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Effects of high power ultrasound treatments on the phenolic, chromatic and aroma composition of young and aged red wine

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Abstract

In this study, the effects of both ultrasonic bath and probe treatments on the phenolic, chromatic and aroma composition of young red wine Cabernet Sauvignon were studied and modeled by artificial neural networks (ANNs). Moreover, the effect of high power ultrasound (HPU) along with antioxidants addition (sulfur dioxide and glutathione) was investigated during 6 months of aging in bottles. Lower amplitude and temperature, shorter treatment duration and particularly lower frequency showed a more favorable and milder effect on the chemical composition of wine. In the case of the ultrasonic probe treatment, similar effect was achieved primarily by a larger probe diameter as well as lower amplitude and treatment duration. Selected ANN models showed the best predictions for the chromatic characteristics followed by total phenolics and anthocyanins. The changes induced by HPU treatment after 6 months of aging were mainly detected in the composition of phenolic compounds (both total and individual), where higher concentration of antioxidants (sulfur dioxide and glutathione) slowed down the decrease rate of these compounds during aging. However, HPU treatment did not influence most of the chromatic characteristics and aroma compounds, except lightness and fatty acids. The obtained results indicated that suitable ultrasound treatment might accelerate some aging reactions and shorten the period of wine aging.

Keywords: high power ultrasound (HPU); wine quality; antioxidants; ultrasonic bath; ultrasonic probe; artificial neural network (ANN).

1. Introduction

High power ultrasound (HPU) is an innovative processing technology that could be used on wines for many applications. For instance, over the last years, many studies have been carried out on the use of ultrasound for wine microbial stabilization [1–5] and for the acceleration of wine aging process [6–9].

Despite the mentioned studies, most of the conducted researches regarding the application of HPU in wine production are related to the effect of the technology on the extraction of different bioactive compounds (phenolics, flavonoids, tannins and others) responsible for wine color, flavor and taste [10–16].

Generally, when it is applied to a wine, HPU causes both physical and chemical effects, which are expected to modify the physicochemical properties and enhance the quality of the product during processing. But first of all, the application of HPU should ensure the preservation of sensory properties of wines and the antimicrobial effect at the same time. The replacement of the antioxidant and antimicrobial effect of sulfur dioxide (SO_2) is still hard to accomplish. However, the combination of HPU together with antioxidants addition (lower SO_2 and glutathione) could be a suitable practice to achieve this purpose, especially regarding the wine stability during aging. In other words, it was reported that the combination of SO_2 and glutathione implicates a respectable protective effect in wines [17]. Additionally, reduced glutathione has been proposed as an alternative method due to its specific antioxidant effects in preserving aroma compounds and preventing oxidation [18].

Recently, García Martín et al. [19] reviewed the effect of ultrasound on the quality properties of red wines. Additionally, other authors reported that different conditions of ultrasound treatment influence the color characteristics and significantly modify the content of total phenolics through stimulation of polymerization reactions that take place during natural aging of wine, without major changes in basic physicochemical parameters such as

pH, total and volatile acidity [9,20,21]. Moreover, some studies showed that ultrasound influences the electrical conductivity of red wine [22], triggers the generation of free radicals into the wine [23] and causes changes in the wine aroma composition and sensory properties (formation of oxidized aromas) [4,24]. However, the results obtained in these studies are still not sufficient to conclude how the use of different ultrasound systems such as ultrasonic baths or ultrasonic immersion probes could lead to different effects on quality properties of wine as well as its characteristics during aging.

Hence, further investigation about the effect of different ultrasound systems and process conditions (i.e., frequency, intensity, treatment duration and temperature) on a wider range of wine quality properties is necessary.

Aside from the aforementioned facts, the stochastic nature of ultrasound process and its dependence on numerous interdependent parameters make it difficult and almost impossible to develop one general and precise mathematical model suitable for investigating the process and product parameters [15]. Therefore, the artificial intelligence based techniques for prediction have attracted increasing attention in recent years, particularly for process modeling. Artificial neural networks (ANNs) are one of the important artificial intelligence methods that can be used to solve the problems that are not suitable for standard statistical methods [25].

Given the above, the aim of this study was (i) to evaluate the effect of HPU treatment applied by an ultrasonic bath and by an ultrasonic probe on the phenolic composition, chromatic characteristics and aroma composition of a young red wine Cabernet Sauvignon; (ii) to evaluate the ability of ANNs to predict aforementioned quality properties of ultrasonic bath and ultrasonic probe treated red wine, and (iii) to study the effect of HPU along antioxidants addition (SO_2 and glutathione) on phenolic, chromatic and aroma composition of red wine during 6 months of storage.

2. Material and methods

2.1. Chemicals

The chemicals used in this work were: Folin-Ciocalteu reagent (Kemika, Zagreb, Croatia), sodium bisulfite (Acros Organics, Geel, Belgium), hydrochloric acid (37%, Carlos Erba, Val del Reuil, Spain), sodium chloride (pro analysis, Carlo Erba, Val del Reuil, Spain), ethanol (96%, Gram-Mol, Zagreb, Croatia), Sodium carbonate anhydrous (99%, T.T.T. Sveta Nedjelja, Croatia), formic acid (98-100 %, T.T.T., Sveta Nedjelja, Croatia), acetonitrile (HPLC grade, J.T. Baker, Deventer, Netherlands), ethanol (HPLC grade, J.T. Baker, Deventer, Netherlands). Malvidin-3-*O*-glucoside chloride, (+)-catechin, (-)-epicatechin, B1 [(-)-epicatechin-(4*ß*-8)-(+)-catechin] and B2 [(-)-epicatechin-(4*ß* -8)-(-)-epicatechin], as well as the aroma reference standards, and L-glutathione reduced (≥98%) were purchased from Sigma Aldrich (St. Louis, USA). The aqueous solution of potassium bisulfite (Bisulfite 15) was purchased from Laffort (Bordeaux, France).

2.2. Wine samples

The work was done with young wine Cabernet Sauvignon (*Vitis vinifera* L.), vintage 2017, from winery Erdutski, Erdut, Croatia. The wine had the following physicochemical characteristics: alcohol 12.8 vol%, total acidity (as tartaric acid) 5.6 g/L, volatile acidity (as acetic acid) 0.4 g/L, pH = 3.5, reducing sugars 5.0 g/L, free SO_2 10 mg/L, and total SO_2 20 mg/L.

2.3. High power ultrasound (HPU) treatments

The ultrasound studies were carried out using two different techniques: ultrasonic bath (experiment 1) and ultrasonic probe (experiment 2). The HPU experiment 1 was carried out using an ultrasonic bath system (Elmasonic P, Elma Schmidbauer GmbH, Singen, Germany), with dimensions of 505 x 300 x 200 mm and maximum capacity of 28 L. The wine (200 mL)

was placed in a round-bottom glass vessel (400 mL), which served as a treatment chamber, and then immersed in the ultrasonic bath. The samples were then treated by ultrasound running at different combinations of process parameters, namely ultrasound frequency (37 and 80 kHz), ultrasound amplitude (40, 60 and 100%), bath temperature (20, 40 and 60 °C) and treatment duration (20, 50, 65 and 90 min), selected based on literature data [7,21,23] and preliminary experiment (data not shown). The sonicator generated the power of 380 W. The ultrasonic energy was delivered from the bottom to the water in the bath with an automatic control of frequency. The control of water temperature inside the bath during the HPU treatments was achieved by cold water cooling of the treatment chamber.

On the other hand, the HPU experiment 2 was carried out using an ultrasonic processor system (Q700, Qsonica Sonicators, Newton, CT, USA) with dimensions of 400 x 400 x 800 mm, which was set at a nominal power of 700 W and a constant frequency of 20 kHz. The HPU probe was centered and dipped 2 cm inside a 400 mL glass vessel containing 300 mL of the sample. To study the effects of the ultrasound treatment, the experimental design considered different process parameters, namely the diameter size of ultrasound probe (12.7, 19.1 and 25.4 mm), ultrasound amplitude (25, 50, 75 and 100%) and treatment duration (3, 6 and 9 min), selected based on literature data [4,20,26] and preliminary experiment (data not shown). The samples were kept at room temperature (25 °C) by cooling the reactor during the treatment. Each HPU treatment in both experimental sets 1 and 2 was conducted in duplicate [144 (72 x 2) and 72 (36 x 2) trials in total]. Finally, after ultrasound exposures, the wine samples were subjected to different analyses in order to evaluate the effects of the treatments on the main wine quality properties. Wine that was not subjected to any HPU treatment was used as control sample in both HPU experiments.

2.4. Storage stability and changes in the chemical composition of red wines processed by HPU

According to the results of both HPU experiments, a second experiment (ultrasonic probe) was chosen for small scale performing at following process conditions: probe diameter of 25.4 mm, ultrasound amplitude of 25% and treatment duration of 6 min. The aim of this was to study the effect of HPU along antioxidants addition (SO_2 and glutathione) on phenolic, chromatic and aroma composition of red wine during 6 months of storage in bottles. Before HPU treatment, three experimental wines were prepared: (i) wine with standard SO_2 concentration (25 mg/L of free SO_2), (ii) wine with low SO_2 concentration and addition of glutathione (10 mg/L of free SO_2 with 20 mg/L of glutathione), and (iii) wine with low SO_2 concentration (10 mg/L of free SO_2). Control wine was untreated wine with standard concentration of SO_2 (25 mg/L of free SO_2). After HPU treatments, the wines were stored for 6 months in 750 mL bottles, sealed with natural cork stoppers and stored in a dark place at 12 °C. HPU treatments were carried out in triplicate and chemical analyses were conducted after 0, 3 and 6 months of aging.

2.5. Analysis of chromatic characteristics

The chromatic characteristics of the wine samples were measured with a Specord 50 Plus spectrophotometer (AnalytikJena, Jena, Germany) using the CIELab space [27]. The values of L^* (lightness), a^* (redness/greenness), b^* (yellowness/blueness), C^* (chroma) and H^* (hue angle) were determined. All measurements were performed in triplicate. The total color difference value (ΔE*) between the control and treated wine samples was calculated by the following equation 1:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Eq. 1

2.6. Spectrophotometric analysis of phenolic compounds

Determination of total phenolics (TP) content was done by the Folin-Ciocalteu method as described in detail by Singleton and Rossi [28]. The results were expressed as mg/L of gallic acid equivalents (mg GAE/L). The total anthocyanins (TA) content was measured by the bisulfite bleaching method as previously described by Ribéreau-Gayon and Stonestreet [29]. The results were expressed as mg/L. Measurement of total tannins (TT) content was carried out according to Ribéreau-Gayon and Stonestreet [30] and the results were expressed as g/L. All these spectrophotometric analyses were carried out in triplicate by a Specord 50 Plus spectrophotometer.

2.7. HPLC analysis of phenolic compounds

The HPLC analyses were performed on an Agilent 1100 Series LC-MSD system (Agilent Technologies, Waldbronn, Germany) with autosampler, binary pump, thermostated column compartment, DAD detector, FLD detector, and single quadrupole mass detector equipped with electrospray ionization interface, coupled to an Agilent Chemstation data analysis software. Wine samples were filtered through a 0.45 μm pore size cellulose acetate syringe filters (Nantong FilterBio Membrane, Nantong City, Jiangsu P.R China) prior to injection.

Free anthocyanins separation in the red wine samples was carried out according to the method previously described by Lorrain et al. [31] by using a Phenomenex Nucleosil C18 (4.6 mm x 250 mm, 5 μm) column. The mobile phase consisted of two solvents, water/formic acid (95:5, v/v) (solvent A) and acetonitrile/formic acid (95:5, v/v) (solvent B) and it was applied at a flow rate of 1 mL/min as follows: 0-25 min, 10-35% B linear; 25-26 min, 35-100% B linear; 26-28 min, 100% B isocratic; 28-29 min, 100-10% B linear. The column was re-equilibrated between runs for 29-35 min under initial gradient conditions. Free anthocyanins were eluted under following conditions: injection volume 20 μL, column temperature 40 °C and detection at 520 nm. The identification and peak assignment of anthocyanins were based on the comparison of their retention times, UV-Vis and mass spectral data with those of

standards [32,33]. The following nine major free anthocyanins were determined: delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, peonidin-3-*O*-acetylglucoside, malvidin-3-*O*-acetylglucoside, peonidin-3-(6-*O*-*p*-coumaroyl) glucoside and malvidin-3-(6-*O*-*p*-coumaroyl) glucoside. The quantification was performed by using an external standard calibration curve of malvidin-3-*O*-glucoside chloride. All analyses were conducted in triplicate and the results were expressed as the sum of the free individual anthocyanins quantified.

The analysis of flavan-3-ols was performed by using a Lichrospher 100-RP18 (4.6 mm x 250 mm, 5 μm) column, according to the method of Ćurko et al. [34] with a slight modification of the solvent gradient conditions. The mobile phase consisted of two solvents, water/formic acid (99:1, v/v) (solvent A) and acetonitrile/formic acid (99:1, v/v) (solvent B) and it was applied at a flow rate of 1 mL/min as follows: 0-11 min, 3-8% B linear; 11-16 min, 8% B isocratic; 16-20 min, 8-10% B linear; 20-27 min, 10% B isocratic; 27-32 min, 10-12% B linear; 32-34 min, 12-14% B linear; 34-45 min, 14-25% B linear; 45-46 min, 25-100% B linear; 46-50 min, 100% B isocratic, 50-51 min, 100-3% B linear. The column was re-equilibrated between runs for 51-55 min under initial gradient conditions. The injection volume was 20 μL and the column temperature was 25 °C. The detection was conducted at 280 nm excitation wavelength and 320 nm emission wavelength with low fluorescence intensity. The identification and peak assignment of flavan-3-ols were based on the comparison of their retention times and mass spectral data with those of standards [35,36]. The following flavan-3-ols were determined: (+)-catechin (C), (-)-epicatechin (EC), dimers B1, B2, B3, B4 and trimer C1. The quantification was performed by using an external standard calibration curve in the case of C, EC, B1, B2. On the other hand, the dimers B3, B4 and trimer C1 were quantified as dimer B1 equivalents. All analyses were conducted in triplicate and the results were expressed as the sum of the free individual flavan-3-ols.

2.8. GC/MS analysis of aroma compounds

Aroma compounds were extracted from the wine by solid-phase microextraction (SPME) and analyzed by gas chromatography coupled with mass spectrometry (GC/MS) using an Agilent Gas Chromatography 6890 series equipped with an Agilent 5973 Inert mass selective detector (Agilent Technologies, Santa Clara, USA) according to the method described by Tomašević et al. [17]. The identification of wine aroma compounds was done with the help of GC/MS using the Enhanced Chemstation software (Agilent Technologies, Santa Clara, CA, USA), and the peak retention times of the total compounds in wine were compared with those of standards as well as their mass spectra were matched with the Nist08 mass library (Wiley & Sons, Hoboken, NJ, USA). The quantification of aroma compounds was carried out by preparing and analyzing calibration curves for each compound using GC/MS at the same extraction and chromatographic parameters as for the wine samples. The identified aroma compounds included esters (ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, diethyl succinate, ethyl acetate, *i*-butyl acetate, *i*-amyl acetate, hexyl acetate and 2-phenylethyl acetate), higher alcohols (amyl alcohol, phenylethyl alcohol, 1-hexanol and *cis*-3-hexenol), fatty acids (hexanoic acid, octanoic acid and decanoic acid) and terpenes (linalool and α-terpineol). All the analyses were conducted in triplicate and the results were expressed as the sum of determined individual aroma compounds, sorted by main aroma groups.

2.9. Data analysis

Overall differences in both HPU experiments were examined using multivariate analysis of variance (MANOVA) testing for the effects of process (input) variables, followed by univariate ANOVAs performed on each dependent variable, as listed in Table 1. The statistical data analysis was performed using Statistica v.10.0 software (StatSoft, Tulsa, USA). To predict total phenolics, total anthocyanins, total tannins, total free anthocyanins, total flavan-3-ols, chromatic characteristics, total esters, total higher alcohols, total fatty acids and

total terpenes in both HPU experiments, artificial neural network (ANN) modeling was applied. The ANN trainings were performed with random separation of data into training, test and validation sets at different ratios. Multiple layer perceptron (MLP) networks trained by Broyden–Fletcher–Goldfarb–Shanno (BFGS) algorithm were selected to develop the prediction models. The performances of the developed models were statistically measured by the root mean squared error (RMSE) and correlation coefficient (R^2). Overall differences in bottled wines were examined using one-way ANOVA. In order to compare variable means and to examine which wines were different, Tukey's HSD test was used as a comparison test when samples were significantly different after ANOVA ($p < 0.05$). All multivariate analyses of experimental data and ANN calculations were carried out using Statistica v.10.0 software (StatSoft, Tulsa, USA).

3. Results and discussion

3.1. Influence of high power ultrasound (HPU) process parameters on the quality properties of red wine

The effects of different HPU process variables (inputs) on the quality properties of red wine (outputs) were studied (Table 1). The results obtained for the phenolic composition, chromatic characteristics and aroma composition of the red wine treated by ultrasonic bath and ultrasonic probe in different conditions were listed (as a supplementary material) in Tables S1-S3, respectively. The summarized results of the analysis of variance are given in Table 2. The ANOVA revealed that all process variables (inputs) and their interactions showed statistically significant effect on analyzed variables (outputs) in both HPU experiments ($p < 0.0001$, $p < 0.001$ $p < 0.01$, Wilk's lambda).

3.1.1. Influence of HPU process parameters on phenolic composition

In ultrasonic bath experiment (Table S1 and Table 2-Experiment 1), ultrasound frequency was the most important variable influencing TP, TA, TT and total free anthocyanins, while the ultrasound amplitude had a greater effect on total flavan-3-ols (higher *F* values). Besides, the largest part of the variation due to an interaction between variables in the phenolic composition of the treated wine was due to frequency × bath temperature (X_2X_3) (Table 3). Generally, a higher value of frequency resulted in a lower content of TP, TA, TT and total free anthocyanins, independently from the other process variables. Similarly, a higher ultrasound frequency along with higher bath temperature also resulted in a lower content of phenolic compounds (Table S1). It was already reported that the ultrasound degradation of phenolic compounds was frequency-dependent and that a low-frequency ultrasound (20 kHz) did not affect the stability of phenolics [37]. Furthermore, it is known that phenol degradation is greater at higher frequencies [38].Specifically, the highest content of TP, TA and total free anthocyanins was observed at the conditions of 40% amplitude, 37 kHz frequency and 60 °C after 50-65 min of sonication, and was the closest to that of the untreated wine (Table S1). On the other hand, the highest content of total flavan-3-ols was achieved under 100% amplitude, 80 kHz frequency and 40 °C after 90 min of sonication (Table S1). Singleton and Draper [24] reported similar results at their work, in which the ultrasound treatments were used to accelerate aging of wine. A similar behavior was also observed by Zhang et al. [21] for the ultrasonic bath treatment of red wine Cabernet Sauvignon. These authors reported that the lowest content of TP was obtained at the highest process conditions (300 W, 100 kHz, 60 °C, 100 min), with the greatest influence of ultrasound frequency and exposure time. Zhang et al. [21] suggested that the high volatility of the wine (due to the ethanol content) promotes the formation of free radicals by cavitation phenomenon which in turn causes oxidative damage, primarily of phenolic compounds. For example, the aforementioned phenomenon could probably induce the degradation of anthocyanins resulting in the opening of the benzene ring

and the formation of a chalcone. During HPU treatment, various physical (cavitation, mechanical effects and micro-mechanical shocks) and chemical effects (formation of free radicals and ions) occur simultaneously or separately, and affect the quality of the treated medium [39]. Also, it is important to highlight that the increase of ultrasound intensity, which is directly correlated to the ultrasound amplitude, results in an increase of sonochemical effects (more violent bubble collapse) [40,41]. Moreover, the transient cavitation bubbles are less numerous at low frequencies, which favor the physical effects instead of the chemical ones [42,43]. On the other hand, higher temperatures induce an increase of vapor pressure, which causes more solvent vapors to enter the bubble cavity and consequently, the sonication effects due to less violent bubble collapse are reduced [44]. The effect of HPU on wine is mainly attributed to acoustic cavitation that creates localized high temperatures and pressures, and consequently induces chemical reactions that naturally occur during wine aging [7,9,45,46]. Masuzawa et al. [47] confirmed an effect of polymerization of phenolic compounds in red wine promoted by ultrasound treatment at low sound pressures. However, some researches indicate a lower degree of chemical decomposition of phenolic compounds when ultrasound is used as extraction method at low frequencies of 20-40 kHz in comparison to conventional processing technologies [15].

Regarding the ultrasonic probe experiment (Table S2 and Table 2-Experiment 2), ANOVA showed that the ultrasound amplitude was the most important variable influencing TP and TT, while the probe diameter and the treatment duration had significantly higher effect on TA and total free anthocyanins (higher *F* values) respectively. Also, the treatment duration showed to be the most important variable affecting total flavan-3-ols. Besides, a decrease in the content of TP, TA, TT, total free anthocyanins and total flavan-3-ols was observed when the probe diameter was reduced. On the other hand, an increase of the ultrasound amplitude or the treatment duration resulted in lower concentrations of phenolic compounds. Moreover, among

the interaction effects, the interaction between the probe diameter and the treatment duration (X_1X_3) was the one that affected in greater extend the phenolic composition of the wine (higher F values), with the exception of total flavan-3-ols. As can be seen in Table S2, the experiments performed with 25.4 mm probe and 25% amplitude during 6 min of sonication resulted in a higher content of TP and TA. In addition, HPU treatment with 19.1 mm probe also resulted in a higher content of total free anthocyanins at identical amplitude and treatment duration (Table S2). On the other hand, the highest content of total flavan-3-ols was obtained with the 19.1 mm probe, but at higher amplitude (75%) after only 3 min of sonication (Table S2). All together, these results demonstrated that there is no clear trend in the overall phenolic composition at different amplitudes and treatment durations of sonication, which could be due to enhanced polymerization/depolymerization, copigmentation, isomerization and decomposition reactions during the ultrasound treatment. So, changes in the phenolic composition are probably related to the already mentioned cavitation phenomenon, which triggers oxidation reactions in wines (phenols are oxidized to quinones, while oxygen is reduced to hydrogen peroxide). Cavitation also produced a variety of chemical reactions by the free radicals generated and that is considered the main cause of the degradation of phenolic compounds. In the same way that in our work, Tiwari et al. [26] observed that the anthocyanins content of red grape juice decreased during prolonged sonication with a 19 mm probe at higher amplitudes. On the other hand, Ferraretto and Celotti [20] found that free anthocyanins in red wines were not modified by ultrasound treatment (200 W output, 20 kHz, 13 mm probe, 30-90%, 1-5 min), whereas the higher process conditions (higher amplitudes and longer exposures) resulted in an increase of flavan-3-ols, namely the monomeric catechins. The possible explanation for the increment of catechin content is that the ultrasound treatment promotes the depolymerization and recombination reactions of phenolic compounds [20]. In addition, the results in the current study also suggest

that application of ultrasound with an appropriate process conditions might accelerate the wine aging reactions.

Similarly to the results of the present work, many studies have confirmed that the HPU treatment using an ultrasonic probe has a higher and localized intensity of ultrasound in comparison to the treatment using an ultrasonic bath, which is characterized by a lower ultrasound or cavitation intensity and an uneven distribution of ultrasound [48]. Generally, higher amplitudes can lead to higher ultrasound intensities, which can promote some undesirable effects (compound degradation). But also, higher amplitudes can cause erosion of the ultrasonic probe, leading to agitation instead of cavitation and a weak distribution of ultrasound through the treated medium [44]. According to the results obtained in both HPU experiments, it is necessary to avoid extreme process conditions (i.e., frequency, amplitude, treatment duration) in order to maintain the phenolic composition of wine.

3.1.2. Influence of HPU process parameters on chromatic characteristics

The effect of the different independent variables of the HPU using an ultrasonic bath on the chromatic characteristics would be ranked in the following order: bath temperature > amplitude > frequency > treatment duration (higher *F* values) according to the results of the ANOVA analysis (Table 2. Experiment 1). Among interaction effects, amplitude × bath temperature (X_1X_3) was the most significant variable in affecting L^*, a^*, b^* and C^* values (Table 2. Experiment 1). The sonicated samples presented slightly different values of the CIELab parameters, when compared with the unsonicated wine (Table S1). Moreover, the values of chromatic characteristics (L^*, a^*, b^* and C^*) varied according to the applied ultrasound conditions, where higher bath temperatures, ultrasound amplitudes as well as treatment durations resulted in slightly lower values of these parameters (Table S1). Contrary, an increase of ultrasound frequency resulted in slightly higher values of L^*, a^*, b^* and C^*, while H^* in general remained constant. For example, the lowest values of chromatic

characteristics were obtained at 100% amplitude and 60 °C after 90 min of sonication (Table S1). In order to determine the total color difference of the wine samples against the control, the parameter ΔE* was calculated (Table S3). For the assessment, it was considered that when the value of ΔE* between two samples is in a range from 2 to 10, the difference in color is clearly perceptible, while in the case of values higher than 10 the colors are more opposite than similar [49]. Also, according to Ramirez-Navas and Rodriguez de Stouvenel [50], all the color differences with ΔE* values higher than 6 are considerable. The values of the total color difference (ΔE*) between treated and control samples were mostly in the range of 2-6, which means there were perceptible differences between these wine colors (Table S3). Furthermore, only the values of ΔE* between the samples sonicated at 100% amplitude and 60 °C during 90 min, as well as at 37 and 80 kHz frequency and 20 °C during 20 min compared to the control sample were higher than 6, being clearly perceptible by the human eye. It is important to consider that the wine color is mainly influenced by the presence of various anthocyanins, the applied winemaking technique and the numerous reactions that take place during natural aging [51]. For anthocyanins is well-known that they are highly unstable and very susceptible to degradation. It is interesting to highlight that, comparing the obtained results, the chromatic characteristics and the anthocyanins were both influenced by the same investigated variables during HPU, namely ultrasound amplitude, frequency and bath temperature. Probably, the localized high temperatures and pressures generated from the acoustic cavitation in the ultrasound treatment initiate some chemical reactions related to the color changes in red wine [9]. Additionally, these extreme physical conditions can also lead to accelerated isomerization of color pigments [52].

When the wine is treated by an ultrasonic probe (Table 2- Experiment 2), it was shown that the probe diameter as well as the interaction probe diameter × treatment duration (X_1X_3) on wine chromatic characteristics were the most significant (higher F values) compared to the

rest of the experimental variables and their interactions. As can be seen from Table S2, the sonicated samples showed slightly different values of the CIELab parameters when compared to the unsonicated wine. Particularly, the lowest values of chromatic characteristics were obtained with 19.1 mm probe at all amplitudes and treatment durations (Table S2). Moreover, as we can observe in Table S3, the total color differences ΔE* between most of the sonicated samples and the control sample were in the range of 0.5-3, all being slightly perceptible. The samples treated with a smaller probe diameter (12.7 mm) for 3 min showed the values of ΔE* around 4-5, which means there were perceptible differences between these samples and control. Nevertheless, there were no considerable color differences between sonicated samples and control (untreated) sample, since obtained ΔE* values were not higher than 6. As previously suggested, these changes in the chromatic characteristics can be related to the changes in the content of anthocyanins, which are known to be responsible for the red color of the wine and to react with catechins during natural aging of wine [7].Then, higher ultrasound powers may cause the breakdown of the existing colored polymeric pigments in the red wine and consequently lead to a decrease in color characteristics [9]. On the contrary, a weaker ultrasound irradiation may initiate and accelerate chemical reactions involving anthocyanins due to ultrasound-generated free radicals and this way positively modify the wine color [9].

3.1.3. Influence of HPU process parameters on aroma composition

Interestingly, from the statistical analysis of aroma composition of ultrasonic bath treated wine (Table 2- Experiment 1), it can be seen that the bath temperature was the most important variable influencing total esters and total fatty acids, whereas ultrasound amplitude had the greatest effect on total higher alcohols and total terpenes (higher *F* values). Additionally, among the interaction effects, the one between bath temperature and treatment duration (X_3X_4), and the one between bath temperature or treatment duration and frequency (X_2X_3, X_2X_4) showed to play the most significant role in affecting the aroma composition of treated

wine (higher *F* values). Besides, the lowest content of total esters and total higher alcohols was observed at the highest bath temperatures (40-60 °C) and treatment durations (65-90 min) (Table S1), probably due to the heating effect of ultrasound energy which could accelerate the evaporation of aroma compounds. Furthermore, an increase in ultrasound amplitude resulted also in a lower content of total esters, total higher alcohols, total fatty acids and total terpenes in sonicated wines, when compared to the untreated wine.

As can be seen in Table S1, the content of total esters and total higher alcohols decreased in the range of 60-100% amplitude and a bath temperature of 40-60 °C at 37 kHz frequency after 90 min of HPU. However, the results of total fatty acids and especially of total terpenes showed no particular trend at all combinations of applied process parameters (Table S1). In an alcoholic beverage such as wine, ultrasound can cause an acceleration of oxidation, polymerization and condensation of alcohols, aldehydes, esters and others compounds [53]. Then, the changes observed in the aroma composition of ultrasound treated wine are probably due to oxidation reactions (occurring as a result of various interactions with free radicals) generated during the HPU [23,54]. Singleton and Draper [24] found that ultrasound bath treatment decreases volatile esters in wines at higher process conditions, relating this to a possible degassing effect of ultrasound. Moreover, Chemat et al. [55] reported a relation between the increase of ultrasound power (higher amplitudes and temperatures) and the degradation of wine phenolics, which could prevent the oxidative degradation of aroma compounds. Due to the complexity of the wine aroma, some wine components were divided into a group of esters, higher alcohols, fatty acids and terpenes. It is known, that the majority of the aroma compounds in wine are fermentation compounds, primarily higher alcohols and esters [56]. Also, the volatile fatty acids and terpenes can contribute significantly to the overall flavor and aroma of wine [57].

Additionally, from Table 2 it can be seen that among the three process variables in ultrasonic probe experiment, the probe diameter was the most significant variable that influenced the content of total esters, total higher alcohols and total terpenes (higher *F* values). Secondly, the treatment duration showed to be the most important variable influencing total fatty acids (higher *F* value). Interestingly, the content of total esters, total higher alcohols and total fatty acids showed first an increase by increasing the probe diameter, achieving highest values using 19.1 mm probe, while afterwards slightly decreased (Table S2). On the other hand, an increase in the probe diameter resulted in a lower content of total terpenes. As can be seen from Table S2, treatments performed with a 12.7 mm probe and 25% amplitude during 6 min provoked lower content of total esters in the wine, while HPU conditions of 100% amplitude and 3 min with the same probe diameter resulted in lower content of total higher alcohols compared to the unsonicated wine. Furthermore, lower content of total fatty acids was observed at 75% amplitude after 9 min of HPU treatment with a 12.7 mm probe. Contrary, the lowest content of total terpenes was achieved at conditions of 25% amplitude after 9 min of HPU treatment with a 25.4 mm probe (Table S2). From these results, it could be suggested that, in general, a smaller probe diameter along with higher amplitudes or longer ultrasound exposures caused a major degradation of the compounds responsible for the wine aroma. These changes could be related to the various mechanisms that can act simultaneously or separately when applying ultrasound, such as the thermal effects of the implosion of cavitation bubbles and consequently the formation of free radicals, mechanical effects of the microstreaming, implosion and shock waves [58,59]. The extreme physical conditions (high temperatures and pressures) that occur inside the bubbles during cavitation collapse at the micro-level [60] are responsible for the observed degradation of aroma compounds. Furthermore, the sonolysis of water as a consequence of cavitation, induces the formation of hydroxyl radicals that can be involved in the degradation,

esterification and ring opening and formation of chalcones [61]. Also, the formation of hydroxyl ions (OH^-) increases linearly with the increase of ultrasound amplitude [62].

Finally, the obtained results demonstrated that the choice of proper ultrasound conditions in both HPU experiments is crucial, in order to avoid the occurrence of excessive oxidation and degradation of phenolic compounds and the compounds responsible for wine aroma, and to maintain the overall wine quality and color.

3.2. ANN modeling of HPU processes

In the present study, ANN models were developed in order to test whether it is possible to predict the content of TP, TA, TT, total free anthocyanins, total flavan-3-ols, total esters, total higher alcohols, total fatty acids, total terpenes, and chromatic characteristics (L^*, a^*, b^*, C^* and H^*) based on HPU process parameters of experiment 1 (ultrasonic bath) and experiment 2 (ultrasonic probe). The data generated from the experimental designs of HPU experiments (Tables S1and S2) were used to figure out the optimal ANNs. Firstly, the total experimental set of each HPU experiment was randomly divided into seven sets for the training, validation and testing of the neural networks. Based on the results of the training process, the separation of data into training, test and validation set as 60:20:20 ratios showed to be the most suitable for both HPU experiments. Among the various structures, models of good performance were developed for both experiments 1 and 2 and their performance parameters are presented in Table 3.

Regarding HPU experiment 1 (ultrasonic bath), nearly all of the selected networks had higher linear correlation coefficient (R^2) for training, test and validation with lower Root mean square error (RMSE) values (Table 3). As can be seen, there are three different ANN regarding the number of neurons in hidden layer (8, 9 and 10) since all of them have 4 neurons in input layer and 14 neurons in output layer. Moreover, the hidden activation and the output activation of the ANNs with the same numbers of neurons in hidden layer were

different. When observing the correlation coefficient for training, for all the five networks, the highest values was observed for ANN 1 ($R^2 = 0.8402$) with the lowest training error (RMSE = 0.0907). The ANN 2 had the highest value for test performance ($R^2 = 0.8137$) with the lowest training error (RMSE = 0.1201). For the validation performance ANN 3 showed the highest performance ($R^2 = 0.7997$) which was slightly higher than ANN 1 ($R^2 = 0.7921$) but in term of validation error ANN 3 showed higher values (RMSE = 0.1310) than ANN 1 (RMSE = 0.1243). Based on these results, ANN 1 was selected as the optimal one for HPU experiment 1 (Table 3).

The results of HPU experiment 2 (ultrasonic probe) demonstrated that almost all of the developed networks had lower linear correlation coefficient (R^2) for training, test and validation with higher RMSE values (Table 3). As indicated in the table, there are four different ANN considering the number of neurons in hidden layer (6, 8, 9 and 10) since all of them have 3 neurons in input layer and 14 neurons in output layer. The hidden activation and the output activation of the ANNs with the same number of neurons in hidden layer were different. Furthermore, the highest value of correlation coefficient for training was observed for ANN 1 ($R^2 = 0.7878$), which also had the lowest training error (RMSE = 0.1354). Also for training performance, ANN 1 had the highest training performance ($R^2 = 0.7607$) as well as the highest validation performance ($R^2 = 0.7771$) with the lowest training and validation errors (RMSE = 0.2346 and RMSE = 0.1126, respectively). Based on these results, ANN 1 was selected as the optimal one for HPU experiment 2 (Table 3).

The performance of the final selected ANN models (4/10/14 and 3/8/14) to predict each of the output variables (TP, TA, TT, total free anthocyanins, total flavan-3-ols, chromatic characteristics, total esters, total higher alcohols, total fatty acids and total terpenes) in experiments 1 and 2 is presented in Table 4. Also, in order to get a clearer picture for each of

the tested parameter in terms of ANN predictions, the results of both HPU experiments are presented as correlation of experimental and model predicted data in Figs. 1 and 2.

Based on the results presented in Table 4, the best correlations between experimental data and the ANN predictions in experiment 1 (ultrasonic bath) for training, test and validation were obtained for chromatic characteristic L^* ($R^2 = 0.9725$, $R^2 = 0.9333$, $R^2 = 0.9852$), followed by C^* ($R^2 = 0.9702$, $R^2 = 0.9143$, $R^2 = 0.9870$), and a^* and b^* which had negligible differences in values. Such good correlations are visible in Figs. 1f, 1g, 1h and 1i. Moreover, it is observed that the correlation coefficients for validation between the measured and predicted data for TP, TA, TT, total free anthocyanins, H^* and total esters were also satisfactory ($0.7773 \leq R^2 \leq 0.8565$) (Table 4). Meanwhile, the least acceptable results of the ANN performance belonged to total flavan-3-ols, total higher alcohols, total fatty acids and total terpenes (Figs. 1e, 1l, 1m and 1n).

Further, regarding HPU experiment 2 (ultrasonic probe), the best correlations between experimental data and the ANN predictions for training, test and validation were again obtained for chromatic characteristic L* with R^2 values of 0.9564, 0.9663 and 0.9882 for training, test and validation (Table 4). The second highest value for validation was observed for chromatic characteristic a^*, followed by b^* and C* values. Moreover, the values of correlation coefficients for validation for TP ($R^2 = 0.9263$) and TA ($R^2 = 0.9580$) were much higher than in the first experiment. Also, the correlation coefficients for validation between the measured and predicted data for TT, total flavan-3-ols, total free anthocyanins and H^* value were satisfactory ($0.8526 \leq R^2 \leq 0.8770$) (Table 4). On the other hand, the least acceptable results (the highest data dispersion) of the ANN performance belonged to total esters, total fatty acids and total terpenes with total higher alcohols at the last place (Figs. 2k, 2m, 2n and 2l).

In general, a good-fitting model should have the R^2 values above 0.90, while the values between 0.70 and 0.90 show that the models can be considered moderately precise. On the other hand, the R^2 values below 0.70 imply that the model can be used for qualitative differentiation without the ability to be used in quantitative prediction [63,64]. As a result, for the ultrasonic bath experiment, the selected ANN 1 model showed the best prediction for monitoring chromatic characteristics (except H*) and also very good prediction for certain parameters such as TP, TA, TT, total free anthocyanins and total esters, while total higher alcohols, total fatty acids and total terpenes did not give satisfactory predictions. For the ultrasonic probe experiment, the ANN 1 showed that chromatic characteristics, TP and TA could be easily predicted but, in the same way than in the first experiment, total higher alcohols, total fatty acids, and total terpenes with addition of total esters had the least acceptable results.

3.3. Effect of HPU treatment along with SO_2 and GSH additions on the phenolic, chromatic and aroma composition of red wine during storage

The effect of HPU treatment along with antioxidants addition (SO_2 and GSH) on the phenolic, chromatic and aroma composition of red wine during 6 months of storage in the bottles is shown in Table 5. Although the analyzed parameters were influenced by the content and type of antioxidants used, a general trend for all wines can be observed. As it can be seen, there is a decreasing trend in the content of TP, TA, total free anthocyanins and total flavan-3-ols with time. After 3 and 6 months of aging, significant differences ($p < 0.05$) were observed among the different treatments indicating that HPU treatment affected both total and individual phenolic compounds, except TT content which remained constant during observed period of storage. Specifically, after 6 months of storage the sonicated samples showed significantly lower content of phenolic compounds when compared with the untreated wine. It is already known that the content of phenolic compounds decrease during storage due to their

potential chemical oxidation, polymerization, condensation and/or precipitation [65]. This tendency was significantly enhanced when ultrasound is applied to wines probably due to specific chemical reactions among phenolic compounds that take place during sonication. Moreover, the lowest concentrations of analyzed phenolic compounds were found in wine with lower content of SO_2. As already well-known the addition of SO_2 in winemaking is essential, in the first place, for preventing microbial spoilage, but also for the management of oxidative aging of wine. This antioxidant removes hydrogen peroxide formed by the oxidation of phenolic compounds and reacts with quinones, reducing them to the catechols, thereby increasing the oxygen consumption rate in wine [66]. Additionally, GSH also influenced, though modest, chemical composition of wine, resulting in slightly higher content of most phenolic compounds (except TT) at the beginning of storage as well as after 6 months compared to wine with lower content of SO_2 aged without GSH (Table 5). This is probably due to the fact that the reduced glutathione has the ability to protect the easily oxidized compounds such as phenolics by reducing oxygen consumption rate [18].

Regarding chromatic characteristics, there is an increasing trend in parameters L^*, a^*, b^*, C^* and H^* of the presented wine samples along storage, changing into more orange and clear color, respectively. At the beginning of storage and after 3 months, significant differences can be observed in parameters L^*, a^*, b^* and C^* among the different treatments of the wine samples. However, after 6 months of storage there were no significant differences in a^*, b^*, C^* and H^* values, except in lightness. Furthermore, sonicated samples were characterized by slightly lower values of chromatic characteristics compared to control wine, indicating that HPU treatment did not affect significantly most of the chromatic characteristics, except lightness. On the other hand, the wines with higher content of antioxidants (sulfur dioxide and glutathione) showed higher values of chromatic characteristics. This could be probably due to the fact that the content of sulfur dioxide is able

to strongly affect the color of red wine by its bleaching effect on the free anthocyanins [67]. Earlier studies showed that the addition of glutathione appeared to have an improving effect on the wine aroma [68], as well as the impact on wine color by increasing chromatic characteristics during aging [69]. After the calculation of the total color difference (ΔE*) between treated and control samples, it can be observed that ΔE* values for the sample with standard SO_2 as well as for the sample with lower content of SO_2 and GSH after 6 months of storage were in the range of 1-4, which means that the color differences in these cases were slightly perceptible. Only treated sample with lower content of SO_2 showed ΔE* value higher than 10, which means there was remarkable color difference compared to the control sample. These observations showed that the total color differences between treated and control samples during aging were primarily influenced by the content of antioxidants (SO_2 and GSH) in wine (Table 5). Regarding aroma composition, a slight decrease in total esters, total fatty acids and total terpenes was observed for all the wines along storage, whereas the content of total higher alcohols slightly increased, independently of treatment applied. In general, during wine aging, the decrease of most aroma compounds can be observed due to various chemical and biochemical reactions such as hydrolysis or oxidation. A well-known is loss of fresh and fruity character of a wine during aging as a consequence of decrease of esters [70]. Furthermore, higher alcohols were generally stable during aging, however their increase could be a result of hydrolysis of esters [71] or oxidation of fatty acids [72]. However, uniform trend was not observed in content of volatile fatty acids during aging, as some compounds can increase while others can decrease or remain stable [73].

There is still lack of information in the scientific literature about the effect of ultrasound on the aroma composition of wine, especially on important aroma groups such as higher alcohols, fatty acids and terpenes. As it can be seen from Table 5, no significant differences among the different treatments of the wine samples were not observed, indicating

that HPU treatment did not affect total esters, total higher alcohols and total terpenes of the wines immediately after the HPU treatment as well as through the whole period of storage. However, after 6 months of storage the sonicated samples presented lower concentrations of total fatty acids when compared with untreated wine, indicating that HPU treatment influenced this group of aroma compounds. Aside that, the effect of antioxidants addition (SO_2 and GSH) was not noticeable on the content of total esters, total higher alcohols and total terpenes, while higher concentration of antioxidants (SO_2 and GSH) resulted in wines with higher content of total fatty acids. In addition, it was reported that GSH in the combination of lower content of SO_2 could slow down oxidation rate of aroma compounds such as volatile thiols, monoterpenes and esters [18,74,75].

4. Conclusions

The ultrasonic bath and probe treatments influenced the chemical composition of young red wine Cabernet Sauvignon. In both cases, the mild ultrasound conditions (lower frequency, amplitude, temperature, treatment duration, and proper probe diameter size) showed in general a more favorable and lighter impact on the phenolic, color and aroma composition of the treated red wine, while on the contrary, higher process conditions resulted in a decrease of aforementioned tested parameters. Respectively, among the four different parameters of ultrasonic bath experiment, the frequency (37-80 kHz) proved to be the most important one influencing chemical composition of red wine, followed by bath temperature (20-60 °C) and amplitude (40-100%). Regarding ultrasonic probe experiment, statistical analysis suggested that the selection of the probe diameter (12.7-25.4 mm) was the most significant parameter affecting red wine chemical composition, followed by treatment duration (3-9 min) and amplitude (25-100%). Moreover, their interaction effects also contributed significantly to a large part of the total variation in the whole data set. When considering ANN prediction for all the 14 parameters in both HPU experiments, the chromatic characteristics had the highest

correlation of experimental and predicted data. For the second HPU experiment (ultrasonic probe) TP and TA showed very good correlation, while in both cases total higher alcohols, total fatty acids, total terpenes and total esters did not have good prediction. HPU treatment influenced the phenolic composition of wine after 6 months of storage in the bottles. Particularly, the lower content of total phenolics, total anthocyanins, total free anthocyanins and total flavan-3-ols was observed in sonicated samples. However, HPU treatment did not affect the content of total tannins. Also, the addition of higher concentration of antioxidants (SO_2 and glutathione) delayed the loss of aforementioned phenolic compounds during aging. Moreover, identical trends noticed for phenolics were observed in lightness (L^*) as well as the content of total fatty acids. On the other hand, HPU treatment after 6 months of aging did not influence the chromatic parameters a^*, b^*, C^* and H^*, as well as the content of total esters, total higher alcohols and total terpenes regardless of the antioxidants addition in wine, since no significant differences among sonicated samples were observed. This shows that HPU can be applied with lower content of SO_2 without causing changes in the aforementioned chromatic and aroma characteristics. Our results indicated that proper HPU treatment might slightly accelerate chemical reactions which naturally occur during aging of red wine.

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Figure captions

Fig. 1. Comparison between experimental data and Artificial Neural Network models predicted data for High Power Ultrasound experiment 1 (ultrasonic bath) for (a) total phenolics – TP, (b) total anthocyanins – TA, (c) total tannins – (TT), (d) total free anthocyanins, (e) total flavan-3-ols, (f) L^*, (g) a^*, (h) b^*, (i) C^*, (j) H^*, (k) total esters, (l) total higher alcohols, (m) total fatty acids, and (n) total terpenes.

Fig. 2. Comparison between experimental data and Artificial Neural Network models predicted data for High Power Ultrasound experiment 2 (ultrasonic probe) for (a) total phenolics – TP, (b) total anthocyanins – TA, (c) total tannins – (TT), (d) total free anthocyanins, (e) total flavan-3-ols, (f) L^*, (g) a^*, (h) b^*, (i) C^*, (j) H^*, (k) total esters, (l) total higher alcohols, (m) total fatty acids, and (n) total terpenes.

Tables

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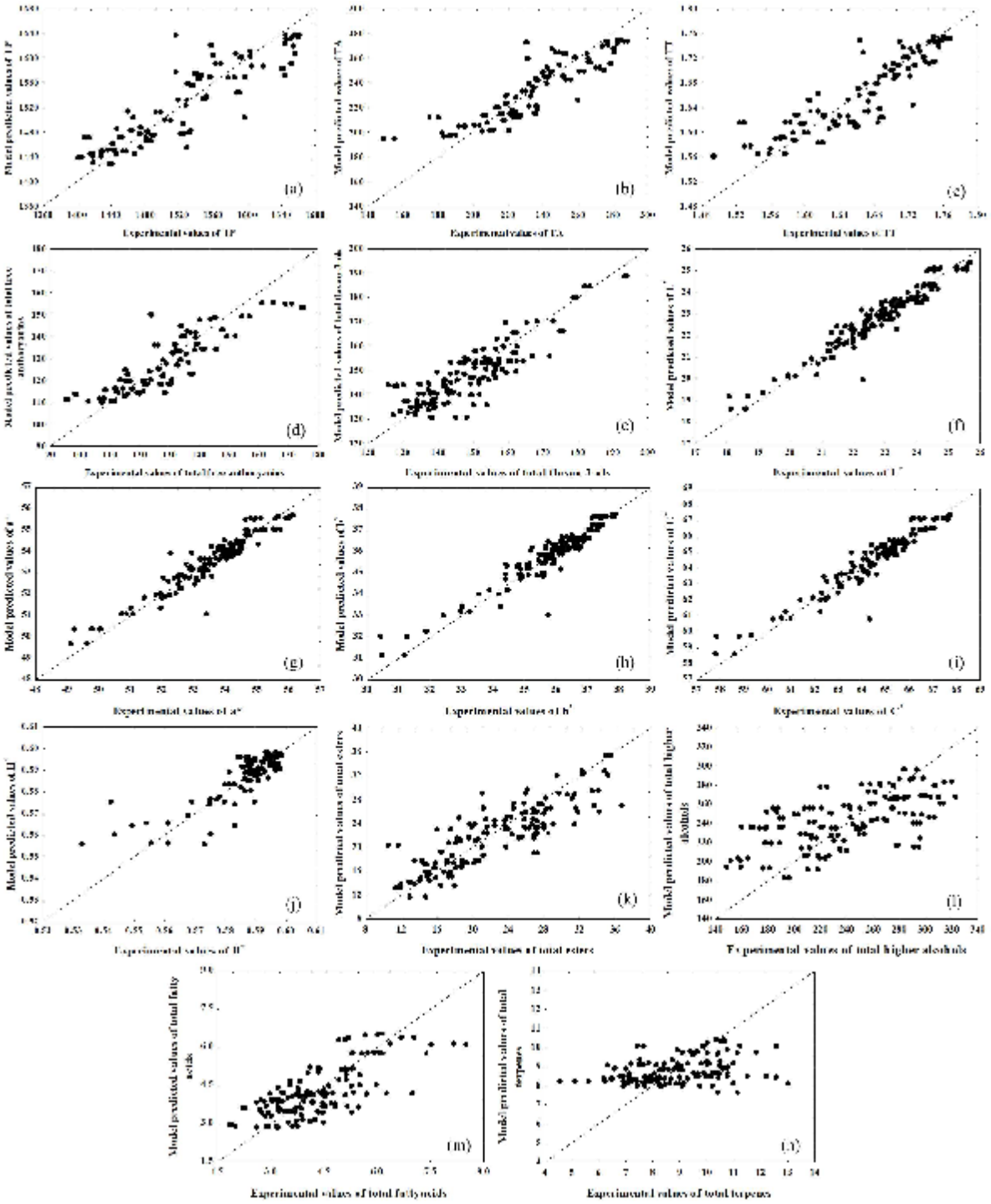
Table 5. Effect of High Power Ultrasound treatment (ultrasonic probe) along with sulfur dioxide (SO_2) and glutathione (GSH) additions on the phenolic, chromatic and aroma composition of red wine during 6 months of bottle aging


(a)
Experimental values of TP
(b)
Experimental values of TA
(c)
Experimental values of TT
(d)
Experimental values of total fatty acid esters
(e)
Experimental values of total flavour 3 ols
(f)
Experimental values of L*
(g)
Experimental values of a*
(h)
Experimental values of b*
(i)
Experimental values of C*
(j)
Experimental values of H°
(k)
Experimental values of total esters
(l)
Experimental values of total higher alcohols
(m)
Experimental values of total fatty acids
(n)
Experimental values of total terpenes

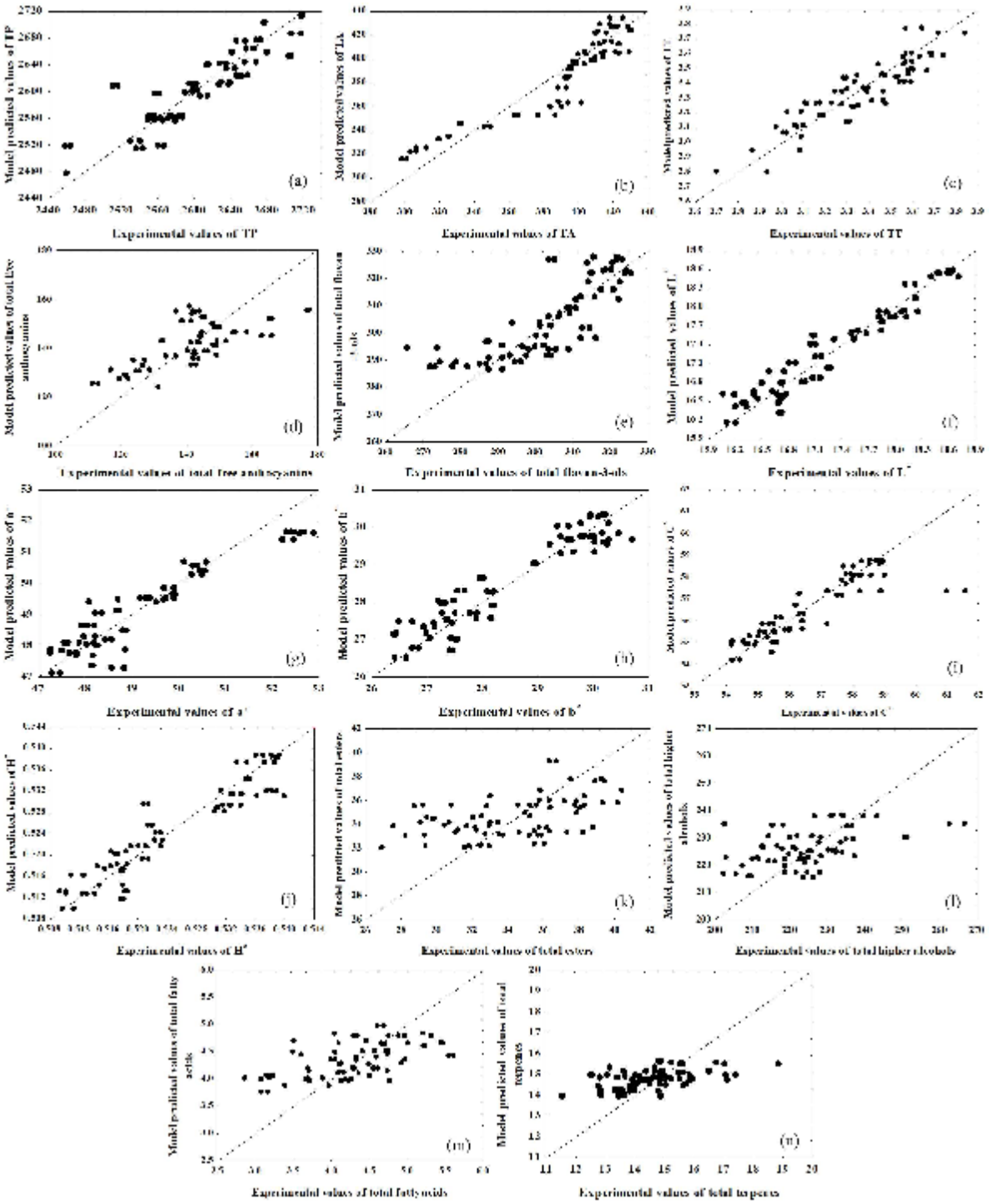

Model predicted values of TP
Experimental values of TP
(a)
Model predicted values of EA
Experimental values of EA
(b)
Model predicted values of TT
Experimental values of TT
(c)
Model predicted values of total free anthocyanins
Experimental values of total free anthocyanins
(d)
Model predicted values of total flavan-3-ols
Experimental values of total flavan-3-ols
(e)
Model predicted values of T*
Experimental values of T*
(f)
Model predicted values of a*
Experimental values of a*
(g)
Model predicted values of b*
Experimental values of b*
(h)
Model predicted values of C*
Experimental values of C*
(i)
Model predicted values of H*
Experimental values of H*
(j)
Model predicted values of total esters
Experimental values of total esters
(k)
Model predicted values of total higher alcohols
Experimental values of total higher alcohols
(l)
Model predicted values of total fatty acids
Experimental values of total fatty acids
(m)
Model predicted values of total terpenes
Experimental values of total terpenes
(n)

Table 1. Experimental design used in the two High Power Ultrasound experiments

	Independent variables (inputs)			
	Amplitude (%)	Frequency (kHz)	Bath temperature (°C)	Treatment duration (min)
Experiment 1	40	37	20	20
Ultrasonic bath	60	80	40	50
	100		60	65
				90

	Probe diameter (mm)	Amplitude (%)	Treatment duration (min)
Experiment 2	12.7	25	3
Ultrasonic probe	19.1	50	6
	25.4	75	9
		100	

*Sum of individual compounds: total free anthocyanins [delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, peonidin-3-*O*-acetylglucoside, malvidin-3-*O*-acetylglucoside, peonidin-3-(6-*O*-*p*-coumaroyl)glucoside and malvidin-3-(6-*O*-*p*-coumaroyl)glucoside)], total flavan-3-ols [(+)-catechin, (-)-epicatechin, dimers B1, B2, B3, B4 and trimer C1], total esters (ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, diethyl succinate, ethyl acetate, *i*-butyl acetate, *i*-amyl acetate, hexyl acetate and 2-phenylethyl acetate), total higher alcohols (amyl alcohol, phenylethyl alcohol, 1-hexanol and *cis*-3-hexenol), total fatty acids (hexanoic acid, octanoic acid and decanoic acid), total terpenes (linalool and α-terpineol).

Table 2. Analysis of variance (*F* values) for High Power Ultrasound experiments 1 and 2

Experiment 1 Ultrasonic bath														
Source	TP	TA	TT	Total free anthocyanins	Total flavan-3-ols	L*	a*	b*	C*	H*	Total esters	Total higher alcohols	Total fatty acids	Total terpenes
Amplitude (X_1)	11466.33[a]	6997.72[a]	8985.55[a]	4163.65[a]	**689.32**[a]	295.11[a]	164.24[a]	197.36[a]	177.74[a]	**60.29**[a]	30.34[a]	**831.52**[a]	4.58[d]	**14.99**[a]
Frequency (X_2)	**30376.99**[a]	**17444.76**[a]	**19404.84**[a]	**18894.49**[a]	29.31[a]	251.74[a]	250.29[a]	167.99[a]	238.86[a]	0.48	0.59	97.01[a]	5.80[d]	0.30
Bath temperature (X_3)	9477.43[a]	5334.66[a]	3929.93[a]	6334.38[a]	106.56[a]	**485.22**[a]	**352.06**[a]	**242.64**[a]	**337.31**[a]	9.00[b]	**603.55**[a]	530.76[a]	**139.84**[a]	3.00
Treatment duration (X_4)	170.95[a]	273.25[a]	220.38[a]	289.33[a]	12.11[a]	138.84[a]	114.82[a]	74.80[a]	94.78[a]	3.65[d]	179.46[a]	497.95[a]	21.42[a]	1.86
X_1X_2	369.91[a]	676.76[a]	492.10[a]	1137.94[a]	53.58[a]	37.64[a]	17.38[a]	24.75[a]	16.21[a]	**22.00**[a]	32.08[a]	46.65[a]	10.75[b]	3.99[d]
X_1X_3	1580.41[a]	972.85[a]	1724.44[a]	1686.47[a]	82.36[a]	**37.97**[a]	**61.44**[a]	**56.72**[a]	**66.79**[a]	8.99[a]	15.22[a]	71.22[a]	7.67[a]	12.74[a]
X_2X_3	**10412.22**[a]	**6952.71**[a]	**5790.01**[a]	**6946.66**[a]	**143.58**[a]	6.49[c]	6.28[c]	7.71[b]	3.17[d]	17.57[a]	41.86[a]	110.05[a]	**47.54**[a]	10.67[b]
X_1X_3	1132.48[a]	988.60[a]	1401.35[a]	1085.46[a]	18.08[a]	6.56[a]	5.69[b]	8.01[a]	5.14[b]	1.44	12.63[a]	136.07[a]	13.32[a]	4.02[c]
X_2X_4	2867.60[a]	1426.89[a]	2125.43[a]	1164.82[a]	9.62[a]	1.44	1.60	1.67	1.44	0.36	**67.03**[a]	94.27[a]	13.92[a]	11.44[a]
X_3X_4	684.81[a]	543.01[a]	861.30[a]	642.37[a]	12.45[a]	28.39[a]	24.76[a]	24.08[a]	27.77[a]	3.16[c]	27.47[a]	**383.58**[a]	27.86[a]	5.08[b]
$X_1X_2X_3$	1917.46[a]	1177.55[a]	1045.64[a]	1640.55[a]	70.54[a]	24.17[a]	31.81[a]	18.82[a]	26.33[a]	12.34[a]	19.61[a]	305.29[a]	6.20[b]	**13.82**[a]
$X_1X_2X_4$	596.44[a]	415.44[a]	270.40[a]	211.76[a]	10.40[a]	3.94[c]	3.61[c]	3.10[c]	2.56[d]	0.43	7.64[a]	313.40[a]	9.99[a]	7.42[a]
$X_1X_3X_4$	1112.54[a]	1059.93[a]	1096.39[a]	878.14[a]	17.61[a]	1.84	1.66	1.97[d]	1.42	0.95	6.44[a]	201.85[a]	6.92[a]	12.23[a]
$X_2X_3X_4$	1070.77[a]	712.54[a]	1168.78[a]	448.43[a]	5.72[b]	3.16[c]	2.43[d]	2.27[d]	1.51	0.28	4.00[c]	222.80[a]	11.20[a]	1.44
$X_1X_2X_3X_4$	841.10[a]	519.76[a]	599.79[a]	506.28[a]	6.94[a]	1.40	1.31	1.26	1.04	0.21	31.70[a]	225.02[a]	6.84[a]	8.43[a]
Experiment 2 Ultrasonic probe														
Source	TP	TA	TT	Total free anthocyanins	Total flavan-3-ols	L*	a*	b*	C*	H*	Total esters	Total higher alcohols	Total fatty acids	Total terpenes
Probe diameter (X_1)	1272.89[a]	**3150.84**[a]	51.19[a]	349.09[a]	3.41[d]	**1228.68**[a]	**403.61**[a]	**697.85**[a]	**432.09**[a]	**816.01**[a]	**15.96**[a]	**56.44**[a]	10.54[b]	**12.67**[b]
Amplitude (X_2)	**1409.70**[a]	215.05[a]	**98.52**[a]	46.45[a]	8.01[b]	8.58[b]	4.20[d]	11.78[a]	11.42[a]	8.50[b]	5.29[c]	49.99[a]	0.56	0.14
Treatment duration (X_3)	325.68[a]	561.04[a]	75.54[a]	**472.84**[a]	**330.18**[a]	255.85[a]	293.39[a]	170.03[a]	79.55[a]	91.92[a]	6.04[c]	32.97[a]	**11.42**[b]	1.98
X_1X_2	26.10[a]	11.05[a]	1.82	21.75[a]	12.14[a]	9.10[a]	1.65	4.63[c]	15.86[a]	14.71[a]	6.59[b]	15.94[a]	1.10	1.89
X_1X_3	**167.95**[a]	**276.18**[a]	**21.43**[a]	**191.18**[a]	13.78[a]	**314.89**[a]	**219.70**[a]	**255.94**[a]	**135.10**[a]	**314.52**[a]	4.93[c]	6.36[b]	2.89[d]	**9.22**[a]
X_2X_3	35.97[a]	5.14[b]	0.43	57.29[a]	**58.09**[a]	12.13[a]	4.39[c]	4.75[c]	15.58[a]	8.05[a]	**9.19**[a]	**31.67**[a]	**3.64**[c]	3.21[d]
$X_1X_2X_3$	35.20[a]	11.46[a]	1.17	45.58[a]	5.45[a]	5.47[a]	1.49	2.65[d]	11.03[a]	7.50[a]	3.47[c]	22.52[a]	2.75[c]	6.10[a]

[a]$p < 0.0001$, [b]$p < 0.001$, [c]$p < 0.01$, [d]$p < 0.05$. The most significant effect (higher *F* values) of process (input) variables and their interactions on each output variable are shown in bold. Error terms for experiments 1 and 2 are df=143 and df=71. Abbreviations: TP, total phenolics; TA, total anthocyanins; TT, total tannins.

Table 3. Performance parameters of Artificial Neural Network (ANN) models of High Power Ultrasound experiments 1 and 2

	Experiment 1 Ultrasonic bath					Experiment 2 Ultrasonic probe				
Network number	**1**	2	3	4	5	**1**	2	3	4	5
Network name[a]	**MLP 4/10/14[b]**	MLP 4/9/14	MLP 4/9/14	MLP 4/8/14	MLP 4/10/14	**MLP 3/8/14[b]**	MLP 3/9/14	MLP 3/6/14	MLP 3/10/14	MLP 3/6/14
Training performance	**0.8402**	0.8148	0.8010	0.7981	0.8042	**0.7878**	0.7715	0.7680	0.7662	0.7183
Training error	**0.0907**	0.1062	0.1147	0.1133	0.1271	**0.1354**	0.1439	0.1391	0.1578	0.1596
Test performance	**0.7919**	0.8137	0.7693	0.7800	0.7902	**0.7607**	0.7302	0.7367	0.7475	0.7330
Test error	**0.1282**	0.1201	0.1402	0.1298	0.1790	**0.2346**	0.2502	0.2410	0.2449	0.2307
Validation performance	**0.7921**	0.7452	0.7997	0.7916	0.7945	**0.7771**	0.7601	0.7343	0.6988	0.7482
Validation error	**0.1243**	0.1525	0.1310	0.1404	0.1782	**0.1126**	0.1417	0.1586	0.1793	0.1511
Hidden activation	**Tanh**	Logistic	Logistic	Tanh	Logistic	**Logistic**	Tanh	Tanh	Tanh	Tanh
Output activation	**Logistic**	Logistic	Tanh	Logistic	Logistic	**Identity**	Identity	Tanh	Exponential	Logistic

[a] Number of input variables/number of neurons in hidden layer/number of output variables. [b] The most suitable ANN is marked bold. Abbreviations: MLP, multilayer perceptron.

Table 4. Performance of the final selected Artificial Neural Network (ANN) model to predict each of the dependent variables (outputs) of High Power Ultrasound experiments 1 and 2

	Experiment 1 Ultrasonic bath			Experiment 2 Ultrasonic probe		
	Correlation coefficient (R^2)			Correlation coefficient (R^2)		
Output variables	Training	Testing	Validation	Training	Testing	Validation
Total phenolics	0.8860	0.8496	0.8565	0.9123	0.8093	0.9263
Total anthocyanins	0.8765	0.8279	0.8525	0.9375	0.9035	0.9580
Total tannins	0.8897	0.8773	0.8387	0.9262	0.8603	0.8576
Total free anthocyanins	0.8608	0.8754	0.8295	0.5959	0.7364	0.8770
Total flavan-3-ols	0.8905	0.8193	0.6899	0.8180	0.7464	0.8526
L^*	0.9725	0.9333	0.9852	0.9564	0.9663	0.9882
a^*	0.9608	0.9093	0.9879	0.9094	0.9529	0.9881
b^*	0.9656	0.9069	0.9885	0.9353	0.9607	0.9554
C^*	0.9702	0.9143	0.9870	0.9258	0.7975	0.9496
H^*	0.8755	0.8215	0.7773	0.9379	0.9717	0.8553
Total esters	0.8482	0.8519	0.8090	0.5359	0.3888	0.3234
Total higher alcohols	0.6297	0.5395	0.4765	0.5792	0.3480	0.0188
Total fatty acids	0.7559	0.6816	0.6253	0.6205	0.6550	0.6579
Total terpenes	0.3807	0.2785	0.3854	0.4394	0.5513	0.6716

Table 5. Effect of High Power Ultrasound treatment (ultrasonic probe) along with sulfur dioxide (SO_2) and glutathione (GSH) additions on the phenolic, chromatic and aroma composition of red wine during 6 months of bottle aging

Months		Wine			
		Control (untreated)	Standard SO_2	Low SO_2 and GSH	Low SO_2
TP (mg/L)	0	2960.42 ± 5.30^{a}	2940.00 ± 8.25^{a}	2790.49 ± 1.87^{b}	2688.75 ± 8.84^{c}
	3	2891.82 ± 9.00^{a}	2841.36 ± 7.07^{b}	2732.73 ± 12.86^{c}	2634.09 ± 14.78^{d}
	6	2820.00 ± 7.71^{a}	2719.55 ± 3.21^{b}	2662.27 ± 9.64^{c}	2544.55 ± 10.29^{d}
TA (mg/L)	0	261.80 ± 4.70^{a}	253.18 ± 2.04^{ab}	243.21 ± 1.55^{b}	217.31 ± 1.42^{c}
	3	259.09 ± 1.11^{a}	245.74 ± 1.61^{b}	237.04 ± 0.37^{b}	184.98 ± 4.21^{c}
	6	243.56 ± 1.20^{a}	233.78 ± 3.02^{b}	186.34 ± 0.11^{c}	153.74 ± 2.97^{d}
TT (g/L)	0	4.49 ± 0.04^{a}	4.46 ± 0.04^{a}	4.40 ± 0.03^{a}	4.33 ± 0.09^{a}
	3	4.45 ± 0.13^{a}	4.35 ± 0.06^{a}	4.24 ± 0.01^{a}	4.25 ± 0.06^{a}
	6	3.75 ± 0.02^{a}	3.69 ± 0.12^{a}	3.65 ± 0.09^{a}	3.57 ± 0.04^{a}
Total free anthocyanins (mg/L)	0	156.55 ± 1.59^{a}	151.89 ± 0.95^{a}	139.28 ± 0.03^{b}	125.14 ± 2.87^{c}
	3	140.88 ± 2.72^{a}	129.71 ± 0.44^{b}	118.63 ± 0.82^{c}	103.93 ± 0.65^{d}
	6	132.61 ± 1.86^{a}	112.83 ± 1.24^{b}	102.64 ± 0.73^{c}	92.53 ± 0.93^{d}
Total flavan-3-ols (mg/L)	0	441.46 ± 3.25^{a}	427.75 ± 0.91^{b}	408.00 ± 3.60^{c}	400.08 ± 0.56^{c}
	3	439.19 ± 1.17^{a}	417.88 ± 2.86^{b}	393.44 ± 2.08^{c}	379.20 ± 1.66^{d}
	6	427.83 ± 3.05^{a}	404.97 ± 0.31^{b}	378.34 ± 3.78^{c}	362.66 ± 3.23^{d}
L^{*}	0	22.28 ± 0.03^{a}	20.93 ± 0.03^{b}	18.84 ± 0.05^{c}	17.30 ± 0.22^{d}
	3	25.56 ± 0.13^{a}	23.81 ± 0.07^{b}	20.99 ± 0.07^{c}	17.45 ± 0.06^{d}
	6	26.90 ± 0.03^{a}	25.48 ± 0.03^{b}	23.95 ± 0.00^{c}	20.39 ± 0.14^{d}
a^{*}	0	52.13 ± 0.06^{a}	51.09 ± 0.07^{b}	46.23 ± 0.08^{c}	47.58 ± 0.30^{d}
	3	54.68 ± 0.16^{a}	53.59 ± 0.10^{b}	50.91 ± 0.10^{c}	48.27 ± 0.07^{d}
	6	54.12 ± 0.03^{a}	53.35 ± 0.06^{a}	53.88 ± 0.03^{a}	50.43 ± 0.39^{b}
b^{*}	0	36.55 ± 0.06^{a}	34.86 ± 0.07^{b}	31.35 ± 0.09^{c}	29.44 ± 0.37^{d}
	3	41.03 ± 0.18^{a}	39.02 ± 0.12^{b}	34.44 ± 0.16^{c}	29.74 ± 0.10^{d}
	6	40.67 ± 0.03^{a}	39.53 ± 0.11^{b}	39.38 ± 0.02^{b}	30.53 ± 0.18^{c}
C^{*}	0	63.67 ± 0.08^{a}	61.85 ± 0.09^{b}	55.86 ± 0.12^{c}	55.95 ± 0.45^{c}
	3	68.36 ± 0.23^{a}	66.29 ± 0.15^{b}	61.47 ± 0.17^{c}	56.69 ± 0.11^{d}
	6	67.70 ± 0.04^{a}	66.40 ± 0.11^{b}	66.73 ± 0.04^{b}	57.46 ± 0.36^{c}
H^{*}	0	0.61 ± 0.00^{a}	0.60 ± 0.00^{a}	0.54 ± 0.00^{b}	0.55 ± 0.00^{b}
	3	0.64 ± 0.00^{a}	0.63 ± 0.00^{a}	0.59 ± 0.00^{b}	0.55 ± 0.00^{c}
	6	0.64 ±0.00^{a}	0.64 ± 0.00^{a}	0.63 ± 0.00^{a}	0.54 ± 0.00^{b}
ΔE*	0	-	2.41 ± 0.18^{b}	8.59 ± 0.22^{a}	9.80 ± 0.43^{a}
	3	-	2.87 ± 0.43^{c}	8.86 ± 0.14^{b}	15.30 ± 0.13^{a}
	6	-	1.98 ± 0.05^{c}	3.23 ± 0.03^{b}	12.61 ± 0.06^{a}
Total esters (mg/L)	0	61.31 ± 8.19^{a}	56.39 ± 7.34^{a}	51.42 ± 1.35^{a}	44.31 ± 0.21^{a}
	3	45.31 ± 1.22^{a}	42.96 ± 0.55^{ab}	41.78 ± 0.16^{b}	36.88 ± 0.27^{c}
	6	34.81 ± 2.53^{a}	31.73 ± 1.80^{a}	31.61 ± 1.11^{a}	30.08 ± 0.55^{a}
Total higher alcohols (mg/L)	0	95.57 ± 1.82^{a}	93.61 ± 2.07^{ab}	88.67 ± 0.85^{ab}	88.20 ± 1.89^{b}
	3	103.96 ± 1.30^{a}	103.69 ± 5.15^{a}	98.83 ± 1.21^{a}	93.59 ± 1.50^{a}
	6	105.50 ± 6.30^{a}	104.10 ± 0.48^{a}	102.63 ± 1.24^{a}	100.38 ± 1.40^{a}
Total fatty acids (mg/L)	0	2.57 ± 0.05^{a}	2.45 ± 0.01^{a}	2.25 ± 0.01^{b}	2.17 ± 0.02^{b}
	3	2.36 ± 0.04^{a}	2.03 ± 0.03^{b}	1.93 ± 0.04^{b}	1.77 ± 0.03^{c}
	6	1.92 ± 0.02^{a}	1.59 ± 0.00^{b}	1.50 ± 0.01^{c}	1.40 ± 0.03^{d}
Total terpenes (μg/L)	0	17.66 ± 0.26^{a}	16.02 ± 0.57^{b}	14.81 ± 0.06^{bc}	13.87 ± 0.03^{c}
	3	13.30 ± 0.12^{a}	13.23 ± 0.35^{a}	12.45 ± 0.76^{a}	11.01 ± 0.91^{a}
	6	9.89 ± 1.26^{a}	8.40 ± 0.17^{a}	7.93 ± 0.18^{a}	7.38 ± 0.03^{a}

Data presented as average value of six analytical repetitions with standard deviation. ANOVA to compare data; different letters indicate statistical differences between wine samples of all treatments at the same time (Tukey's test, $p < 0.05$). Abbreviations: TP, total phenolics; TA, total anthocyanins; TT, total tannins.

Highlights

- Ultrasound (USN) bath and probe influenced chemical composition of young red wine
- Mild USN conditions affected less phenolic, color and aroma
- USN bath (37-80 kHz/20-60 °C/40-100%) with highest impact on chemical composition
- USN probe (12.7-25.4 mm/3-9 min/25-100%) had the most important effect
- High power USN influenced wine phenolic composition after 6 mo storage in bottles