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INFLUENCE OF HIGH-POWER ULTRASOUND TREATMENT ON RED WINE QUALITY PARAMETERS

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Abstract

The objective of this study was to assess the impact of applying high-power ultrasonic treatment (HPU) on crushed Merlot grapes, at a laboratory scale, on the phenolic matrix of red wines, particularly anthocyanins, which are crucial for color, stability, and sensory profile. The ultrasonic treatment (US) was carried out using two amplitudes, 70% and 90%, and three treatment times, namely, 3, 4, and 5 minutes, while maceration was conducted via sequential extraction after 3, 5, and 7 days. After a bottling period of three months, there was a decrease in total polyphenol content observed compared to the content found at the end of maceration. Treatment with ultrasound caused significant variations in the optical density at 420, 520, and 620 nm, and in the content of monomeric anthocyanins. All the sonicated samples, including those extracted after three days of maceration, exhibited significantly higher color intensity values than the maximum color intensity value in the untreated samples. It is noteworthy that the change in color was a positive outcome of this treatment. The Random Forest algorithm was used to identify the most distinct variables among wines. The most significant variable was found to be the total polyphenol content, followed by antioxidant capacity and the color intensity of the wines. The algorithm grouped all the samples into 5 clusters based on three fixed factors that influenced their characteristics: amplitude, treatment time, and maceration duration. Based on these results, it can be inferred that the effects of ultrasound treatment vary significantly depending on the parameters used.

Key words*: high-power ultrasound treatment, red wines, bioactive compounds, chromatic characteristics*

INTRODUCTION

One of the world's earliest known alcoholic beverages is wine. Red wine can be considered a major dietary source of polyphenols due to its generally high polyphenol content (Castaldo et al., 2019). Essential substances called polyphenols give wine its color, flavor, and aroma as well as some possible health advantages (Banc et al., 2014). Red wine's phenolic content can be broadly classified into two groups: non-flavonoids, which include phenolic acids like benzoic, caffeic, and cinnamic acids and stilbenoids like resveratrol, and 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 (Gutiérrez-Escobar et al., 2021). Nonetheless, flavonoids account for the majority of wine's phenolic component. Of these, the grape's stems, seeds, and skins account for up to 90% of the phenolic content found in red wine (Nemzer et al., 2021). One-year-old red wine's polyphenol composition is composed of approximately 60–80% polymeric polyphenols, 10-15% anthocyanidins, 5–10% dimer procyanidins, 5-8% catechins, 3-6% phenolic acids, less than 1% flavonols, and less than 0.3% resveratrol, among other particular components (Buljeta et al., 2023). Red wine's phenolic components are known to provide a number of health advantages, including anti-inflammatory, cardioprotective, anticarcinogenic, neuroprotective, and gut microbiota impacts (Nardini, 2022). Many variables, including grape variety and age, pre-fermentation techniques, fermentation and aging circumstances, and technological procedures, influence the kind of polyphenols present in red wine (Luzzini et al., 2021). It is also crucial to remember that these elements may interact in intricate ways to affect how a particular wine's ultimate polyphenol profile is determined. Accordingly, the precise function and influence of polyphenols can change based

on things like the kind of polyphenols present, how long they macerate for, and the desired qualities of the finished product (Gómez-Plaza et al., 2020). Wines with good chromatic quality must have a good dispersion of the phenolic compounds from the grape to the must or must-wine. This extraction process, which is based on the disintegration of the grape skin and seed cell walls, is carried out during the maceration step to attain the release of the intended compounds into the medium. As such, the quality of the wine is influenced by the length of the maceration process (Alencar et al., 2018). 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) are some of the advanced techniques for extracting plant bioactive compounds from foods and food-related matrices. These improved procedures are 32-36% more efficient, using roughly 15 times less energy and yielding higher-quality extracts (Sridhar et al., 2021). A technique known as high-power ultrasound (HPU) makes use of sound waves with frequency higher than 20 kilohertz (Ali et al., 2023; Hussain et al., 2023; Ranjha, Irfan, et al., 2021). The fundamental way that ultrasound affects a fluid is by increasing the hydrostatic pressure that already exists in the medium with acoustic pressure (Patist & Bates, 2011). The HPU's extraction capacity and the subsequent physico-chemical quality of the wines have been the subject of highly encouraging results in previous studies on red grapes and wines of various types (Q. A. Zhang et al., 2023).

Research has shown that ultrasound can be a valuable tool in winemaking, particularly in the extraction of polyphenols from grapes (Gómez-Plaza et al., 2020; Plaza et al., 2019). Studies have found that ultrasound treatment of grape marc can enhance the accumulation of polyphenols and anthocyanins, as well as influence the quantity of monomeric fraction of anthocyanins (Khmelev et al., 2015). High-power ultrasounds have been shown to modify the physical characteristics of grape skin, facilitating phenolic extraction and improving wine chromatic characteristics (Pérez-Porras et al., 2021). The application of high-power ultrasounds during red wine vinification has been found to be effective in increasing the extraction of polyphenols, particularly in certain grape cultivars (Bautista-Ortin et al., 2017). These findings suggest that ultrasound can play a significant role in enhancing the quality and characteristics of wine. The International Organization of Vine and Wine decided after much investigation that this technology might be used in wineries in 2019 (OIV - International organisation of vine and wine, 2021).

However, the following concerns regarding basic research and technological implementtation in industry need to be addressed. The different research groups used very different ultrasonic equipment, studied very different parameters, and obtained very different results, so they cannot be used as a standard for future research.

The objective of this study was to assess the impact of applying ultrasonic treatment (US) on crushed Merlot grapes, at a laboratory scale, on the phenolic matrix of red wines, particularly anthocyanins, which are crucial for color, stability, and sensory profile. Furthermore, to identify the most distinguishing variables among wines, we utilized the Random Forest clustering algorithm that indicates the most significant variables for grouping the samples in descending order.

MATERIALS AND METHODS

Grapes

The experimental study used about 100 kg of Merlot-varietal red grapes from the 2019 harvest. At the Pietroasa-Istrita Viticulture and Winemaking Research and Development Station in Buzau, Romania, the grapes were gathered by hand. The grapes were harvested at 29.8 °Brix, which is considered technological maturity, and were then promptly transferred to the lab for processing. For the investigation, only sound grape bunches selected at random were used.

Ultrasonic instrument

All experimental procedures were sonicated using SONIX VCX750, a probe-style ultrasonic apparatus from Sonics and Materials Inc. in Newtown, USA (SONIX VCX750, Sonics and Materials Inc., Newtown, USA). One way to express the amplitude is as a percentage, with values between 10% and 100%. The device operates at a frequency of 20 kHz and has an ultrasonic power of 750 W. The 20-100 kHz range is the most often utilized frequency range for extraction techniques.

Preparation of the sample

Samples of 1000 grams of 2019 vintage Merlot red grapes were prepared for laboratory testing. Sixty specimens, each weighing 100 grams, were randomly selected, de-stemmed, manually ground, and subjected to ultrasonic probe treatment. After sonication, we created six 1000 g samples, each containing ten 100 g specimens. The characteristics of each sample are represented by the average of the conclusions drawn from its ten instances. Ten specimens weighing 100 g each were used as the untreated control sample (C) since they had not undergone ultrasonic treatment. The specimens were evaluated independently. To treat the specimens, a 100 mL Pyrex glass beaker with a counter current water-cooling jacket was used. The cooling water was maintained at a consistent temperature of 19 °C. The acoustic amplifier was positioned 20 mm above the bottom of the container to ensure consistent treatment. The untreated sample (Control, C) and the ultrasound-treated samples underwent microvinification. After maceration, samples were sequentially extracted at 3 (D3), 5 (D5), and 7 (D7) days, pressed, and then underwent alcoholic fermentation. To ensure winemaking control, 20 g of Viniferm *Saccharomyces cerevisiae* Agrovin, Spain, fermentation yeast was added per 100 liters. The temperature was monitored daily during alcoholic fermentation, with variations ranging from 19.7 to 21.3°C. After completing alcoholic and malolactic fermentation, the samples were separated from the yeasts and clarified using 30 g of bentonite per 100 liters. Following clarification, the samples were cold-stabilized at 9°C before being bottled. All experiments were conducted in this manner.

Total polyphenolic content and anthocyanins determination

The Folin-Ciocalteu spectrophotometric method was used to calculate the total polyphenolic content (TPC), which was then expressed as micrograms of gallic acid equivalents per milliliter (µg GAE/mL) using gallic acid as a reference (Singleton et al., 1999). A 0.5 mL sample was treated with 1.25 mL of Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), which had been diluted 1:10 (v/v) with distilled water. After the mixture had been incubated for five minutes at room temperature, one milliliter of Na_2CO_3 60 g/L was added. After 30 minutes of incubation at 50 °C, the sample absorbance at 750 nm was measured using a UV-VIS spectrophotometer (Specord 205, Analytik Jena Inc., Jena, Germany). The calibration curve was made using gallic acid as the standard, which was utilized in values ranging from 5 to 250 g GAE/mL.

Monomeric anthocyanins (MA) were found using the pH differential method (Lee et al., 2005). To make two dilutions of the same sample, 1 mL of wine was combined 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). The absorbance at 520 and 700 nm was measured against deionized water after 15 minutes at room temperature. The results were expressed in milligrams (mg CGE/L) of cyanidin-3-glucoside equivalents per liter. The total anthocyanin content of the samples was calculated using a molecular weight of 449.2 g/mol and a molar absorbance coefficient of 26,900 L/mol x cm.

Ferric reducing antioxidant power (FRAP) assay

The FRAP test methodology was built upon the Benzie and Strain approach (Benzie & Strain, 1996). Two stock solutions were used: 20 mM $FeCl_3$ x $6H_2O$ solution in 300 mM acetate buffer (pH = 3.6) and 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl. The working solution was prepared by mixing acetate buffer, TPTZ solution, and 10 mL of $FeCl_3$ x $6H_2O$ solution. It was then heated to 37 °C before to use. Prior to analysis, a 0.5 mL aliquot of the diluted wine samples was allowed to react with 2.5 mL of the working

solution for 30 minutes at 37 °C. The wine samples had been diluted 1:50 (v/v) with distilled water.

Chromatic characteristics

The intensity of color (IC) and hue (N) of the samples were determined using a spectrophotometric method. The intensity of color was calculated by summing the absorptions at wavelengths 420, 520, and 620 nm with a 1 cm optical path. The hue was expressed as the ratio of absorbance at 420 nm to absorbance at 520 nm (OIV (International organisation of vine and wine), 2021).

Statistical analysis

The results, which were all measured in triplicate, are presented as mean ± standard deviation (SD). Statistical analysis was performed using DESIGN-EXPERT® VERSION 13 software from Stat-Ease Inc. (Stat-Ease Inc., 2020). To assess statistically significant differences between samples, we used one-way ANOVA with post hoc analysis employing the HSD Tukey test. The Pearson correlation coefficient was computed using JASP software version 0.17.1 (JASP Team, 2023) for TPC, MA, FRAP value, IC, and N. The wines were grouped based on the parameters of ultrasound treatment using Random Forest Clustering (JASP Team, 2023). This algorithm partitions data into distinct clusters, with each observation belonging to only one group.

RESULTS AND DISCUSSIONS

Following destemming and crushing, the samples treated with ultrasound and the control sample underwent microvinification. The sequential extraction method was used to carry out the maceration operation over a period of 3, 5, and 7 days.

After fermentation and bottling, the purpose was to assess the impact of high-power ultrasound treatment parameters: amplitude (A) and treatment time (t) on wine quality parameters, specifically bioactive compounds such as TPC (total polyphenol content), MA (monomeric anthocyanin content), FRAP value, color intensity (IC), and hue (N) in the sonicated samples in comparison to the untreated sample (C). The study analyzed the above parameters on untreated samples (C) after 3 (C3), 5 (C5), and 7 (C7) days of maceration. Additionally, the study examined samples that underwent ultrasound treatment at 70% and 90% amplitude for 3, 4, and 5 minutes after 3, 5, and 7 days of maceration. The samples were labeled as follows: SW70/3 (A: 70%; t: 3 min), SW70/4 (A: 70%; t: 4 min), SW70/5 (A: 70%; t: 5 min), SW90/3 (A: 90%; t: 3 min), SW90/4 (A: 90%; t: 4 min), and SW90/5 (A: 90%, t: 5 min). Each sample was replicated three times.

Analytical determinations performed on the samples treated with ultrasound and control samples (untreated), subjected to different durations of maceration of 3, 5, and 7 days, respectively, and microvinified and bottled for three months showed interesting results (Table 1). As these are red wines, it is important to consider the potential impact of ultrasonic treatment on the coloring matter.

The sonicated samples showed distinct chromatic features and higher concentrations of bioactive chemicals from the beginning of the maceration than the untreated samples. This difference persisted until the end of the maceration (Maier et al., 2023; Margean et al., 2020).

Effects of ultrasound treatment on wine parameters

After a bottling period of three months, both the wines made from crushed grapes subjected to ultrasonic treatment and the control wines showed a decrease in total polyphenol content compared to the content found at the end of maceration. However, the TPC values found in the sonicated samples remained higher than those of the controls, regardless of the duration of maceration (Table 1).

The total polyphenol content registered a maximum for all samples at the end of maceration and a decrease after bottling for all samples, with values ranging from 1.12% for sample SW70/3 extracted after 5 days (1169.23 µg GAE/mL at the end of maceration) to 64.62% for sample C3 (870.32 µg GAE/mL at the end of maceration).

High-power ultrasound applied to grapes or during winemaking has been shown in previous research to produce a greater extraction yield of

TPC (Ferraretto et al., 2011; Pérez-Porras et al., 2021). Other authors have reported no significant degradation of polyphenols after sonication of red wine (Natolino & Celotti, 2022).

Table 1. Effects of the ultrasonic treatment on the parameters of the wine

Sample	Maceration (days)	TPC (µg GAE/mL)	MA (mg CGE/L)	FRAP (µM Fe^{2+}/mL)	IC	N
C3	3	307.91±2.36	63.19±1.6	39.1±2.4	4.8±0.3	0.56±0.02
SW70/3		893.47±19.6	129.51±3.6	55.97±0.9	16.86±0.88	0.6±0.03
SW70/4		876.24±12.1	153.15±3.9	57.70±2.25	16.87±1.1	0.6±0.01
SW70/5		1 115.26±15.4	131.07±2.8	55.22±1.7	16.65±2.3	0.6±0.01
SW90/3		946.24±15.2	137.59±6.5	61.57±2.3	15.93±1.1	0.57±0.02
SW90/4		1 648.75±24.9	152.04±4.6	69.00±2.3	17.56±0.83	0.61±0.08
SW90/5		1 333.94±16.98	158.63±2.2	68.13±2.04	15.98±1.47	0.54±0.01
C5	5	415.76±3.8	95.99±3.7	54.64±2.6	5.63±0.76	0.57±0.02
SW70/3		1 156.14±11.9	138.79±2.6	52.77±1.6	11.6±1.2	0.57±0.05
SW70/4		1 094.92±13.1	171.06±2.5	52.07±2.1	8.47±1.5	0.72±0.09
SW70/5		1 304.79±12.4	125.51±6.3	57.46±2.8	14.36±1.4	0.64±0.02
SW90/3		1 159.05±11.3	123.68±5.6	57.61±3.0	11.36±1.2	0.67±0.04
SW90/4		1 243.58±10.8	126.74±4.9	68.52±4.9	11.49±1.3	0.67±0.05
SW90/5		1 605.02±21.7	122.86±4.8	56.06±2.7	15.13±0.9	0.63±0.05
C7	7	439.08±8.9	119.18±6.1	46.65±3.7	6.44±1.1	0.58±0.1
SW70/3		1 043.58±18.4	121.63±3.8	58.89±2.1	13.66±1.1	0.64±0.07
SW70/4		1 269.82±13.8	156.28±4.2	61.39±1.8	9.06±0.8	0.65±0.03
SW70/5		1 418.47±21.3	121.66±3.6	68.04±2.9	14.76±1.2	0.65±0.07
SW90/3		1 261.07±19.2	159.61±4.1	68.84±1.9	13.53±1.0	0.68±0.02
SW90/4		1 296.05±23.1	200.36±2.7	59.91±1.8	11.92±1.2	0.70±0.04
SW90/5		1 596.28±25.4	132.43±2.8	61.36±1.9	16.54±0.7	0.67±0.01

Untreated sample (C); SW70/3 (A: 70%; t: 3 min), SW70/4 (A: 70%; t: 4 min), SW70/5 (A: 70%; t: 5 min), SW90/3 (A: 90%; t: 3 min), SW90/4 (A: 90%; t: 4 min), and SW90/5 (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).

Moreover, high-power ultrasound has been shown to have a significant impact on the phenolic structure of red wines, particularly in accelerating the aging process (Ferraretto & Celotti, 2016). It can also enhance the extraction of polyphenols from grapes during winemaking, with varying effects depending on the grape variety (Gambacorta et al., 2017). However, the specific effects on polyphenols in wine during storage are still being explored (Q. Zhang & Wang, 2017b).

As can be observed in Table 1, the treatment with HPU caused significant variations in the optical density (420, 520, and 620 nm) and in the monomeric anthocyanin content. After a period of three months of bottling, there was a significant decrease in the color intensity values of the wines for all the samples, compared to the values obtained at the end of the maceration, with the greatest decrease being recorded for the sample SW90/5 extracted after 5 days (24.85 at the end of maceration).

However, based on the data analyzed, it is evident that the ultrasonic treatment had a positive effect on the color content. All ultrasound samples, including those extracted after 3 days of maceration, recorded significantly higher color intensity values compared to the untreated samples (C7), ranging from 31.52% (SW70/4 sample, extracted after 5 days) to 172.67% (SW70/4 sample, extracted after 3 days). Other authors found that ultrasound treatment affects the evolution of color properties and major phenolic compounds in wine during storage, showing similar effects in treated and untreated wines, with quicker changes observed in treated wines (Q. Zhang & Wang, 2017a). According to additional research, it was found that the control samples that were macerated for 48 hours had the lowest color intensity. However, the differences from the other control wines were not statistically significant. Furthermore, the color intensity of the sonicated samples macerated for 48 hours was

not significantly different from the control wine that was exposed to the skin for 7 days (Pérez-Porras et al., 2021).

The monomeric anthocyanin content was also measured for all samples immediately after maceration stage. After three months of bottling, the quantities present in the wine samples were smaller, with a decrease between 27.35% and 73.03%. The smallest decrease was observed in the case of sample SW90/4, which was extracted after 7 days (275.78 mg CGE/L at the end of maceration). This decrease in monomeric anthocyanin content is likely caused by factors such as polymerization with other phenolic compounds and oxidation reactions.

The influence of ultrasonic treatment on the monomeric anthocyanin content of wine samples is similar to its effect on their color content. The untreated samples had the highest content of monomeric anthocyanins in the C7 sample. However, the sonicated samples showed significant variations in the monomeric anthocyanin content compared to the untreated samples (Table 1). The increase ranged from 2.05% (sample SW70/3, extracted after 7 days) to 68.1% (sample SW90/4 min, extracted after 7 days). According to (Dalagnol et al., 2017), ultrasound-assisted extraction was found to accelerate the rate of anthocyanin extraction. It is worth noting that free anthocyanins are the primary source of red color in young red wines, despite the instability of monomeric anthocyanins. To stabilize these anthocyanins, one of the main methods is to condense them with tannins to generate stable anthocyanin/tannin adducts. It has been estimated that approximately 25% of the anthocyanins may undergo polymerization with flavonoid molecules towards the end of the alcoholic fermentation process. Following a year, this percentage is observed to increase to over 40% (He et al., 2012a, 2012b).

The antioxidant capacity increased for all samples after the bottling period compared to the values found at the end of maceration. The increase in antioxidant activity ranged from 3.52% for sample SW90/5, which was extracted after 7 days (59.27 µmol Fe^{2+}/mL at the end of maceration), to 47.1% for sample SW70/4 min, which was extracted after 5 days (52.48 µmol Fe^{2+}/mL at the end of maceration). Nevertheless, it appears that the sonicated samples may have contained a notable quantity of phenolic compounds as a result of the US treatment, which led to elevated FRAP values.

Previous studies have indicated that the phenolic composition of wine can be influenced by the winemaking process. Moreover, a significant correlation has been found between the phenolic composition and the 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 individual phenolic compounds present in the wine, rather than the total phenolic content (Banc et al., 2020).

The shade of wines after three months of bottling varies between 0.54 for sample SW90/5, extracted after 3 days, and 0.72 for sample SW70/4, extracted after 5 days (Table 1). A smaller shade indicates a larger red component compared to yellow, while high shades indicate an orange color. It is likely that anthocyanins degrade and condense with tannins, forming complexes that contribute to color stabilization.

Correlation of maceration duration, amplitude level, and sonication time

The statistical analysis of the data reveals that the investigated parameters were affected by three factors: amplitude level, ultrasonic treatment time, and maceration duration, either independently or in combination. The analysis of variance conducted on the analytical parameters for different maceration durations, amplitude, and treatment time conditions showed significant differences in the total content of polyphenols, monomeric anthocyanins, antioxidant capacity, color intensity, and hue of wines. The evolution of the analyzed parameters appears to be influenced by changes in maceration duration, percentage of amplitude, and treatment time. It is worth noting that the correlation coefficient (R) is 0.791 for CTP and 0.208 for AM. Additionally, the antioxidant activity also appears to vary, with a correlation coefficient of 0.518. The adjusted R^2 used for the three predictors: maceration duration, amplitude and treatment time shows that they can predict: 60.4% of the variation in results obtained for

CTP; 46.3% of the variation in results obtained for AM; 22.4% of the variation in FRAP results; 26.2% of the variation in results obtained for IC; and 22.3% of the variation in results obtained for hue. Previous research has also established a direct proportionality between the amplitude of the transducer and the intensity of the ultrasound. The sonochemical effects of the ultrasound were observed to increase with increasing intensity and amplitude (Mason & Lorimer, 2002). ANOVA shows that the model is significant, and the predictors introduced into the model, both alone and in the case of interaction between them, significantly influence ($p<0.05$) the total content of polyphenols, monomer anthocyanins, and FRAPs (Table 2).

Table 2. Exploring the impact of various factors on wine parameters

	p-value				
Factors	**TPC**	**MA**	**FRAP**	**IC**	**N**
Amplitude (%)	<0.001	<0.001	<0.001	<0.05	0.564
US treatment time (min)	<0.001	<0.001	<0.05	<0.001	<0.05
Maceration time (days)	<0.001	<0.001	<0.001	<0.001	<0.001
Maceration time *Amplitude	<0.001	<0.001	<0.001	<0.05	0.162
Maceration time *US treatment time	<0.001	<0.001	<0.001	<0.001	0.549
Amplitude *US treatment time	<0.001	<0.001	<0.001	<0.05	0.288
Maceration time *Amplitude *US treatment time	<0.001	<0.001	<0.001	0.976	0.164

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).

The color intensity appears to be minimally impacted by the interaction between amplitude level, treatment time, and maceration duration. However, it is worth noting that the hue of wines is significantly influenced by treatment time and duration of maceration.

Identifying the most distinguishing variables between wines by grouping them

Random Forest clustering is a hard-partitioning algorithm that divides data into multiple clusters (groups), with each observation belonging to only one group (JASP Team, 2023). This clustering approach uses the Random Forest algorithm in an unsupervised manner, with the output variable 'y' set to NULL.

The wine dataset contains the results of analytical determinations of wines produced according to the traditional technology (control samples) and wines obtained from crushed grapes treated with ultrasound before the maceration stage. Two types of wine are represented in the 63 samples (21 samples analyzed in triplicate), with the results of 5 analytical determinations recorded for each sample. The variables recorded are TPC (µg GAE/mL), MA(mg CGE/L), FRAP (µM Fe^{2+}/mL), IC and N.

The purpose of using the Random Forest algorithm is to identify the most distinct variables among wines. Specifically, the Random Forest cluster model is optimized against its BIC value, which can be inspected in the Elbow Curve Diagram.

Table 3 and Table 4 present summary and performance statistical data, such as the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) of the model.

Table 3. Statistical data on cluster grouping

Number of clusters	Number of samples	R^2	AIC	BIC	Silhouette
5	63	0.638	162330	215910	0.220

Determination coefficient (R^2); Akaike Information Criterion (AIC); Bayesian Information Criterion (BIC); The model is optimized with respect to the BIC value.

AIC is an estimator of prediction error and provides a means for model selection. BIC is a criterion for model selection from a finite set of models. The preferred model is the one with the lowest BIC. BIC is closely related to the AIC criterion and is partly based on the probability function. When the models match, adding parameters can increase the probability, but this can lead to overlap. Both BIC and AIC introduce a penalty term for the number of parameters in the model to solve this problem. However, the penalty term is higher in BIC than in AIC. The silhouette value of the model ranges from -1 to 1, with 1 representing a perfect score. The sum of squares of each

cluster indicates the spread within the cluster, while R^2 indicates the amount of variance explained by the model (Table 4). Silhouette scores describe the degree of separation between groups.

Table 4. Grouping and sizes of clusters

Cluster	1	2	3	4	5
Dimension (number of samples)	9	11	16	14	13
Sum of squares within the group	19170	5998	36059	28188	22917
Silhouette score	0.468	0.498	0.003	0.108	0.177

Table 5 displays the Random Forest algorithm's ranking of variables in descending order of importance for grouping samples. The most significant variable is the total polyphenol content, followed by antioxidant capacity and the color intensity of the wines.

Table 5. Importance of variables in sample grouping

Parameter	Importance value
Total polyphenol content	13796
Antioxidant capacity	12996
Color intensity	12952
Monomeric anthocyanins	12628
Hue	10089

The Elbow diagram indicates the point at which adding another cluster would be unnecessary, also known as the kink in the curve. The red dot represents the minimum BIC value, which is the metric optimized by the model (Figure 2).

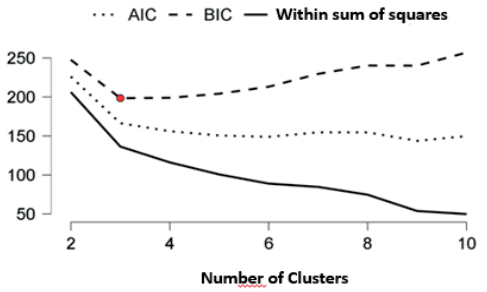

Figure 2. Elbow Diagram

Figure 3 displays the two-dimensional t-SNE graph, which provides a comprehensive overview of the various characteristics determined for the samples. However, the disadvantage is that the axes become uninterpretable. This t-SNE graph illustrates how the different clusters are grouped together. Moreover, Figure 3 displays the sample grouping into clusters based on three fixed factors that influenced their characteristics: amplitude, treatment time, and maceration duration.

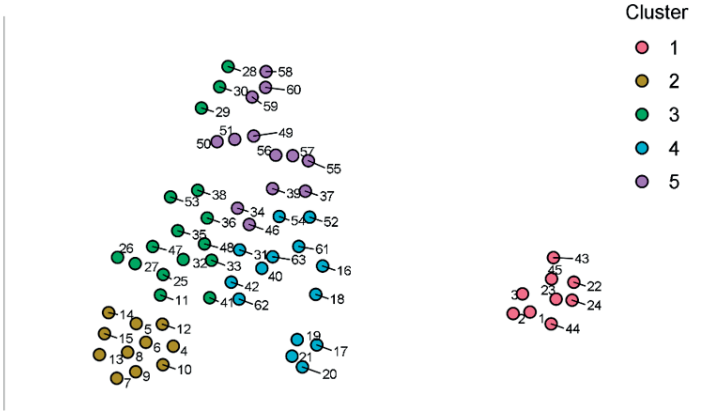

Figure 3. Two-dimensional t-SNE plot illustrating clustering of samples

Compared to all samples that underwent ultrasonic treatment, the control samples, regardless of maceration duration, recorded significantly lower values for all determined

parameters (refer to Table 1). These control samples were grouped in a separate cluster (Cluster 1).

A cluster (Cluster 2) was identified among the samples treated with ultrasound. This cluster was influenced by both the lower amplitude (70%) used during treatment, regardless of the treatment time (3, 4, or 5 minutes), and the increased amplitude (90%) used during a short treatment time (3 minutes). As a result, significantly lower values were recorded for all determined parameters compared to the other samples subjected to ultrasonic treatment. Cluster 3 confirms the positive influence of amplitude and treatment time on the properties of wines. It also shows the influence of maceration duration. The samples in Cluster 3 were extracted after 5 days, which is 2 days longer than the samples in Cluster 2. The same cluster also includes another sample that underwent maceration for 7 days but was treated with lower amplitude (70%) for only 3 minutes.

Cluster 4 and Cluster 5 group samples that underwent ultrasonic treatment with increased amplitude (90%) for 4 or 5 minutes, regardless of the duration of maceration, and additionally two samples that were treated with lower amplitude (70%) for 4 or 5 minutes, but with a maceration duration of 7 days. Clusters 4 and 5 recorded the highest values for all analyzed parameters of the wine samples obtained.

It can be inferred from these results that the effects of ultrasound treatment are subject to significant variation based on the parameters employed.

CONCLUSIONS

After a bottling period of three months, a decrease in the total polyphenol content was observed compared to the content found at the end of maceration, both in the wines made from crushed grapes treated with ultrasound and in the control samples, with the values found in the ultrasound-treated samples remaining higher than those of the control samples, regardless of the duration of maceration-fermentation of the latter. However, within each series (D3, D5 and D7), the decrease was influenced by the variation of the ultrasonic treatment parameters. The positive effect of the ultrasonic treatment on the color content can be observed since all the ultrasonically treated samples, including those extracted after 3 days of maceration, showed significantly higher color intensity values than the maximum color intensity value of the control samples (C7), ranging from 31.52% (sample SW70/4, extracted after 5 days) to 172.67% (sample SW70/, extracted after 3 days). In conclusion, the ultrasonic treatment significantly modified the color of the wine, an effect that is very beneficial to the quality, especially for young wines, where color is one of the most important evaluation factors. The monomeric anthocyanin content of the sonicated samples showed significant variation compared to the maximum of the untreated samples, with an increase ranging from 2.05% (sample SW70/3 min, extracted after 7 days) to 68.1% (sample SW90/4 min, extracted after 7 days). The maximum anthocyanin content of the untreated samples was determined for the one extracted after 7 days. The data, subjected to statistical analysis, show that amplitude level, ultrasound treatment time and maceration duration significantly ($p<0.05$) influence the total polyphenol content, monomeric anthocyanins and antioxidant capacity. In addition, colour intensity is not significantly influenced by the interaction between amplitude level, treatment time and maceration time, while wine hue is significantly influenced only by treatment time and maceration time. Furthermore, to identify the most distinguishing variables among wines, we utilized the Random Forest clustering algorithm. The Random Forest algorithm indicates the most significant variables for grouping the samples in descending order. Our results display that the total polyphenol content is a distinguishing feature, followed by antioxidant activity and the color intensity of the wines. All untreated samples, regardless of maceration time, consistently recorded significantly lower values for all determined parameters, in contrast to all samples exposed to ultrasound treatment, and were grouped in a separate cluster. The ultrasound-treated samples were categorized into four clusters, confirming that the effects of ultrasound treatment vary considerably depending on the parameters used (amplitude and time).

However, further research is needed to fully understand the implications of ultrasound on wine color and organoleptic characteristics, as well as the economic feasibility and scalability of implementing high-power ultrasound in winemaking processes.

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