends of some analytical indices highlighted after sonication treatments.

2. Materials and methods

2.1. Chemicals

The chemicals used were of analytical reagent grade and included Folin-Ciocalteau, sodium carbonate, potassium bisulfite, potassium metabisulfite, gallic acid, tartaric acid, hydrochloric acid malvidin-3-glucoside, (+)-catechin, 4-(dimethylamino)-benzaldehyde, bovine serum albumin (BSA), and ferric sulfate. All chemicals were purchased from Sigma-Aldrich Co. (Milan, Italy). The solvents used, including ethanol, methanol, and butanol, were purchased from Carlo Erba (Milan, Italy) and were of analytical grade or the highest available purity. https://www.sigmaaldrich.com/catalog/substance/4dimethylaminobenzaldehyde1491910010711.

2.2. Wine sample

A young red wine, Cabernet Sauvignon (Vitis vinifera L.), vintage 2019, from a winery in the Veneto region (Italy) was chosen and subjected to sonication treatments. The wine had the initial physicochemical characteristics of alcohol 12.5%, pH = 3.49, total acidity 5.3 g/L, and reducing sugars 3.8 g/L.

2.3. Ultrasound treatments

All the sonication treatments were carried out in an ultrasonic sonifier (ultrasonic processors UP200St, Hielscher Ultrasonics Gmbh, Teltow, Germany) equipped with a titanium alloy flat tip probe (13 mm diameter) (Sonotrode S26d14, Hielscher Ultrasonics Gmbh, Teltow, Germany). Aliquots of 100 mL of red wine were processed in a continuous mode at a constant frequency of 26 kHz. The energy input was controlled by setting the amplitude of the sonicator probe and the total nominal output was 200 W. The ultrasound probe was placed at the center of a 150 mL beaker and submerged about 20 mm under the surface of the sample. The beaker was placed into an ice bath to avoid an excessive increase in temperature, which was continuously monitored using a temperature controller. The process conditions, including temperature, amplitude, time, power, and energy, were continuously monitored and registered by the integrate operation software of an ultrasonic processor. Different levels of amplitude (30, 60, and 90%) and sonication times (2, 6, and 10 min) were studied.

The experimental conditions are summarized in Table 1. All the experiments were carried out in triplicate.

2.4. Analytical methods

2.4.1. Total polyphenol content (TPC)

The total polyphenols content (TPC) in the untreated and sonicated samples was determined using Folin–Ciocalteau, reagent as reported by Arnous et al. (2001), with slight modifications. Briefly, the reaction mixture contained 100 μL of diluted sample, 500 μL of the Folin–Ciocalteau reagent, 4 mL of water, and 2 mL of a sodium carbonate–water solution (15% w/v). After 2 h of reaction at room temperature, the absorbance was read at 750 nm using a UV–Vis spectrophotometer (Shimadzu UV-1650, Italy) to calculate the TPC. Gallic acid was employed as the standard. A calibration curve was made with standard solutions of gallic acid in the range of 50–500 mg/mL (R^2 = 0.99). The results were expressed as the mg gallic acid equivalent (GAE) per liter (mg $_{GAE}$/L).

Table 1
Experimental conditions of ultrasound treatments.

Exp.No°	Sample code	Amplitude (%)	t_{US} (min)
1	US1	30	2
2	US2	30	6
3	US3	30	10
4	US4	60	2
5	US5	60	6
6	US6	60	10
7	US7	90	2
8	US8	90	6
9	US9	90	10

2.4.2. Anthocyanin content

The anthocyanins content was determined as reported by Ribéreau-Gayon and Stonestreet (1965). One milliliter of sample was mixed with 1 mL of HCl/EtOH solution (0.1% *v/v*) and 20 mL of HCl/H_2O solution (2% v/v). Then, 2.5 mL of sample mixture was added to 1 mL of deionized H_2O and other 2.5 mL of sample mixture with 1 mL of potassium bisulphite solution (20% *w/v*). After 15 min of reaction, the absorbance of each solution was measured at 520 nm using a UV–Vis spectrophotometer (Shimadzu UV 1650, Milano, Italy), using distilled water as a control. The anthocyanin content, expressed as milligrams of malvidin-3-glucoside equivalent per liter (mg/L), was calculated considering a calibration curve, obtained with different solutions of malvidin-3-glucoside as a standard.

2.4.3. Flavan-3-ols content

The flavan-3-ols content was determined according to the method proposed by Zironi et al. (1992). The chromogen reagent was prepared with 1 g of 4-(dimethylamino)-cinnamaldehyde (DAC) dissolved in 250 mL of 37% HCl and 750 mL of methanol. Next, 1 mL of diluted sample (1:25 *v/v*) was added to 5 mL of DAC solution. Then, the absorbance was read at 640 nm using a UV–Vis spectrophotometer (Shimadzu UV 1650, Tokyo, Japan) against a blank prepared by substituting the sample with 1 mL of 10% ethanol solution. A calibration curve was prepared with several standard solutions of (+)-catechin and measurements were carried out at 640 nm. All analyses were performed in triplicate. Results were expressed as milligrams of (+)-catechin equivalents per liter (mg/L).

2.4.4. Condensed tannins

As reported by Bate-Smith (1954), two reaction mixtures were prepared by mixing 2 mL of diluted sample and 6 mL of hydrochloric acid–butanol solution. Four milliliters of reaction mixture was placed in a water bath at 100 °C for 20 min. After cooling, the absorbance of the two mixtures at 550 nm was measured using a UV–Vis spectrophotometer (Shimadzu UV 1650, Tokyo, Japan). The concentration of condensed tannins was then calculated using the following equation:

$$TA = D_{Abs} \cdot DF \cdot 0.1736 \quad (1)$$

where TA is the concentration of tannins, expressed as grams per liter (g/L), and DF is the dilution.

2.4.5. Polymerized pigments index (PPI)

Polymerized Pigment Index (PPI) represents the contribution of condensed tannins to the red color and the polymerized form of anthocyanins insensitive to bleaching by sulfur dioxide (Glories, 1978). Two reaction mixtures were prepared as follows: (A) 5 mL of sample was mixed with 45 mL of tartaric buffer (pH = 3.2) and 0.2 mL of potassium metabisulfite solution (20% w/v); (B) 5 mL of sample were mixed with 45 mL of tartaric buffer (pH = 3.2) and 0.2 mL of water. After 5 min, the optical density of each solution was read at 420 and 520 nm in a UV–Vis spectrophotometer (Shimadzu UV 1650, Tokyo, Japan) against water as a blank. The PPI was then calculated using the equation:

$$PPI = \frac{OD420_{(A)} + OD520_{(A)}}{OD420_{(B)} + OD520_{(B)}} \quad (2)$$

2.4.6. HCl index

The HCl index method is based on the instability of procyanidins in concentrated HCl medium, and their speed of precipitation depends on the degree of polymerization (Glories, 1978). Sample solutions were prepared by mixing 10 mL of sample, 15 mL of HCl (12 N), and 5 mL of water. The obtained mixture was then diluted 25 times and the optical density (d0) at 280 nm was measured immediately using a UV–Vis spectrophotometer (Shimadzu UV 1650, Tokyo, Japan). The same measurement of optical density at 280 nm (d1) was performed after 24 h after centrifuging the sample mixture at 3000 g for 10 min and diluting the supernatant solution 25 times. The HCl index is given by the equation:

$$HCl\ index = \frac{(d_0 - d_1)}{d_0} \times 100 \quad (3)$$

2.4.7. Astringency index

Astringency evaluation is based on the reactivity of tannins against bovine serum albumin (BSA). This method is more fast and reliable then the gelatin index.

Briefly, the sample was diluted 50 times and the initial turbidity was measured using a nephelometer (NTU 1). Subsequently, 20 mL of diluted sample were mixed with 1.5 mL of BSA solution (0.4 g/L) and vigorously homogenized. After 45 min of reaction, the turbidity was measured (NTU 2). The astringency index (AI) was then calculated as follows:

$$AI = \frac{(NTU\ 2 - NTU\ 1)}{0.4} \quad (4)$$

2.4.8. Dynamic light scattering (DLS) measurement

Dynamic light scattering measurements were carried out with a Nicomp 380 ZLS Nanoparticle Size Analyzer (Particle Sizing Systems, Santa Barbara, CA) equipped with a 10 mW He–Ne laser at a wavelength of 633 nm. Measurement occurred at 90° from the incident beam and gave an estimation of the particle mean diameter distribution, expressed in nanometers (nm).

DLS measurements were performed at 20 °C for a period of 5 min. All assays were performed in triplicate.

2.5. Absorbed energy density determination

The absorbed energy density (AED) parameter indicates the total heating energy experienced by a unit volume of sample under ultrasonic cavitation and is expressed as Joules per milliliter (J/mL). The AED can be calculated easily using the following equation:

$$AED = \frac{Q}{V} \quad (5)$$

where Q is the total heat absorbed during the sonication treatment (J) and V is the sample volume (mL). The total heat absorbed (Q) can be calculated from the temperature profiles of the sample during ultrasound treatment using the calorimetric method, as follows:

$$Q = m_s * c_p * \Delta T \quad (6)$$

where m_s is the total mass of sample (g), c_p is the specific heat capacity (J/kg°C), and T (°C) is the temperature change induced by the ultrasound treatment.

2.6. Mathematical modelling

The analytical parameters, which were significantly influenced by ultrasound treatments, were correlated with the absorbed energy density (AED). The trends obtained were mathematically described using three different models: power law function, logistic, and Peleg's model. In Table 2, the equations related to each model and the descriptions of all the mathematical parameters are reported. Generally, ΔY is the variation in the analytical parameter; AED is the absorbed energy density (J/mL); and Y_0 and Y_i are, respectively, the initial value of the analytical parameter and the value at point i.

2.7. Statistical analysis and model evaluation

All the experimental trials and analytical determinations were performed in triplicate and the results are represented by their mean ± standard deviation (SD). The Minitab 17 software (Minitab Inc., State

Table 2
Mathematical model equations and constants.

Model	Equation	Model parameters
Power law function model	$\Delta Y = k \cdot AED^n$ or $Y_i = Y_0 \pm k \cdot AED^n$	k = constant related to the variation rate n = exponential term (<1)
Logistic model	$Y_i = \frac{K}{(1 - a \cdot e^{-b \cdot AED})}$	K, a, b = estimated model coefficients
Peleg's model	$Y_i = Y_0 - \frac{AED}{(a + (b \cdot AED))}$	a, b = estimated model coefficients

College, PA, USA) was used for statistical analysis with one-way analysis of variance (ANOVA, with Tukey's HSD multiple comparison), and the level of significance was set at $p < 0.05$.

The values of the model parameters and graph plots were calculated using Matlab 2019b (MathWorks, Inc., USA). The agreement between the experimental values was assessed by means of correlation coefficients (R^2 and R^2-adj) and the normalized root means squared deviation (NRMSD) criterion, which is defined as:

$$NRMSD = \frac{RMSD}{exp_{max}} = \frac{\sqrt{(1/n) \cdot \sum_{p=1}^{n} (exp_p - mod_p)^2}}{exp_{max}} \quad (9)$$

where n is the number of experimental points composing a graph curve (n = 81), exp_p is the experimental value at point p, mod_p is the model value at point p, and exp mac is the maximum within the n experimental values. Higher values of R_2 and lower values of NRMSD denote that the model fits the experimental values well.

3. Results and discussion

3.1. Effect of ultrasounds on polyphenol profile

In Table 3, the results of total polyphenol content (TPC), total tannins (TA), flavan-3-ols (FL), anthocyanins content (AA), and polymerized pigments index (PPI) of untreated (Control) and sonicated samples at different levels of amplitude (30, 60 and 90%) and sonication time (2, 6 and 10 min) are shown.

As reported, no significant differences can be highlighted between the untreated (Control) and sonicated samples, considering the total polyphenol, tannin, flavan-3-ol, and anthocyanin contents. The increase in amplitude from 30 to 90% and sonication time from 2 to 10 min did not highlight any change in the main phenolic compounds. The ultrasound treatment carried out in this work permits to maintain the initial polyphenols content detected on the untreated sample and no degradative phenomena can be highlighted. The same results have been reported in our previous work, where other red wines were sonicated (Celotti et al., 2020; Ferraretto & Celotti, 2016). Other authors, instead, have reported that ultrasound induced a decrease in phenolic compounds, color, and aroma composition, especially at higher process conditions, and lighter modifications at mild levels of frequency, amplitude, and probe diameter (Lukić et al., 2019).

A possible explanation for the results reported in Table 3 could be found considering the temperature changes due to acoustic cavitation. In Table 4, the sample temperatures at the end of the sonication treatment at different levels of amplitude and sonication time are reported. The initial temperature was fixed at 16 °C for all samples and, as can be seen, the maximum temperature achieved was 48 °C at 90% of amplitude and 10 min (US9). Sólyom et al. (2014) have reported that thermal treatments at 80 °C for up to 250 min of a grape marc extract did not induce any degradation effect on phenolic compounds. The maintenance of the polyphenol profile is ensured also if the temperature has been increased up to 150 °C, but with a treatment time not higher than 1 min. Other studies have highlighted that polyphenol can be preserved from thermal degradation processes at temperatures of up to 50 °C (Dzah et al., 2020).

Wine aging is a complex process that involves several compounds, with changes on color and sensorial properties of red wine. Polyphenols are the main compounds that can undergo several reactions, such as degradation, condensation, structural changes, polymerization, and oxidation. In the literature, it has been reported that significant effects could be induced by ultrasound cavitation on these aging processes, according to amplitude, frequency, and sonication time (Lukić et al., 2019). If accurately modulated, ultrasound could enhance the condensation reaction rate between anthocyanin and tannins, decreasing the conventional times of wine color evolution (García Martín et al., 2016; Masuzawa et al., 2000) and potentially reducing the red wine aging period. Several analytical methods can be exploited to give valuable information on aging reactions. Polymerized pigment index (PPI) represents an analytical tool that indicates the degree of condensation

Table 4
Wine temperatures after sonication treatments ($T_{fin.}$) and acoustic energy density (AED) values.

Sample	Amplitude (%)	t_{US} (min)	$T_{fin.}$ (°C)	AED (J/mL)
US1	30	2	17.7 ± 0.6	3.96 ± 0.02
US2	30	6	20.7 ± 0.6	11.88 ± 0.04
US3	30	10	21.3 ± 0.7	17.16 ± 0.02
US4	60	2	27.0 ± 1.3	46.20 ± 0.12
US5	60	6	37.3 ± 0.6	74.48 ± 0.08
US6	60	10	38.3 ± 0.7	83.16 ± 0.21
US7	90	2	33.0 ± 1.0	62.04 ± 0.15
US8	90	6	46.3 ± 0.6	120.12 ± 0.47
US9	90	10	48.0 ± 1.1	122.76 ± 0.38

Table 3
Effect of amplitude and sonication time (t_{US}) on total polyphenol content (TPC), tannins (TT), flavan-3-ols (FL), anthocyanins (AA) and polymerized pigment index (PPI).

Sample	Amplitude (%)	t_{US} (min)	TPC (mg_{GAE}/L)	TT (g/L)	FL (mg/L)	AA (mg/L)	PPI (—)
Control	–	–	1812 ± 55 a*	1.83 ± 0.03 a	256 ± 22 a	81.46 ± 1.77 a	83.38 ± 0.24 b
US1	30	2	1805 ± 67 a	1.84 ± 0.04 a	223 ± 11 a	75.54 ± 3.13 a	83.13 ± 3.08 ab
US2	30	6	1772 ± 37 a	1.85 ± 0.11 a	231 ± 7 a	83.86 ± 1.31 a	83.23 ± 2.99 ab
US3	30	10	1799 ± 32 a	1.95 ± 0.12 a	225 ± 25 a	83.13 ± 2.33 a	84.76 ± 1.16 ab
US4	60	2	1793 ± 28 a	1.94 ± 0.13 a	231 ± 18 a	76.53 ± 9.35 a	84.44 ± 1.13 ab
US5	60	6	1873 ± 22 a	1.87 ± 0.09 a	248 ± 18 a	72.92 ± 2.14 a	82.71 ± 3.06 ab
US6	60	10	1894 ± 48 a	1.89 ± 0.07 a	242 ± 11 a	81.35 ± 2.20 a	87.00 ± 3.76 ab
US7	90	2	1857 ± 41 a	1.87 ± 0.03 a	237 ± 32 a	81.32 ± 3.46 a	83.33 ± 1.91 ab
US8	90	6	1860 ± 41 a	1.90 ± 0.10 a	250 ± 14 a	84.35 ± 4.34 a	85.09 ± 3.92 ab
US9	90	10	1874 ± 48 a	1.94 ± 0.16 a	248 ± 13 a	89.95 ± 5.98 a	85.07 ± 0.73 a

* Each data represent the mean of three replicates ± standard deviation.
Values with different letter within column indicate significative difference ($p < 0.05$).

between tannins and anthocyanins. As shown in Table 2, no effect of sonication treatments on the PPI index can be highlighted between untreated and sonicated samples at conditions below 90% and 10 min. Only at higher ultrasound conditions (90% and 10 min) can a slight increase in PPI be detected, from 83.38 ± 0.24 (Control) to 85.07 ± 0.73 (US9). Acoustic cavitation can generate radical species in water medium, which can enhance the polymerization and condensation reactions of chemical species (Kentish & Ashokkumar, 2011; McKenzie et al., 2019).

In a previous work, different results regarding the effect of sonication on copigmentation reactions have been reported (Celotti et al., 2020). In some cases, the cavitation phenomena showed a positive effect and, in others, no differences could be highlighted. The effect of ultrasounds could be affected by the ratios between different polyphenol classes and higher tannin-anthocyanins ratio could positively affect the copigmentation reactions.

3.2. Effect of ultrasound on HCl and astringency index

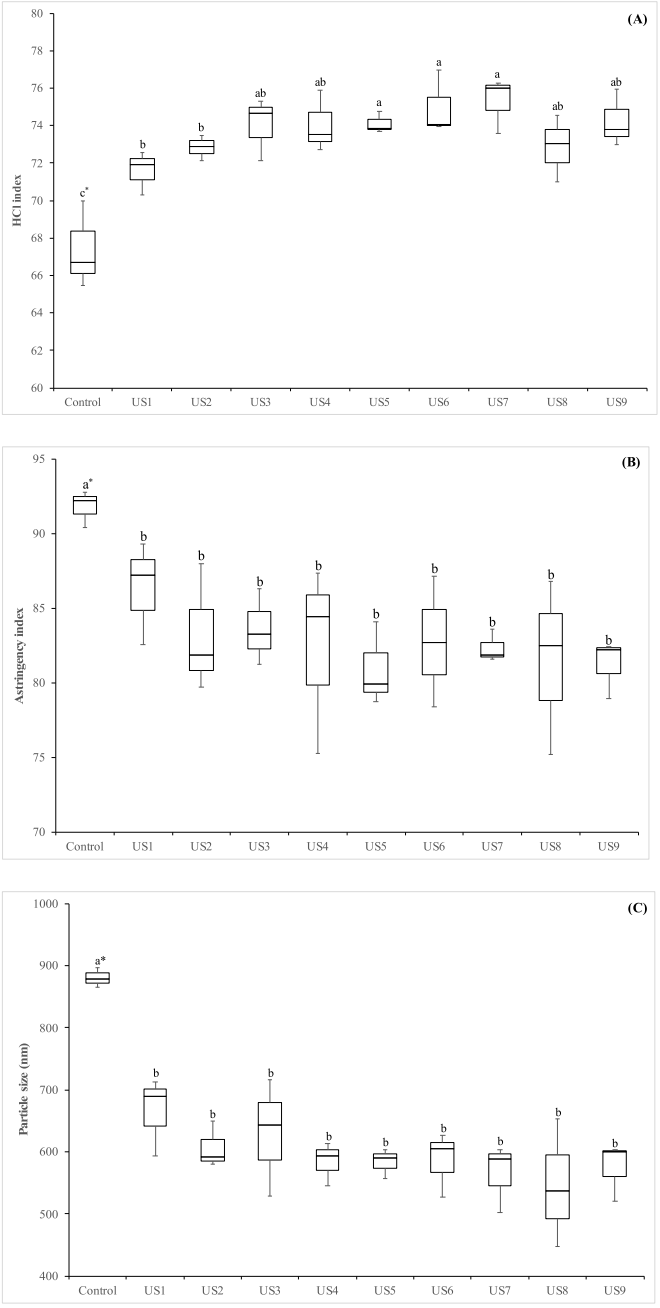
During wine aging, tannins undergo to structural modifications, such as polymerization reactions, and an increase in polymerization degree can be highlighted. The HCl index is a useful method for detecting some structural changes; higher values indicate a higher polymerization degree of tannins. Fig. 1(A) shows box plots of the HCl index of untreated and sonicated samples at different levels of amplitude and sonication time.

As can be seen, ultrasound enhances the HCl index for all the sonicated samples from 68.06 ± 1.72 of the untreated sample, to 73.78 ± 1.52 as the mean value of treated samples. No clear trend could be highlighted between increasing amplitude and sonication time, though it is possible to observe a significant increase in the HCl index between sonicated samples at 30% and 2 min (71.59 ± 1.06) and at 90% and 10 min (74.25 ± 1.53).

The use of ultrasound as an external stimulus for promoting polymerization reactions has received increasing attention in recent years. Water represents the most well-studied liquid for sonochemical fragmentation, and can generate a wide range of radical and non-radical species including hydroxyl radicals, peroxyl radicals, and hydrogen peroxide. Any species dissolved in water may then undergo a chemical reaction with these generated reactants (McKenzie et al., 2019). Generally, in food processing a depolymerization effect of ultrasounds on several polymeric compounds, such as carbohydrates, proteins, and starch, has been reported (Bhargava et al., 2021). Interestingly, some authors have reported a constructive and destructive effect of ultrasound on polymer reactions, depending on the operating conditions adopted (Kubo et al., 2018). A formation of narrowly dispersed polymers by degradative reactions induced by ultrasounds, following radical-induced chain growth mechanism, has also proposed (Xia et al., 2002).

Tannins, especially proanthocyanidins, are related to the perception of astringency. In Fig. 1(B), box plots of the astringency index, evaluated by the bovine serum albumin (BSA) method, for untreated and sonicated samples are depicted. As can be seen, the ultrasound treatment showed a decrease in astringency for all amplitudes and sonication times, from 91.8 ± 1.2 for untreated wine to 82.7 ± 3.7 as mean value for sonicated samples. No differences can be detected comparing the treated samples and no significant effects were detected with an increase in the amplitude and time.

The increase in polymerization degree generally induces an increase in the perception of astringency (Sun et al., 2013). Despite this, the ultrasound treatments showed an increase in HCl index and, in addition, a decrease in the perception of astringency. The intensity of astringency sensation is related to the chemical structural characteristics of proanthocyanidins, involving not only the degree of polymerization but also galloylation, B-ring trihydroxylation. and stereochemistry (Ma et al., 2014). As has been reported, conformational arrangements and



*Different letters indicate significant differences (p<0.05).

Fig. 1. HCl index (A), Astringency index (B), and Particle size (C) - box plots of untreated (Control) and sonicated samples at different levels of amplitude (30, 60 an 90%) and sonication time (2, 6 and 10 min).
*Different letters indicate significant differences ($p < 0.05$).

aggregation processes of larger tannins to more condensed or folded structures can "hide" the hydrophobic functional groups responsible for tannin–protein affinity (McRae & Kennedy, 2011). High-power ultrasound could induce a conformational change in proanthocyanidins structures. The conformational modifications induced by the cavitation phenomena of several compounds, such as proteins, pectin, and other polysaccharides have been reported (Qiu et al., 2019; Vera et al., 2019).

3.3. Effect of ultrasound on particle size

Wine contains many types of colloidal substances, such as tannins

and polysaccharides, which contribute to stability and final sensorial perceptions. Condensed tannins are among the most abundant macromolecules in red wine and they can aggregate and form colloidal dispersions, with hydrodynamic diameters in magnitudes of a few hundred to over a thousand nanometers (Li et al., 2019). Colloidal status and its evolution can be monitored by the determination of particle sizing using several methods, such as dynamic light scattering. In Fig. 1(C), box-plots of the mean particle size of untreated (Control) and sonicated samples at different levels of amplitude and sonication time are reported.

It is notable that ultrasound treatments induced a decrease in mean particle size, from 880 ± 16 of untreated sample to 590 ± 62, as mean value of sonicated samples. The increase in amplitude and sonication time did not affect significatively the mean particle size and no significant differences can be highlighted.

The particle size reduction observed could be due to the cavitational forces of the ultrasonic treatment exerted by the probe, as well as microstreaming and turbulent forces (Jambrak et al., 2009). During sonication, aggregates are violently agitated, colliding frontally and tangentially, resulting in smaller broken particles with a narrower size distribution (Lu et al., 2002). The particle size reduction could be related to conformational changes induced by ultrasound waves, as well as the decrease in astringency shown in Fig. 1(B), but more detailed investigations are necessary.

3.4. Mathematical modelling and scale-up considerations

In laboratory investigations, it is generally desirable that an ultrasonic process be transferrable to an industrial-scale production environment. During the scale-up, it is essential to make sure that all processing conditions remain the same: this will ensure that the final product quality is unchanged while the productivity rate is increased. Scaling up ideally requires an understanding of the appropriate design parameters (Prado et al., 2017). The energy dissipated per volume of treated sample is an intensive parameter that it is independent of the equipment scale. The energy introduced in the system by acoustic cavitation can be a useful guide for scale-up considerations. The level of energy introduced into the system can be expressed as the acoustic energy density (AED in J/mL) and can be determined by calorimetric methods based on the temperature variation induced by acoustic cavitation. Plotting AED against the HCL index, astringency, or particle size, as depicted in Fig. 2, it is notable that the experimental data can be mathematical well described by all the models considered. The criteria adopted to evaluate how well the empirical model represent the experimental data were the magnitudes of the coefficients of determination (R^2 and R^2-adj) and normalized root-mean-square deviation (NRMSD). Higher values of R^2 and R^2-adj and lower values of NRMSD denote a better goodness of fit and suggest that the model represents the experimental values well (Table 5). Peleg's model best described the HCl and Astringency index, based on a high R^2 (0.9662–0.9724) and low NRMSD (%) (0.46–0.62), confirming the model's accuracy and suitability for describing the effect of acoustic energy. Instead, the power law function model best described the particle size trend against AED (R^2 = 0.9793; NRMSD (%): 1.76).

The AED parameter has been successfully adopted for the scaling up of ultrasound- and microwave-assisted extraction processes as a reference for calibrating the optimum nominal power or percentage amplitude and treatment time (Chan et al., 2017). In view of the positive effects of ultrasound on some wine analytical indices and the good fitting results of the power law model, it is conceivable that this scale-up method could also be useful for determining the optimum operational conditions of ultrasonic systems for various scales of the US wine treatment process. Considering Fig. 2, it is notable that at AED levels of 15–25 J/mL it is already possible to obtain 80–85% changes in wine properties at the maximum levels of energy densities considered in this work.

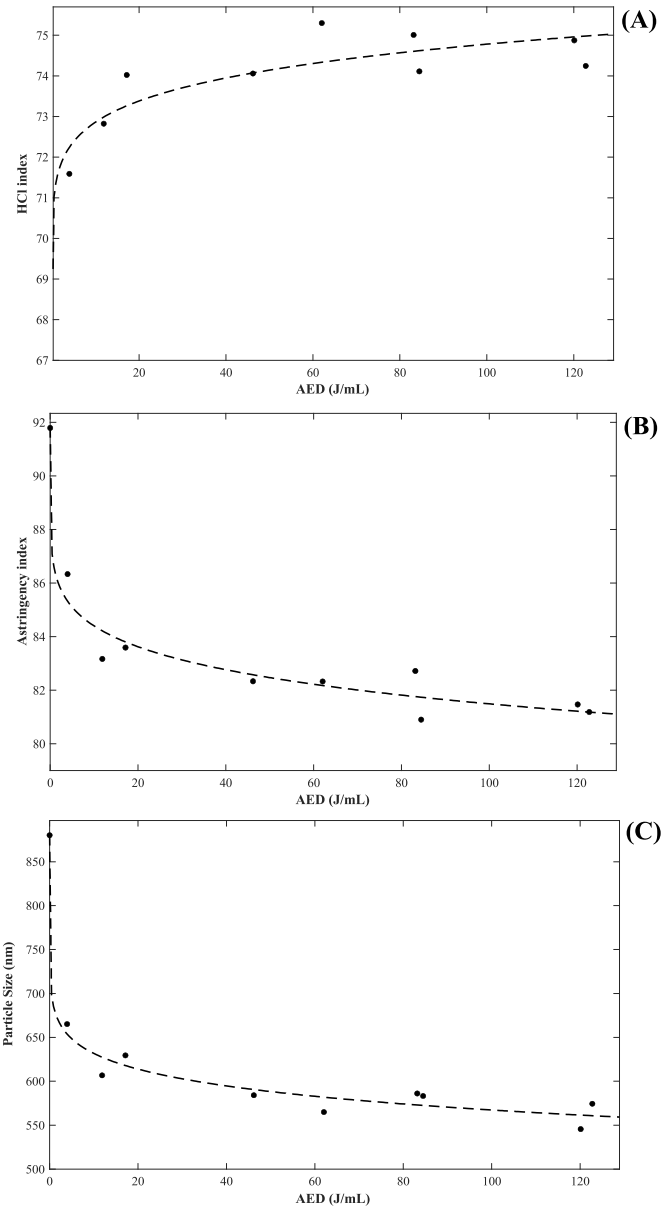


Fig. 2. Comparison between experimental mean values (•) and simulated (—) power law model for HCl index (A), Astringency index (B) and particle size (C) at different levels of acoustic energy densities (AED, J/mL).

4. Conclusions

Ultrasound can be considered one of the most promising technologies for several purposes in winemaking processes and it was recently officially approved for use in crushed grape treatments. The effect of amplitude and sonication time was studied and no changes in the main polyphenol contents were detected. The sonication treatments carried out at the operating conditions adopted in the present work enabled us to maintain the initial phenolic profile and without degradative effects occurring.

Significant results were obtained considering the HCl index, astringency index, and particle size. The increase in the amplitude and sonication time induced an increase in the polymerization of tannins, as demonstrated by the HCl index, and a significant decrease in the perception of astringency, probably due to the conformation changes in the proanthocyanidin structure induced by acoustic cavitation. Furthermore, a decrease in particle size was detected due to the

Table 5
Mathematical models, coefficients and regressed statistical parameters for HCl index, Astringency index and Particle size.

N°	Model name		Model coefficients				Statistical parameters		
			Astringency index	HCl index	Particle size		Astringency index	HCl index	Particle size
1	Power law	K	4.08	5.33	197.4	R^2	0.9415	0.9594	0.9793
		n	0.1298	0.1437	0.1001	R^2-adj.	0.9248	0.9478	0.9734
						NRMSD (%)	0.84	0.81	1.76
2	Logistic model	K	81.94	74.52	580.4	R^2	0.9561	0.9493	0.9551
		a	0.1056	−0.1017	0.3388	R^2-adj.	0.9436	0.9349	0.9423
		b	0.1607	0.1747	0.2165	NRMSD (%)	0.78	0.60	23.26
3	Peleg's model	K	91.79	67.39	880	R^2	0.9724	0.9662	0.9757
		a	0.3378	0.4406	0.006439	R^2-adj.	0.9645	0.9619	0.9727
		b	0.09584	0.1331	0.003202	NRMSD (%)	0.62	0.46	16.00

potential shear stress effect of sonication waves on colloidal particles.

The effect of acoustic cavitation at different levels of amplitude and time can be better described by Peleg's model considering the acoustic energy density (AED). Good fittings results were achieved for the HCl index, astringency, and particle size. AED is an intensive parameter and can be useful for the optimization of operative sonication conditions and for the potential up-scaling of ultrasound technology at a commercial level.

Ultrasound could be applied to finished red wines with the aim of accelerating aging reactions; however, more investigations, sensory evaluations, and market research studies are needed to better understand the potential industrial feasibility of this technology for this specific purpose.

CRediT authorship contribution statement

Andrea Natolino: Investigation, Formal analysis, Data curation, Writing – original draft, Roles/Writing – original draft, Writing – review & editing, All authors have read and agreed to the published version of the manuscript. **Emilio Celotti:** Conceptualization, Funding acquisition, Supervision, Validation, Writing – review & editing, All authors have read and agreed to the published version of the manuscript.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Arnous, A., Makris, D. P., & Kefalas, P. (2001). Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. *Journal of Agricultural and Food Chemistry, 49*(12), 5736–5742. https://doi.org/10.1021/jf010827s

Avizcuri, J. M., Sáenz-Navajas, M. P., Echávarri, J. F., Ferreira, V., & Fernández-Zurbano, P. (2016). Evaluation of the impact of initial red wine composition on changes in color and anthocyanin content during bottle storage. *Food Chemistry, 213*, 123–134. https://doi.org/10.1016/j.foodchem.2016.06.050

Bate-Smith, E. C. (1954). Leuco-anthocyanins. 1. Detection and identification of anthocyanidins formed from leuco-anthocyanins in plant tissues. *Biochemical Journal, 58*(1), 122–125. https://doi.org/10.1042/bj0580122

Bhargava, N., Mor, R. S., Kumar, K., & Sharanagat, V. S. (2021). Advances in application of ultrasound in food processing: A review. *Ultrasonics Sonochemistry, 70*(105293). https://doi.org/10.1016/j.ultsonch.2020.105293

Carpena, M., Pereira, A. G., Prieto, M. A., & Simal-Gandara, J. (2020). Wine aging technology: Fundamental role of wood barrels. *Foods, 9*(9), 1160. https://doi.org/10.3390/foods9091160

Catania, A., Lerno, L., Sari, S., Fanzone, M., Casassa, F., & Oberholster, A. (2021). Impact of micro-oxygenation timing and rate of addition on color stabilization and chromatic characteristics of cabernet sauvignon wines. *Lebensmittel-Wissenschaft und -Technologie, 149*(111776). https://doi.org/10.1016/j.lwt.2021.111776

Celotti, E., Osorio Barahona, M. S., Bellantuono, E., Cardona, J., Roman, T., Nicolini, G., & Natolino, A. (2021). High-power ultrasound on the protein stability of white wines: Preliminary study of amplitude and sonication time. *Lebensmittel-Wissenschaft und -Technologie, 147*(111602). https://doi.org/10.1016/j.lwt.2021.111602

Celotti, E., Stante, S., Ferraretto, P., Román, T., Nicolini, G., & Natolino, A. (2020). High power ultrasound treatments of red young wines: Effect on anthocyanins and phenolic stability indices. *Foods, 9*(10), 1344. https://doi.org/10.3390/foods9101344

Chan, C. H., See, T. Y., Yusoff, R., Ngoh, G. C., & Kow, K. W. (2017). Extraction of bioactives from Orthosiphon stamineus using microwave and ultrasound-assisted techniques: Process optimization and scale up. *Food Chemistry, 221*, 1382–1387. https://doi.org/10.1016/j.foodchem.2016.11.016

Ćurko, N., Ganić, K. K., Tomašević, M., Gracin, L., Jourdes, M., & Teissedre, P.-L. (2021). Effect of enological treatments on phenolic and sensory characteristics of red wine during aging: Micro-oxygenation, sulfur dioxide, iron with copper and gelatin fining. *Food Chemistry, 339*(127848). https://doi.org/10.1016/j.foodchem.2020.127848

Dzah, C. S., Duan, Y., Zhang, H., Wen, C., Zhang, J., Chen, G., & Ma, H. (2020). The effects of ultrasound assisted extraction on yield, antioxidant, anticancer and antimicrobial activity of polyphenol extracts: A review. *Food Bioscience, 35*(100547). https://doi.org/10.1016/j.fbio.2020.100547

Ferraretto, P., & Celotti, E. (2016). Preliminary study of the effects of ultrasound on red wine polyphenols. *CyTA - Journal of Food, 14*(4), 529–535. https://doi.org/10.1080/19476337.2016.1149520

del Fresno, J. M., Loira, I., Morata, A., González, C., Suárez-Lepe, J. A., & Cuerda, R. (2018). Application of ultrasound to improve lees ageing processes in red wines. *Food Chemistry, 261*, 157–163. https://doi.org/10.1016/j.foodchem.2018.04.041

Gao, X., Feng, T., Liu, E., Shan, P., Zhang, Z., Liao, L., & Ma, H. (2021). Ougan juice debittering using ultrasound-aided enzymatic hydrolysis: Impacts on aroma and taste. *Food Chemistry, 345*(128767). https://doi.org/10.1016/j.foodchem.2020.128767

Gao, X., Liu, E., Zhang, J., Yang, L., Huang, Q., Chen, S., Ma, H., Ho, C.-T., & Liao, L. (2020). Accelerating aroma formation of raw soy sauce using low intensity sonication. *Food Chemistry, 329*(127118). https://doi.org/10.1016/j.foodchem.2020.127118

García Martín, J. F., & Sun, D.-W. (2013). Ultrasound and electric fields as novel techniques for assisting the wine ageing process: The state-of-the-art research. *Trends in Food Science & Technology, 33*(1), 40–53. https://doi.org/10.1016/j.tifs.2013.06.005

García Martín, J. F., Zhang, Q.-A., & Feng, H. (2016). Ultrasound for accelerating the wine ageing process from physicochemical point of view. In J. F. García Martín (Ed.), *Applications of ultrasound in the beverage industry*. Nova Publishers.

Garcia-Estevez, I., Perez-Gregorio, R., Soares, S., Mateus, N., & De Freitas, V. (2017). Oenological perspective of red wine astringency. *OENO One, 51*(2). https://doi.org/10.20870/oeno-one.2017.51.2.1816

Gavahian, M., Chu, R., & Ratchaneesiripap, P. (2021). An ultrasound-assisted extraction system to accelerate production of Mhiskey, a rice spirit-based product, inside oak barrel: Total phenolics, color, and energy consumption. *Journal of Food Process Engineering*. https://doi.org/10.1111/jfpe.13861

Glories, Y. (1978). Recherches sur la matière colorante des vins rouges. *Université de Bordeaux II*. https://books.google.it/books?id=WGfuOgAACAAJ.

Gogate, P. R., Sutkar, V. S., & Pandit, A. B. (2011). Sonochemical reactors: Important design and scale up considerations with a special emphasis on heterogeneous systems. *Chemical Engineering Journal, 166*(3), 1066–1082. https://doi.org/10.1016/j.cej.2010.11.069

Harder, M. N. C., Silva, L. A. C. S., Pires, J. A., Scanholato, M., & Arthur, V. (2013). Physical-chemical evaluation of wines subjected to gamma irradiation for aging. *Food Science and Technology, 1*(3), 62–65. https://doi.org/10.13189/fst.2013.010304

He, F., Liang, N.-N., Mu, L., Pan, Q.-H., Wang, J., Reeves, M. J., & Duan, C.-Q. (2012a). Anthocyanins and their variation in red wines I. Monomeric anthocyanins and their color expression. *Molecules, 17*(2), 1571–1601. https://doi.org/10.3390/molecules17021571

He, F., Liang, N.-N., Mu, L., Pan, Q.-H., Wang, J., Reeves, M. J., & Duan, C.-Q. (2012b). Anthocyanins and their variation in red wines II. Anthocyanin derived pigments and their color evolution. *Molecules, 17*(2), 1483–1519. https://doi.org/10.3390/molecules17021483

Jambrak, A. R., Lelas, V., Mason, T. J., Krešić, G., & Badanjak, M. (2009). Physical properties of ultrasound treated soy proteins. *Journal of Food Engineering, 93*(4), 386–393. https://doi.org/10.1016/j.jfoodeng.2009.02.001

Kalkan Yıldırı, H., & Dündar, E. (2017). New techniques for wine aging. *BIO Web of Conferences, 9*. https://doi.org/10.1051/bioconf/20170902012, 2012.

Kentish, S., & Ashokkumar, M. (2011). The physical and chemical effect of ultrasound. In H. Feng, G. V. Barbosa-Cánovas, & J. Weiss (Eds.), *Ultrasound technologies for food and bioprocessing* (pp. 7–11). Springer.

Kubo, M., Kondo, T., Matsui, H., Shibasaki-Kitakawa, N., & Yonemoto, T. (2018). Control of molecular weight distribution in synthesis of poly(2-hydroxyethyl methacrylate) using ultrasonic irradiation. *Ultrasonics Sonochemistry, 40*, 736–741. https://doi.org/10.1016/j.ultsonch.2017.08.011

Li, S., Wilkinson, K. L., Mierczynska-Vasilev, A., & Bindon, K. A. (2019). Applying Nanoparticle tracking analysis to characterize the polydispersity of aggregates resulting from tannin–polysaccharide interactions in wine-like media. *Molecules, 24*(11), 2100. https://doi.org/10.3390/molecules24112100

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

Lu, Y., Riyanto, N., & Weavers, L. K. (2002). Sonolysis of synthetic sediment particles: Particle characteristics affecting particle dissolution and size reduction. *Ultrasonics Sonochemistry, 9*(4), 181–188. https://doi.org/10.1016/S1350-4177(02)00076-7

Ma, W., Guo, A., Zhang, Y., Wang, H., Liu, Y., & Li, H. (2014). A review on astringency and bitterness perception of tannins in wine. *Trends in Food Science & Technology, 40*(1), 6–19. https://doi.org/10.1016/j.tifs.2014.08.001

Masuzawa, N., Ohdaira, E., & Ide, M. (2000). Effects of ultrasonic irradiation on phenolic compounds in wine. *Japanese Journal of Applied Physics, 39*(Part 1, No. 5B), 2978–2979. https://doi.org/10.1143/JJAP.39.2978

McKenzie, T. G., Karimi, F., Ashokkumar, M., & Qiao, G. G. (2019). Ultrasound and sonochemistry for radical polymerization: Sound synthesis. *Chemistry - A European Journal, 25*(21), 5372–5388. https://doi.org/10.1002/chem.201803771

McRae, J. M., & Kennedy, J. A. (2011). Wine and grape tannin interactions with salivary proteins and their impact on astringency: A review of current research. *Molecules, 16*(3), 2348–2364. https://doi.org/10.3390/molecules16032348

Oliveira, J., Brás, N. F., da Silva, M. A., Mateus, N., Parola, A. J., & de Freitas, V. (2014). Grape anthocyanin oligomerization: A putative mechanism for red color stabilization? *Phytochemistry, 105*, 178–185. https://doi.org/10.1016/j.phytochem.2014.05.006

Patist, A., & Bates, D. (2011). Industrial applications of high power ultrasonics. In H. Feng, G. Barbosa-Canovas, & J. Weiss (Eds.), *Ultrasound technologies for food and bioprocessing* (pp. 599–616). New York: Springer. https://doi.org/10.1007/978-1-4419-7472-3_24.

Prado, J. M., Veggi, P. C., & Meireles, M. A. A. (2017). Scale-Up Issues and Cost of Manufacturing Bioactive Compounds by Supercritical Fluid Extraction and Ultrasound Assisted Extraction. In G. V. Barbosa-Canovas, G. Maria Pastore, K. Candogan, I. G. Medina Meza, S. Caetano da Silva Lannes, K. Buckle, R. Y. Yada, & A. Rosenthal (Eds.), Global Food Security and Wellness (pp. 377-433). Springer, New York. https://doi.org/10.1007/978-1-4939-6496-3_20.

Qiu, W. Y., Cai, W. D., Wang, M., & Yan, J. K. (2019). Effect of ultrasonic intensity on the conformational changes in citrus pectin under ultrasonic processing. *Food Chemistry, 297*(125021). https://doi.org/10.1016/j.foodchem.2019.125021

Ribéreau-Gayon, P., & Stonestreet, E. (1965). Determination of anthocyanins in red wine. *Bulletin de la Societe Chimique de France, 9*, 2649–2652.

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

Sólyom, K., Solá, R., Cocero, M. J., & Mato, R. B. (2014). Thermal degradation of grape marc polyphenols. *Food Chemistry, 159*, 361–366. https://doi.org/10.1016/j.foodchem.2014.03.021

Sun, B., de, S. M., Leandro, C., Caldeira, I., Duarte, F. L., & Spranger, I. (2013). Reactivity of polymeric proanthocyanidins toward salivary proteins and their contribution to young red wine astringency. *Journal of Agricultural and Food Chemistry, 61*(4), 939–946. https://doi.org/10.1021/jf303704u

Tao, Y., Sun, D.-W., Górecki, A., Błaszczak, W., Lamparski, G., Amarowicz, R., Fornal, J., & Jeliński, T. (2016). A preliminary study about the influence of high hydrostatic pressure processing in parallel with oak chip maceration on the physicochemical and sensory properties of a young red wine. *Food Chemistry, 194*, 545–554. https://doi.org/10.1016/j.foodchem.2015.07.041

Tiwari, B. K., Patras, A., Brunton, N., Cullen, P. J., & O'Donnell, C. P. (2010). Effect of ultrasound processing on anthocyanins and color of red grape juice. *Ultrasonics Sonochemistry, 17*(3), 598–604. https://doi.org/10.1016/j.ultsonch.2009.10.009

Tredoux, A. G. J., & Silva Ferreira, A. C. (2012). Fortified wines: Styles, production and flavour chemistry. In *Alcoholic beverages* (pp. 159–179). Elsevier. https://doi.org/10.1533/9780857095176.2.159.

Vera, A., Valenzuela, M. A., Yazdani-Pedram, M., Tapia, C., & Abugoch, L. (2019). Conformational and physicochemical properties of quinoa proteins affected by different conditions of high-intensity ultrasound treatments. *Ultrasonics Sonochemistry, 51*, 186–196. https://doi.org/10.1016/j.ultsonch.2018.10.026

Wei, X., Ju, Y., Ma, T., Zhang, J., Fang, Y., & Sun, X. (2021). New perspectives on the biosynthesis, transportation, astringency perception and detection methods of grape proanthocyanidins. *Critical Reviews in Food Science and Nutrition, 61*(14), 2372–2398. https://doi.org/10.1080/10408398.2020.1777527

Xia, H., Wang, Q., Liao, Y., Xu, X., Baxter, S. M., Slone, R. V., Wu, S., Swift, G., & Westmoreland, D. G. (2002). Polymerization rate and mechanism of ultrasonically initiated emulsion polymerization of n-butyl acrylate. *Ultrasonics Sonochemistry, 9*(3), 151–158. https://doi.org/10.1016/S1350-4177(01)00118-3

Yu, Z., Su, Y., Zhang, Y., Zhu, P., Mei, Z., Zhou, X., & Yu, H. (2021). Potential use of ultrasound to promote fermentation, maturation, and properties of fermented foods: A review. *Food Chemistry, 357*, 129805. https://doi.org/10.1016/j.foodchem.2021.129805

Zhang, Q.-A., & Wang, T.-T. (2017). Effect of ultrasound irradiation on the evolution of color properties and major phenolic compounds in wine during storage. *Food Chemistry, 234*, 372–380. https://doi.org/10.1016/j.foodchem.2017.05.022

Zironi, R., Buiatti, S., & Celotti, E. (1992). Evaluation of a new colorimetric method for the determination of catechins in musts and wines. *Wein-Wissenschaft, 47*, 1–7.