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Effect of High-Power Ultrasound Treatment on Bioactive Compound Content and Chromatic Characteristics of Red Wines

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RESEARCH ARTICLE

Abstract

The objective of this work was to investigate, at a laboratory scale, the effect of ultrasound treatment (frequency 20 kHz) on the extraction of total phenolic compounds and monomeric anthocyanins from crushed Merlot grapes in order to reduce maceration time. Antioxidant capacity expressed as ferric reducing antioxidant power (FRAP) and chromatic properties were also investigated. Microvinification was performed on samples both sonicated with a probe-type ultrasonic instrument and untreated (control) and the maceration operation was carried out with sequential extraction (after 3, 5 and 7 days) to evaluate the effect of two factors such as amplitude (A), 70 and 90% and treatment time (t), 3, 4 and 5 minutes on the extraction kinetics of bioactive compounds and chromatic characteristics of the samples. The results showed that throughout the maceration process, the sonicated samples exhibited higher levels of bioactive compounds and chromatic characteristics compared to the control, with the values obtained correlating with maceration time, US treatment time and amplitude. Improvement in all investigated characteristics is evident for ultrasound treated samples compared to untreated ones. Our study provided useful data concerning the impact of ultrasound on the bioactive properties and sensory attributes of red wine, thus offering a theoretical basis for the implementation of this technique in winemaking.

Keywords: ultrasound, red winemaking, maceration, phenolic compounds, chromatic properties.

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INTRODUCTION

Wine is one of the oldest traditional alcoholic beverages in the world. Red wine is generally high in polyphenols and can be regarded as a significant dietary source of polyphenols (Castaldo et al., 2019). Polyphenols are essential compounds that contribute to the color, flavor, aroma, and potential health benefits of wine (Banc et al., 2014). The phenolic content in red wine can be broadly divided into two categories: flavonoids - which include anthocyanins and tannins that contribute to the color and mouthfeel of the wine, flavan-3-ols (or catechins), flavonols, and their derivatives, and non-flavonoids – which include stilbenoids such as resveratrol and phenolic acids such as benzoic, caffeic, and cinnamic acids (Gutiérrez-Escobar et al., 2021). However, most of the phenolic content of wine falls under the classification of flavonoids. Of these, up to 90% of the phenolic content in red wine comes from the stems, seeds, and skins of the grape (Nemzer et al., 2021). In terms of specific compounds, polyphenol composition in one-year-old red wine includes around 60-80% polymeric polyphenols, 10-15% of anthocyanidins, 5-10% dimer procyanidins, 5-8% of catechins, 3-6% phenolic

acids, less than 1% of flavonols, and less than 0.3% of resveratrol (Buljeta et al., 2023). Phenolic compounds in red wine are known to have several health benefits: antioxidant properties, anti-inflammatory effects, cardioprotective effects, anticarcinogenic effects, neuroprotective effects, effects on gut microbiota (Nardini, 2022). The type of polyphenols found in red wine depends on several factors, such as environmental factors, grape variety and maturity, pre-fermentation practices, fermentation and aging conditions, or technological practices (Luzzini et al., 2021). It is also important to note that these factors can interact in complex ways to determine the final polyphenol profile of a given wine. Thus, the specific role and impact of polyphenols can vary depending on factors such as the type of polyphenols present, the duration of maceration, and the intended characteristics of the final product (Gómez-Plaza et al., 2020a). A good diffusion of the phenolic compounds from the grape to the must or must-wine is required to produce wines with good chromatic quality. In order to achieve the release of the desired chemicals into the medium, this extraction procedure is carried out during the maceration step and is based on the breakdown of the cell walls of the grape skin and seeds. Consequently, the length of the maceration process affects the wine's quality (Alencar et al., 2018a).

Advanced techniques for extracting plant bioactive compounds from foods and food-related matrices include ultrasound-assisted extraction (Aadil et al., 2015; Yusoff et al., 2022), microwave-assisted extraction (KHAN et al., 2022), cold plasma (Ahmadian et al., 2023; Heydari et al., 2023), supercritical fluid extraction (Molino et al., 2020), pressurized liquid extraction (Zia et al., 2022), high-voltage electric discharge (Molino et al., 2020), pulse electric field extraction (Ranjha, Kanwal, et al., 2021), microfluidization (Mukhtar et al., 2022) and enzyme-assisted extraction (Noranizan et al., 2020). These advanced techniques are 32–36% more efficient with approximately 15 times less energy consumption and producing higher-quality extracts (Sridhar et al., 2021).

High-power ultrasound (HPU) is a technology that utilizes sound waves with frequencies greater than 20 kilohertz (Ali et al., 2023; Hussain et al., 2023; Ranjha, Irfan, et al., 2021). The basic effect of ultrasound on a fluid is to add acoustic pressure to the hydrostatic pressure already present in the medium (Patist and Bates, 2011).

Previous research on red grapes and wines of different varieties has yielded very encouraging results regarding the HPU's extraction capacity and the resulting physico-chemical quality of the wines (Zhang et al., 2023). Plaza et al. found that utilizing ultrasound during the grape fermentation process produced wine with favorable color properties, a reduced impregnation time, and a heightened extraction of phenolic compounds from the grapes (Gómez-Plaza et al., 2020b; Plaza et al., 2019). Similarly, Romero-Díez et al. found that the application of ultrasound enhanced the anthocyanin extraction rate from wine lees, resulting in a reduced extraction time (Romero-Díez et al., 2019). Ferraretto et al found an improvement in the extraction of polyphenolic compounds from grapes, resulting in a reduction of the classical maceration time. Additionally, using ultrasound-assisted yeast lysis resulted in the release of various fractions into wine (Ferraretto et al., 2013). Numerous research determined the

International Organization of Vine and Wine (OIV, 2021) to approve this technology for use in wineries in 2019. However, the following issues with utilizing basic research and implementing the technology in industry still require resolution. The different research groups used very different ultrasound equipment, tested very different parameters, and produced very different results, so they cannot serve as a standard for future research.

The objective of this work was to evaluate, at laboratory scale, the effect of ultrasound treatment (frequency 20 kHz) on facilitating the extraction of phenolic compounds from crushed grapes Merlot variety, and subsequent on having shorter maceration times. To achieve this, total phenolic compounds (TPC), which are frequently linked to the organoleptic characteristics of finished red wines and their stability, were first assessed. Monomeric anthocyanins (MA), which make a decisive contribution to the specific colour of red wines, were also measured as well as chromatic characteristics (colour intensity and hue). In addition, antioxidant capacity was assessed by ferric reducing antioxidant power (FRAP) assay. Our study enables a comprehensive and systematic inquiry into the process that underlies the impact of ultrasound on the quality measurements and sensory attributes of wine, thereby offering a theoretical basis for the commercial implementation of ultrasound during winemaking.

MATERIALS AND METHODS

Materials

Approximately 100 kg of red grapes, Merlot variety, from the 2019 vintage were used in the experimental research. The grapes were hand-harvested at Prahova, Romania (Pietroasa-Istrita Viticulture and Winemaking Research and Development Station). The grapes were quickly brought to the lab for processing after being harvested at technological maturity when they reached 29.8 °Brix. Only sound grape bunches that were chosen at random for the experiment were used.

Ultrasonic equipment

Sonication was done on all experimental experiments utilizing a probe-type ultrasonic equipment (SONIX VCX750, Sonics and Materials Inc., Newtown, USA). The amplitude can be specified as a percentage ranging from

10% to 100%. The device has a 750 W ultrasonic power and a frequency of 20 kHz. The most common frequencies used in extraction methods range between 20 and 100 kHz.

Sample preparation

For the laboratory testing, samples of 1000 g of Merlot red grapes, vintage 2019, were made. Grape samples (60 specimens of 100 g each), were randomly collected, destemmed, hand-crushed, and then treated with a probe-type ultrasonic instrument. After sonication, we formed 6 samples of 1000 g each (10 specimens of 100 g. each). After destemming and crushing, microvinification was performed on the untreated sample (Control, C) and ultrasound treated samples. The arithmetic mean of the determinations made on the 10 specimens of each sample represents the characteristics of the respective sample. An untreated control sample (C) consisting of 10 specimens of 100 g each was tested separately because it had not been subjected to ultrasonic treatment (Figure 1). The specimens were processed in a 100 mL Pyrex glass beaker with a counter current water-cooling jacket. The cooling water was kept at a constant temperature of 19 °C. The acoustic amplifier was placed in the container at a normal distance of 20 mm from the bottom. The maceration-fermentation duration of the tests was set at 3 days (D3), 5 days (D5) and 7 days (D7), respectively. In this way, all extraction experiments were carried out.

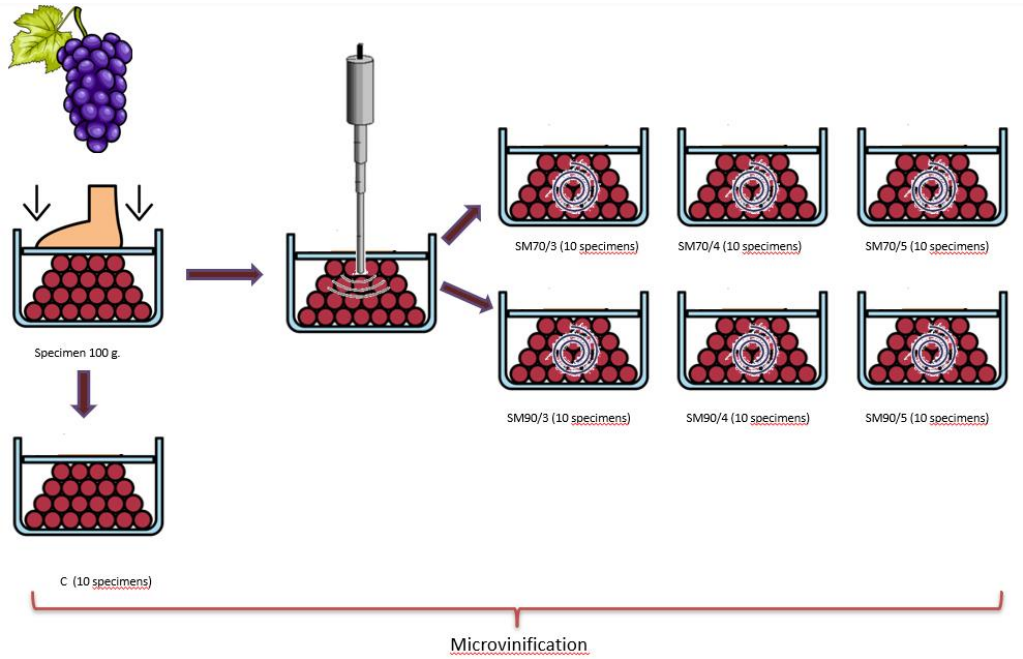

Figure 1. Sample and specimen preparation for laboratory testing. Sonicated samples (SM) at 70 and 90% amplitude for 3, 4, and 5 minutes: SM70/3 (A: 70%; t: 3 min), SM70/4 (A: 70%; t: 4 min), SM70/5 (A: 70%; t: 5 min), SM90/3 (A: 90%; t: 3 min), SM90/4 (A: 90%; t: 4 min), SM90/5 (A: 90%, t: 5 min).

Methods

Total polyphenolic content and anthocyanins determination

Using gallic acid as a reference, the total polyphenolic content (TPC) was calculated using the Folin-Ciocalteu spectrophotometric method and represented as micrograms of gallic acid equivalents per millilitre (g GAE/mL) (Singleton, Orthofer, and Lamuela-Raventos, 1999). Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), 1.25 mL, was applied to a 0.5 mL sample after being diluted 1:10 (v/v) with distilled water. 1 mL of Na_2CO_3 60 g/L was added after the mixture had been incubated for 5 minutes at room temperature. The sample absorbance at 750 nm was measured using a UV-VIS spectrophotometer (Specord 205, Analytik Jena Inc., Jena, Germany) after 30 min of incubation at 50 °C. Gallic acid was used as the standard, with concentrations ranging from 5 to 250 g GAE/mL, to create the calibration curve.

The pH differential approach was used to identify monomeric anthocyanins (MA) (Lee, Durst, and Wrolstad, 2005). In brief, 1 mL of wine was mixed with 14 mL of potassium chloride buffer (0.025 M, pH 1.0) and 14 mL of sodium acetate buffer (0.4 M, pH 4.5) to make two dilutions of the same sample. After 15 minutes at room temperature, absorbance at 520 and 700 nm was measured against deionized water. The findings were given in

milligrams of cyanidin-3-glucoside equivalents per liter (mg CGE/L). Using a molar absorbance coefficient of 26,900 L/mol x cm and a molecular weight of 449.2 g/mol, the total anthocyanin content of the samples was determined.

Chromatic characteristics

The chromatic characteristics described by the intensity of color (IC) and hue (N)of the samples were determined by a spectrophotometric method in which the intensity of color is given by the sum of absorptions using a 1 cm optical path and radiation of wavelengths 420, 520 and 620 nm, and the hue is expressed as the ratio of absorbance at 420 nm to absorbance at 520 nm (OIV,2021).

Ferric reducing antioxidant power (FRAP) assay

The Benzie and Strain method (Benzie and Strain, 1996) served as the foundation for the FRAP test methodology. As stock solutions, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl and 20 mM $FeCl_3$ x $6H_2O$ solution in 300 mM acetate buffer (pH = 3.6) were utilized. Acetate buffer, TPTZ solution, and 10 mL of $FeCl_3$ x $6H_2O$ solution were combined to create the working solution, which was then heated to 37 °C before use. The wine samples were diluted 1:50 (v/v) with distilled water before being analyzed, and a 0.5 mL aliquot of the diluted samples was then allowed to react with 2.5 mL of the working solution for 30 minutes at 37 °C.

Statistical Analysis

All measurements were performed in triplicate, and the results are presented as the mean ± standard deviation (SD). DESIGN-EXPERT® VERSION 13 software (Stat-Ease Inc. 2022) was used for statistical analysis. One-way ANOVA with post hoc analysis using the HSD Tukey test was used to assess significant differences between samples. JASP software version 0.17.1 (JASP Team 2023) was used to calculate the Pearson correlation coefficient between TPC, MA, FRAP value, IC, and N.

RESULTS AND DISCUSSIONS

After destemming and crushing, microvinification was carried out on the control sample and the samples treated with ultrasound. The maceration operation was carried out with sequential extraction (after 3, 5 and 7 days) to evaluate the effect of the two factors: amplitude (A) and treatment time(t) on the extraction kinetics of polyphenols and anthocyanins during maceration, ferric reducing antioxidant power (FRAP) value, color intensity and hue in the sonicated samples compared to the untreated sample (C).

The effects of high-power ultrasound treatment on extraction kinetics are discussed in terms of bioactive compounds such as TPC total polyphenol content (mg GAE/L), MA monomeric anthocyanin content (mg CGE /L), FRAP value (µM Fe^{2+}/mL wine), color intensity (IC) and hue (N).

All the above parameters were studied on untreated samples (C) after 3 (C3), 5 (C5), and 7 (C7) days of maceration, and on the samples subjected to ultrasound treatment at 70 and 90% amplitude for 3, 4, and 5 minutes, after 3, 5, and 7 days of maceration as follows: SM70/3 (A: 70%; t: 3 min), SM70/4 (A: 70%; t: 4 min), SM70/5 (A: 70%; t: 5 min), SM90/3 (A: 90%; t: 3 min), SM90/4 (A: 90%; t: 4 min), and SM90/5 (A: 90%, t: 5 min), each with three replicates. Since the possible increase in the temperature of the samples, as a result of the treatment, is an important factor on the possible effects of the treatment on the final quality of the product, it was monitored during the tests. The temperature at which the maceration took place was monitored daily and was in the range of 23-25°C.

The extraction of phenolic compounds is influenced by the maceration time, which affects both the total polyphenol content of the wine and the interactions between the different phenolic fractions (Bautista-Ortin et al., 2017). Maceration is a solid-liquid extraction and it has been observed that anthocyanins and tannins, two polyphenolic substances that characterize red wines, have different affinities for the different solvents, particularly water and ethanol, with which the exocarp comes into natural contact during maceration (Setford et al., 2017). The amount of anthocyanins at the end of maceration depends on the strength of the extractive processes linked to the level of degradation of cellular structures on the one hand, and the phenomenon of fixing by adsorption on the other (Ferraretto et al., 2013).

Analytical determinations performed on crushed grapes treated with ultrasound and subjected to different maceration times on the skins of 3 days (D3), 5 days (D5) and 7 days (D7) respectively, compared to the control samples that followed the classic maceration, gave interesting results (Table 1).

At the beginning of the maceration, the sonicated samples had higher levels of bioactive compounds (Maier et al., 2023) and chromatic characteristics compared to the untreated sample, a difference that was also found at the end of the maceration.

Table 1. Effect of ultrasonic treatment on samples at the end of skin maceration

Sample	Maceration (days)	TPC (µg GAE/mL)	MA (mg CGE/L)	FRAP (µmol Fe^{2+}/mL)	IC	N
C3	3	870.32±10.72	105.30±4.02	33.73±1.11	6.61±1.21	1.14±0.07
SM70/3		1 407.78±11.09	240.59±3.97	43.79±0.99	23.39±1.75	1.24±0.09
SM70/4		1 425.02±10.54	250.82±2.54	44.69±1.23	22.12±2.05	1.27±0.07
SM70/5		1 715.31±9.63	224.51±2.45	43.96±1.19	21.46±1.97	1.21±0.04
SM90/3		1 491.13±14.21	240.46±3.01	51.69±2.56	22.73±2.06	1.24±0.05
SM90/4		1 778.54±11.58	239.89±3.65	61.51±3.01	22.42±2.37	1.20±0.10
SM90/5		1 925.12±12.31	275.33±2.45	62.43±2.78	20.29±1.13	1.12±0.09
C5	5	965.16±10.54	206.87±5.11	35.40±1.69	8.35±1.54	0.73±0.04
SM70/3		1 169.23±11.23	234.28±6.02	47.37±3.47	9.54±1.50	0.69±0.03
SM70/4		1 378.94±10.62	241.94±5.41	52.48±2.56	12.17±1.21	0.77±0.03
SM70/5		1 795.79±9.36	318.14±3.98	52.52±2.53	19.46±1.74	0.81±0.02
SM90/3		1 522.74±11.45	302.83±2.54	52.52±2.55	13.63±1.34	0.74±0.04
SM90/4		1 562.98±10.35	337.15±3.87	57.66±3.04	13.93±1.49	0.71±0.06
SM90/5		1 689.44±14.21	382.54±3.41	51.19±2.98	24.85±1.89	0.91±0.05
C7	7	778.34±9.01	220.05±2.58	35.15±1.65	8.68±1.46	0.74±0.07
SM70/3		1 209.46±10.36	191.97±4.05	40.85±2.55	16.98±2.03	0.79±0.03
SM70/4		1 467.44±12.54	234.08±3.09	57.28±3.14	11.58±1.92	0.81±0.05
SM70/5		1 618.21±11.87	215.04±2.96	58.49±2.85	17.31±1.47	0.81±0.02
SM90/3		1 536.61±11.25	288.83±5.32	60.35±2.45	16.13±1.25	0.75±0.08
SM90/4		1 434.61±9.89	275.78±4.58	51.45±1.95	14.5±1.07	0.78±0.07
SM90/5		1 909.63±12.26	335.57±4.56	59.27±1.99	21.36±2.07	0.79±0.04

Note: Untreated sample (C); SM70/3 (A: 70%; t: 3 min), SM70/4 (A: 70%; t: 4 min), SM70/5 (A: 70%; t: 5 min), SM90/3 (A: 90%; t: 3 min), SM90/4 (A: 90%; t: 4 min), and SM90/5 (A: 90%, t: 5 min); Total polyphenolic content (TPC); Monomeric anthocyanins content (MA); Ferric reducing antioxidant power (FRAP); Intensity of color (IC); Hue (N); The results are expressed as the mean value of the three replicates ± the standard deviation (SD).

Effects of Ultrasound Treatment on Total Polyphenol Content

At the end of maceration, the TPC varied from 870.32 micrograms gallic acid equivalents per millilitre (GAE/mL) to 1925.12 µg GAE/mL, as shown in Table 1. The lowest amount of TPC was found for the untreated sample C after 3 days of maceration (C3), while the higher amount of TPC was found for the SM90/5 sample also after 3 days of maceration. It can be seen that there is an increase, generally proportional to the treatment conditions, for the TPC of the sonicated samples compared to the untreated sample C from 1.5 to 2 times in the case of 3 days maceration; from 1.01 to 2.06 times in the case of 5 days maceration; and from 1.11 to 2.45 times in the case of 7 days maceration. This shows that sonication has a positive effect on the extraction of phenolic compounds, as reported in other studies (Natrella et al. 2023, Lukić et al. 2019, Ranjha et al. 2020). In addition, the effect of ultrasound on grapes during the vinification process was investigated by (Ferraretto et al., 2013). These authors found that the disruption of the cell wall caused by pressure cycling and cavitation induced by ultrasound, which led to a reduction in the duration of traditional maceration, improved the extraction of polyphenolic chemicals from grapes. Other authors (Pérez-Porras et al., 2021) also reported the lowest amount of TPC for the control sample compared to sonicated samples at 20 and 28 kHz. According to the data, amplitude appears to play a significant role in the extraction of total phenols.

Effects of Ultrasound Treatment on Monomeric Anthocyanins

The MA content for sonicated samples varied from 105.3 mg cyanidin 3-glucoside equivalents (CGE)/L to 382.54 mg CGE/L (Table 1). Similar to TPC, the lowest amount of MA was found for untreated sample C (C3) after 3 days of maceration (D3), while the higher MA content was found for SM90/5 sample extracted after 5 days of maceration (D5). It can be observed that there is an increase, generally proportional with the treatment conditions, from 1.43 to 2.61 times in the case of 3-day maceration; from 1.13 to 1.85 times in the case of 5 days; and from 0.12 to 1.52 times in the case of 7 days for the monomeric anthocyanins of the sonicated samples compared with untreated sample C. According to research made by Dalagnol et al. 2017, ultrasound-assisted extraction increased the rate at which anthocyanins were extracted. Although monomeric anthocyanins are not very stable, free anthocyanins are

the main source of red color in young red wines. Condensation with tannins to form stable anthocyanin/tannin adducts is one of the main strategies for their stabilization. At the end of alcoholic fermentation, about 25% of the anthocyanins are predicted to have polymerized with flavonoid molecules; after one year of aging, the percentage increases to more than 40% (He et al., 2012a, 2012b).

Effects of Ultrasound Treatment on Antioxidant Capacity

The maximum FRAP value, 62.43 μmol Fe2+/mL was observed in the sonicated samples SM90/5 extracted after 3 days of maceration. The lowest FRAP value, 33.73 μmol Fe2+/mL was observed for control sample also after 3 days of maceration. Moreover, the highest value of antioxidants was found in the same sample as for TPC and MA, respectively the sample sonicated at 90% amplitude for 5 minutes and extracted after 3 days of maceration (D3). It can be seen that the ferric reducing antioxidant power of the sonicated samples increases in proportion to the treatment conditions, from 1.3 to 1.85 times in the case of 3-day maceration; from 0.32 to 1.1 times in the case of 5 days; and from 1.16 to 1.72 times in the case of 7 days compared to the untreated sample C. It can be observed that the presence of a significant amount of phenolic compounds in the sonicated samples as a result of the US treatment led to high FRAP values.

Other studies have reported that the phenolic composition is greatly influenced by the winemaking process and have shown a high correlation between phenolic composition and ferric reducing antioxidant power of samples (Lingua et al., 2016). It has also been concluded that the antioxidant capacity of wines is more closely related to the related to the nature of the individual phenolic compounds found in the wines rather than to the total phenolic content of the wines (Banc et al., 2020).

Effects of Ultrasound Treatment on Chromatic Characteristics

The IC varied from 6.61 to 24.85 at the end of maceration process. The maximum IC was observed in the sonicated samples SM90/5 extracted after 5 days of maceration and the lowest IC was observed for control sample after 3 days of maceration. It can be seen that the IC of the sonicated samples increases in proportion to the treatment conditions, from 3.07 to 3.54 times in the case of 3-day maceration; from 1.14 to 3 times in the case of 5 days; and from 1.67 to 2.5 times in the case of 7 days compared to the untreated sample C. Other research (Pérez-Porras et al., 2021) found that the control samples macerated for 48 hours had the lowest color intensity. However, the differences from the other control wines were not statistically significant. Moreover, the color intensity of the sonicated samples macerated for 48 hours was not significantly different from the control wine exposed to the skin for 7 days.

Evaluating the Effects of Ultrasound Treatment on Maceration Time

Analyzing each of the samples according to the different maceration periods on the skins, it can be observed that the TPC increases during the maceration process, reaching a maximum on the fifth day and then decreasing. Other authors found that wines made with a 6-day maceration period had the highest levels of phenolic compounds (Ivanova et al., 2012) while others reported that by the 15th day, maceration continued to promote increased phenolic compounds (Alencar et al., 2018b). The monomeric anthocyanin content also increases during the maceration process, reaching a maximum on the seventh day. The previous studies have highlighted the fact that, in general, the antocyanins content and the color intensity increase during the maceration period, reaching a maximum on the fifth or sixth day (Busse-Valverde et al., 2011), and then decrease as a result of hydrolysis or oxidation reactions, participate in cycloaddition processes with metabolites produced by the yeasts, are absorbed by the yeasts and precipitated in the lees, or are condensed with catechins (González-Neves et al., 2008; Setford et al., 2017). As a result of the increase in the content of extracted bioactive compounds, the FRAP value increases, reaching a maximum on the fifth day and then decreasing. The color intensity increases similarly to the anthocyanins, reaching a maximum on the seventh day.

Considering the average duration of maceration of 7 days, we compared the results obtained for the total polyphenol content, the monomeric anthocyanin content, the values obtained for the ferric reducing antioxidant power and the color intensity of the untreated sample extracted after 7 days with each of the samples subjected to ultrasound treatment after only 3 days of maceration (Figure 2). Regarding the duration of maceration-fermentation, the advance, even after 4 days of maceration, is evident, both for the total content of polyphenols and monomeric anthocyanins, as well as for the FRAP value and color intensity, compared to the control sample.

Comparing the total polyphenol content of the samples, it can be seen that the samples treated with ultrasound and macerated for only three days (D3) recorded the highest TPC values (except for sample A 90%, 3 min, which recorded its maximum value on the seventh day). This was also the case when the control sample was macerated for four more days (D7).

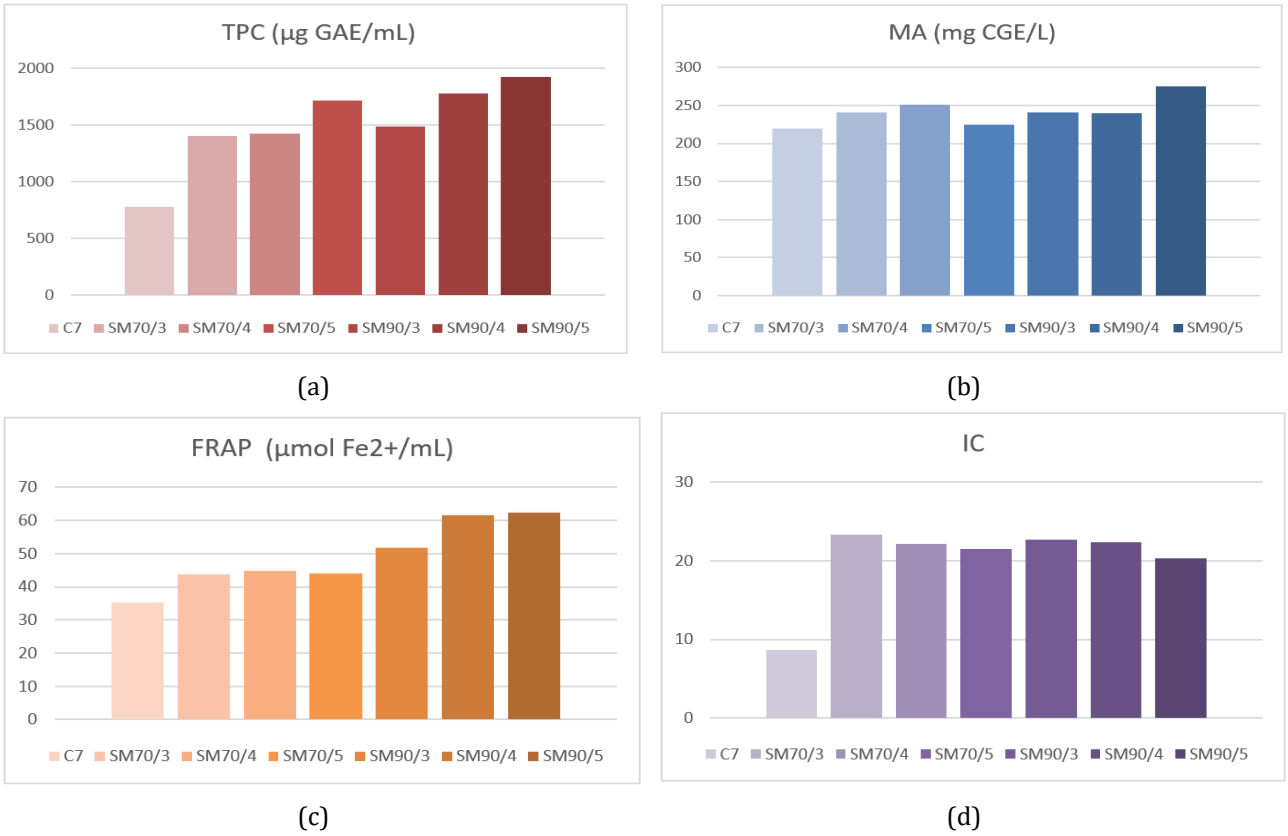

Figure 2. Comparison between the bioactive compounds and the chromatic characteristics of the sonicated samples after 3 days of maceration in comparison to the untreated sample after 7 days of maceration: (a) Total phenolic content; (b) Monomeric anthocyanins; (c) Ferric reducing antioxidant power (FRAP); (d) Intensity of color.

Similarly, the samples treated with ultrasound and macerated for three days (D3) and five days (D5), respectively, recorded the highest values of monomeric anthocyanin content in the conditions in which the control sample was macerated for four and two days, respectively (D7). Also, the highest values of color intensity were observed for samples treated with ultrasound and macerated for only three days (D3), except for sample A 90%, 5 min, whose maximum was recorded on day five.

Therefore, it is clear that the ultrasonic treatment of the crushed grapes caused a breakdown of the cellular structures, which facilitated the extraction of phenolic compounds during the maceration process. In addition, the duration of maceration-fermentation on the lees modified the final chromatic characteristics of the wine samples. As a result, it is clear that one of the issues now being addressed by wineries is the likelihood that ultrasonic treatment has advantageous extractive capabilities that allow time savings in the maceration phase.

Our results were in agreement with those of others who have included ultrasound-assisted extraction in the list of new technologies that have been developed with the aim of reducing the extraction time (Carrera et al., 2012; El Darra et al., 2013) and increasing the yield (Constantin and Istrati, 2022). Besides, Bautista-Ortin et al. 2017 studied the effect of ultrasonic treatment on the maceration stage and determined the color characteristics of the wine, as well as anthocyanin and tannin concentrations. The authors found that the use of ultrasound reduced the maceration time and increased the concentration of tannins and volatile compounds in the resulting red wine.

Correlation between maceration time, amplitude level and ultrasonic treatment time

The statistical analysis showed that the parameters studied were influenced by three factors: maceration time, amplitude level and ultrasonic treatment time, with single or interaction effects. The analysis of variance carried out on the analytical parameters in relation to the different durations of maceration, as well as the conditions of amplitude and treatment time, revealed significant differences in terms of the TPC, MA and FRAP, IC and N of the samples (Table 2). The model presented shows, for the observed parameters, values of coefficients of determination (R^2>0.477) that indicate the validity of the method.

Table 2. Analysis of variance of the regression coefficients for the different parameters

				Durbin-Watson			
Model summary	**R**	**R^2**	**Adjusted R^2**	**Autocorrelation**	**Statistic**	**p-value**	**F-value**
CTP	0.781	0.610	0.587	0.778	0.946	<0.001	26.109
AM	0.703	0.494	0.463	0.540	0.975	<0.001	16.249
FRAP	0.690	0.477	0.445	0.564	0.970	<0.001	15.171
IC	0.603	0.480	0.476	0.572	1.037	<0.001	9.518
N	0.806	0.649	0.628	0.287	1.401	0.009	30.854

Note: Correlation coefficient (R); Determination coefficient (R^2); Significance of each parameter (p-value); $p<0.05$ statistically significant and $p<0.001$ statistically highly significant; Ratio between the model sum of squares and the residual error (F-value). The larger the F-value, the better the model; Total polyphenolic content (TPC); Monomeric anthocyanins content (MA); Ferric reducing antioxidant power (FRAP); Intensity of color (IC); Hue (N).

It can be seen that the extraction kinetics increase with increasing maceration time, amplitude percentage and treatment time with a correlation coefficient (R) of 0.781 in the case of TPC and 0.703 in the case of MA, while the FRAP value increases with a correlation coefficient of 0.690. The adjusted R^2 used for the three predictors: maceration time, amplitude and treatment time, shows that they can predict: 58.7% of the variation in the results obtained for CTP; 46.3% of the variation in the results obtained for MA; 44.5% of the variation in the results obtained for FRAP. The Durbin-Watson index checks for correlations between residuals that may invalidate the test.

It should be greater than 1 and less than 3, ideally around 2. In our case, the value of the index for all three parameters is very close to 1, so we can consider it to be in the range that validates the test. Previous research also concluded that the amplitude of the transducer was directly proportional to the intensity of the ultrasound. The intensity of the ultrasound increased along with the amplitude, which increased the sonochemical effects of the ultrasound (Mason and Lorimer, 2002).

The ANOVA shows that the model is significant and that the predictors included in the model, both individually and in the case of interaction between them, significantly influence ($p<0.001$) the content of total polyphenols, monomeric anthocyanins and FRAP. As for the color intensity, it is not significantly influenced by the interaction between the three predictors of the model, while the shade is not influenced by either the amplitude level or the treatment time, being influenced only by the duration of maceration (Table 3).

Table 3. Effect of treatment amplitude and time, and maceration duration on TPC, MA, FRAP, IC and N

	p-value				
Factors	**TPC**	**MA**	**FRAP**	**IC**	**N**
Maceration time (days)	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Amplitude (%)	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.276*
Ultrasound treatment time (min.)	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.251*
Maceration time ∗Amplitude	*<0.001*	*<0.001*	*<0.001*	*0.003*	*0.107*
Maceration time ∗Ultrasound treatment time	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Amplitude ∗Ultrasound treatment time	*<0.001*	*<0.001*	*<0.001*	*0.038*	*0.302*
Maceration time ∗Amplitude ∗Ultrasound treatment time	*<0.001*	*<0.001*	*<0.001*	*0.394*	*0.280*

Significance of each parameter (p-value); $p<0.05$ statistically significant and $p<0.001$ statistically highly significant; Total polyphenolic content (TPC); Monomeric anthocyanins content (MA); Ferric reducing antioxidant power (FRAP); Intensity of color (IC); Hue (N).

It can be observed that there is a different response for similar treatment conditions (significant differences between R^2). The tests performed show an increase in the total content of polyphenols and monomeric anthocyanins and FRAP correlated with the maceration time, treatment time and % amplitude.

CONCLUSIONS

Our data provide strong evidence that high-power ultrasound treatment applied to crushed Merlot grapes improved the extraction process of polyphenolic compounds during the maceration stage. The improvements recorded in

total polyphenolic content, monomeric anthocyanin content, ferric reducing antioxidant power and color characteristics are correlated with maceration time, ultrasound treatment time and % amplitude. In most cases, samples treated with ultrasound and left to macerate for only three days recorded the highest value of investigated characteristics, even though the control sample was left to macerate for a period of seven days. The analysis of variance of the results obtained after the period of maceration shows that the model is significant and the predictors introduced in the model (amplitude%, treatment time and duration of maceration), both individually and in the case of interaction between them, significantly influence ($p<0.05$) the total content of polyphenols, monomeric anthocyanins and antioxidant capacity. As regards the intensity of color, it is not significantly influenced by the interaction between the three predictors of the model, while hue is influenced by neither by the amplitude level nor by the treatment time, being influenced only by the duration of maceration. High-power ultrasound treatment can improve both phenolic compound extraction and ferric reducing antioxidant power, but also has the effect of reducing maceration time by 2 or even 4 days, which is a promising result for one of the challenges currently facing wineries. Therefore, ultrasound treatment could save time during the maceration phase, thus representing a solution for optimizing management in a winery.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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