c. Lista portofoliului de lucrări științifice relevante

Candidat abilitare IPA : Conferențiar dr. ing. Mirabela Ioana LUPU

1. Lupu MI., Bădărău C.L., Padureanu, V**.,** Canja C.M., *Effects of Thermal and Ultrasound Treatments on L Ascorbic Acid of Grapes Juice*, Revista de Chimie, nr.4, vol.70, 1258-1261, 2019. https://doi.org/10.37358/RC.19.4.7104.
2. Margean A, Lupu, M.I., Ersilia Alexa, Vasile Padureanu, Cristina Maria Canja, Ileana Cocan, Monica Negrea, Gavrila Calefariu, Mariana-Atena Poiana, *An overview of effects induced by pasteurization and high-power ultrasound treatment on the quality of red grape juice,* Molecules, 2020, 25(7). https://doi.org/10.3390/molecules25071669
3. Maier, A.; Pădureanu, V.; Lupu, M.I.; **Canja, C.M.;** Badarau, C.; Padureanu, C.; Alexa, E.; Poiana, M.-A. *Optimization of A Procedure to Improve the Extraction Rate of Biologically Active Compounds in Red Grape Must Using High-Power Ultrasound*. Sustainability 2023, 15, 6697. https://doi.org/10.3390/su15086697
4. Maier, A; Padureanu, V ; Lupu, M I; Canja, C M; Branescu, GR; Padureanu, C; Poiana, MA. *Effect of High-Power Ultrasound Treatment on Bioactive Compound Content and Chromatic Characteristics of Red Wines,* Bulletin Of University Of Agricultural Sciences And Veterinary Medicine Cluj-Napoca-Food Science And Technology, Volume 81 Issue 1, pp. 56-67, 2024. https://doi.org/10.15835/buasvmcn-fst:2023.0021
5. Maier, A; Padureanu, V ; Lupu, M I; Canja, C M; Branescu, GR; Padureanu, C; Poiana, MA, *Influence of High-Power ultrasound treatment on red wine quality parameters*, Scientific papers. Seria B, Horticulture, vol. LXVIII, no. 1, 2024. https://horticulturejournal.usamv.ro/pdf/2024/issue_1/Art40.pdf
6. Margean A., Padureanu V., Lupu M.I., *Application of ultrasound in winemaking process*, Actual Tasks on Agricultural Engineering-Zagreb, Opatija, 2018. https://www.cabidigitallibrary.org/doi/pdf/10.5555/20183183948
7. Padureanu, C; Badarau, CL; Maier, A; Padureanu, V; Lupu, MI; Canja, CM; Branescu, GR; Bujor, OC; Matei, F; Poiana, MA; *Ultrasound Treatment Influence on Antioxidant Properties of Blueberry Vinegar*. Fermentation. 2023; 9(7):600. https://doi.org/10.3390/fermentation9070600
8. Lupu, M.I.; Canja, C.M.;Padureanu, V.; Boieriu, A.; Maier, A.;Badarau, C.; Padureanu, C.; Croitoru, C.; Alexa, E.; Poiana, M.-A. Insights on the Potential of Carob Powder (Ceratonia siliqua L.) to Improve the Physico-Chemical, Biochemical and Nutritional

Properties of Wheat Durum Pasta. Appl. Sci. 2023, 13, 3788. https://doi.org/10.3390/app13063788

9. Lupu M.I., Canja C.M., Maier A., Padureanu V., Branescu G.R, Manolică A.M., The impact of the infusion method of chokeberry powder in white tea, Carpathian journal of food science and technology, 16(4), 2024. https://doi.org/10.34302/crpjfst/2024.16.4.3

Conf. dr. ing. Lupu Mirabela Ioana

Effects of Thermal and Ultrasound Treatments on L Ascorbic Acid of Grapes Juice

MIRABELA IOANA LUPU, CARMEN LILIANA BADARAU*, VASILE PADUREANU, CRISTINA MARIA CANJA
Transilvania University of Brasov, Faculty of Food and Tourism, 148 Castelului Str., 500014, Brasov, Romania

Improving the nutritional quality for non-alcoholic beverages (especially the content of valuable natural antioxidants) by using modern ultrasound treatments represent a challenge for more and more food scientists. The objective of this research work was to estimate the effect of some ultrasound treatments on the total L ascorbic acid content and on several physicochemical characteristics of grape juice stored for different time periods. In order to determine this influence, the sonication parameters chosen were the amplitude (60%, 80%) and the treatment times (18, 36 min.). The samples treated by direct sonication (amplitude 80%, 36 min.) and stored 17 days had higher values of total L ascorbic acid content.

Keywords: L ascorbic acid, grapes juice, ultrasound treatments

Fruit intake is recommended as part of a healthy diet and a practical way to do so is in form of juice. When fruit juices are produced on an industrial scale, the inactivation of microorganisms and enzymes is mandatory in order to guarantee safety and stability.

Thermal processing has been quite useful for that purpose. However, excessive heating induces physical and chemical reactions that negatively affect the sensorial and nutritional properties of this kind of food [1]. The application of ultrasound-assisted extraction for plant materials and its opportunities in the food industry has proved many advantages [2-5].

Ultrasound is reported to have a minimal effect on the quality of fruit juices [6].

One of the most important micronutrient present in the fruits juice is the L ascorbic acid (vitamin C). Three main biological functions have been identified for compound: enzyme cofactor, free radical scavenger and donor/ acceptor of electrons. Humans have lost ability to synthesize L ascorbic acid and depend on the diet to acquire the necessary amounts required to maintain good health. Deficiency of the vitamin C causes the disease scurvy characterized by spots on the skin, spongy gums and bleeding from mucous membranes. Is caused by deficient synthesis of collagen in which L ascorbic acid is cofactor [7-9]. Although, nowadays, scurvy is considered rare in developed nations, the vitamin C intake of significant part of the population of some of these countries may be below recommended daily acceptance (80mg per day in European Union EC) [10-12]. About 13% of the population in the USA or 1 in 7 young adults in Canada have been reported to be deficient in vitamin C with certain groups such as smokers, pregnant women and people of low socioeconomic status at a higher risk of deficiency is common. L ascorbic acid is particular important because it can reduce the chelating effect that some compound could have on iron, increasing its bioavailability [11]. Data reported by other researchers [13] revealed that the ultrasound treatments applied to different jus could inactivate microorganisms and increase antioxidants compounds. Therefore, the purpose of this study was to estimate the effect of ultrasound treatment on the level content of L ascorbic acid and on some physicochemical properties (*p*H, density, total soluble solids, titratable acidity, tartaric acid) of grapes juice during different storage periods.

Experimental part

Material and methods

Samples preparation

The experimental variants of juices were obtained from grape varieties Riesling (cultivated in the vineyard Dealu Mare, Romanian region and harvest at the end of September 2017). For obtain the juice, the grapes were first removed from the bunch and crushed with a crusher destemmer (Enoventa Tecnologie Enologiche, Italy). The next step was pressing the juice and solid particles with a hydraulic press machine (LU.C.M.E. Elettromeccanica, Italy). The juice was left for clearing for 4 h before starting the treatments.

Juice samples were treated by ultrasound (VCX-750, Sonics & Materials, Inc. Newtown, CT, USA) at 750 W, with constant frequency of 20 kHz at 60 and 80% amplitude for time periods of 18 or 36 min using a sample of 500 mL grape juice. This combination of amplitude and time was established according results of previous study, where a reduced of microbial count and release of antioxidant compounds was obtained [13].

Untreated juice was selected as control and pasteurized juice in a water bath at 70°C for 30 min [13], was also included to compare results. Samples were stored at 4°C and analyzed after 1, 4, 7, 10 and 17 storage days.

Determination of parameters quality, polyphenol and vitamin C content

The *pH* was measured with a potentiometer (Consort C1010, Consort, Belgium).

The *density* (g/L) was measured with a densitometer standardized at 20°C.

Total soluble solids content (°Brix) was determined using a refractometer (Brix/ ATC FG-113, Hangzhou Chinchan Trading Co., China).

Titratable acidity was performing on aliquots of 10 mL placed into a 250 mL beaker and titration with sodium hydroxide 0.1N (Sigma-Aldrich, Dublin, Ireland). With a pipette, on a porcelain plate for titration, a drop of juice was removed and mix with two drops of indicator red phenol 0.02%. The titration is continued drop to drop, after each addition of the sodium hydroxide solution, until the indicator turns in pink-orange. The results are expressed as g tartaric acid/L. The titratable acidity (A_T) was calculated using the value of sodium hydroxide 0.1N solution volume (V), as follows:

* email: carmen.badarau@unitbv.ro, Phone: 0730594687

$$A_T = \frac{V \cdot 0.0075 \cdot 1000}{10} = 0.75 \cdot V \quad g\ tartaric\ acid/L$$

The semnifications of symbols used in these relations are:

Tartaric acid content (g/L) was determined following a colorimetric sequential technique (HI83748, Hanna Instruments).

The total phenols content was measured with an optical system of Hanna's HI 83000 series colorimeters. Photometric chemical analysis is based on the possibility to develop an absorbing compound from a specific chemical reaction between sample and reagents.

Extraction of L ascorbic acid (L-AA) was carried out by following steps (indicated by the manufacturer instructions - Megazyme Ltd., Sigma Aldrich, USA, cod product MAK074): adding 10 mL of 6% (w/v) aqueous solution of meta phosphoric acid and 40 mL 1-octanol to 10 mL sample (variants of grapes juice treated and non-treated); vortex this mixture for 1 min; adjust to *p*H 3.5-4; quantitatively transferring to a 20 mL volumetric flask; centrifuge at 13.684 g (14.000 rot/min.) for 5 min. (a part of the mixture was taken in 1.5 mL tubes).

Determination of the L ascorbic acid content was carried out using the supernatant and an enzymatic method (L-ascorbic acid test kit, Megazyme Ltd., Sigma Aldrich, USA, cod product MAK074) following the instructions of the manufacturer. This method is based on the color change of the absorption caused by the reduction of 3-(4.5-dimethylthiazol-2)-2.5-diphenyltetrazolium bromide before and after ascorbic oxides is added, the concentration of L ascorbic acid being proportional to this change. The absorbance was measured at 570 nm (Tecan SunRise micro plate reader, software Magellan). The determination was made in 5 repetitions at different time periods of the samples storage. The content of the L-AA was expressed as mg L Ascorbic Acid per liter of juice (mg L-AA/L).

Results and discussions

All the grapes juice treated by ultrasound had higher values of L ascorbic acid content compared to the pasteurized and control samples (table 1). The juices samples treated by sonication at 80% amplitude for 36 min presented the highest value of vitamin C content (64.498 ± 4.022 mg L-AA/L). Pasteurized treatments led to a significant decrease of L ascorbic acid content (compared to the untreated control) and after 17 storage days the juice had the lowest content in this phytochemical compound (23.990 ± 6.509 mg L-AA/L).

Different researchers reported the most important mechanisms for explain the evolution and variations of vitamin C induced by the ultrasound during the storage. Maybe, the vitamin C breakdown is prevented by the elimination of dissolved oxygen (essential for ascorbic acid degradation), during the cavitations produced by the ultrasounds treatment [14]. Tiwari et al. [6] considered that the extreme physical conditions existing inside the cavitations bubbles collapsing in a micro scale and caused some chemical reactions that occur either in the same time or in isolation. Sonication cavities can be filled using water vapor and different gases dissolved in the juice, such as N_2 which may aid oxidation reactions promoted by interactions with free radicals formed during sonication [15]. This oxidation could cause that ascorbic acid converts to dehydroascorbic acid [16] explaining the variations during storage.

Significant differences regarding the vitamin C content were observed between the variants of treatment (table 1). So, the simple correlation coefficient Pearson revealed significantly higher value regarding the vitamin C content (as compared to the different treatment variants) (table 2). Our results revealed that ultrasound treatments, especially at 80% amplitude for 36 min. had a grate effect on vitamin C content during storage. Ultrasound can be considered as an alternative emerging technology for grapes juice preservation, for increase the level of vitamin C content, maintaining the antioxidant properties. However,

Table 1

TOTAL L-ASCORBIC ACID CONTENT OF THE GRAPES JUICE TREATED AND UNTREATED AFTER DIFFERENT STORAGE PERIODS (DATA ARE MEAN OF THE VALUES ± STANDARD DEVIATION)

Variant of the treatment	Storage days				
	1	4	7	10	17
Juice untreated	43.880±3.738 (fgh)*	42.425±7.397 (gh)	41.455±1.918 (gh)	42.182±2.921 (gh)	39.272±4.236 (h)
Pasteurized	25.688±5.001 (i)	24.961±7.357(i)	24.476±5.941(i)	25.446±5.584 (i)	23.990±6.509 (i)
A60%,18min	52.370±6.302 (de)	49.217±5.259 (efg)	51.642±4.896 (def)	52.855±5.117 (cde)	54.553±3.679 (cde)
A60%,36min	57.464±4.728 (abcde)	54.796±3.209 (cde)	55.038±2.766 (bcde)	55.281±6.220 (bcde)	57.464±3.254 (abcde)
A80%,18min	55.523±5.328 (bcde)	53.098±6.279 (cde)	54.068±6.220 (cde)	56.251±3.473 (abcd)	58.677±4.881 (abcd)
A80%,36min	57.949±3.986 (abcd)	59.162±4.666 (abcd)	61.102±4.406 (abc)	63.528±4.059 (ab)	**64.498±4.022 (a)**

*Values not followed by the same letter are significantly different (P=0.05) according to Duncan's test.

Table 2

THE CORRELATION BETWEEN THE CONTENT OF L ASCORBIC ACID (IN GRAPES JUICE TREATED AND UNTREATED AFTER DIFFERENT DAYS) AND THE VARIANT OF TREATMENT

Variables	Statistical indicators	Days after treatments	Variant of the treatment
Vitamin C content (mg/L)	Correlation coefficient Pearson	0.042	**0.722****
	Significance threshold	0.612	0.000
	N	150	150

** Correlation is significant for p<0.01.

N =150 (5 periods of storage x 6 variants x 5 repetitions)

Table 3

MEAN (±SD) VALUES OF PH, TOTAL SOLUBLE SOLIDS, TOTAL ACIDITY, TARTARIC ACID AND TOTAL PHENOL CONTENT OF GRAPES JUICES (DIFFERENT VARIANTS OF TREATMENTS) DURING STORAGE

Type of analysis	Storage days	Treatments					
		Grape juice untreated (control)	Pasteurized juice	A60% 18 min	A60% 36min	A80% 18 min	A80% 36min
pH	1	3.633±0.006 ^{az}	3.593±0.011 ^{bz}	3.543±0.012 ^{cz}	3.536±0.015 ^{cz}	3.543±0.006 ^{cz}	3.507±0.015 ^{dz}
	4	3.613±0.006 ^{ay}	3.590±0.010 ^{bz}	3.537±0.006 ^{cyz}	3.537±0.006 ^{cz}	3.546±0.006 ^{cz}	3.503±0.006 ^{dz}
	7	3.600±0.006 ^{ax}	3.573±0.006 ^{by}	3.527±0.006 c y	3.517±0.006 ^{cy}	3.523±0.006 ^{cy}	3.487±0.006 ^{dy}
	10	3.597±0.006 ^{ax}	3.563±0.006 ^{by}	3.507±0.006 ^{cx}	3.493±0.006 ^{dx}	3.507±0.006 ^{cx}	3.463±0.006 ^{ex}
	17	3.583±0.006 ^{aw}	3.543±0.006 ^{bx}	3.493±0.006 ^{cw}	3.483±0.006 ^{cx}	3.487±0.006 ^{cw}	3.447±0.006 ^{cw}
Total soluble solids [° Brix]	1	24.37±0.058 ^{az}	24.40±0.000 ^{az}	23.73±0.058 ^{bz}	23.67±0.115 ^{bz}	23.33±0.115 ^{cz}	23.43±0.058 ^{cz}
	4	23.37±0.044 ^{ay}	23.04±0.574 ^{aby}	22.96±0.015 ^{abcy}	22.78±0.021 ^{bcy}	22.55±0.044 ^{cy}	22.63±0.015 ^{bcy}
	7	20.46±0.557 ^{ax}	20.63±0.142 ^{ax}	19.62±0.047 ^{bx}	19.46±0.051 ^{bx}	19.42±0.026 ^{bx}	19.51±0.026 ^{bx}
	10	18.70±0.025 ^{aw}	17.91±0.050 ^{bw}	16.61±0.026 ^{cw}	16.14±0.555 ^{cdw}	16.35±0.042 ^{dw}	16.45±0.035 ^{cdw}
	17	13.81±0.015 ^{av}	12.89±0.015 ^{bv}	11.46±0.036 ^{cv}	11.35±0.031 ^{dv}	11.13±0.112 ^{ev}	11.29±0.015 ^{dv}
Total acidity [g/L]	1	5.40±0.100 ^{az}	5.77±0.058 ^{bz}	5.13±0.153 ^{cz}	4.98±0.104 ^{cz}	4.48±0.029 ^{dz}	4.74±0.228 ^{ez}
	4	5.07±0.058 ^{ay}	5.33±0.058 ^{by}	4.85±0.050 ^{cy}	4.57±0.061 ^{dy}	4.40±0.010 ^{ey}	4.35±0.025 ^{ey}
	7	4.82±0.005 ^{ax}	4.95±0.050 ^{bx}	4.58±0.047 ^{cx}	4.41±0.010 ^{dx}	4.31±0.010 ^{ex}	4.17±0.038 ^{fx}
	10	4.61±0.015 ^{aw}	4.72±0.012 ^{bw}	4.39±0.015 ^{cw}	4.28±0.017 ^{dw}	4.14±0.047 ^{ew}	4.06±0.015 ^{fw}
	17	4.26±0.053 ^{bv}	4.40±0.025 ^{av}	4.03±0.026 ^{cv}	4.03±0.042 ^{cv}	3.80±0.010 ^{ev}	3.93±0.032 ^{dv}
Tartaric acid [g/L]	1	3.57±0.153 ^{dw}	3.68±0.035 ^{cdv}	4.67±0.031 ^{av}	3.79±0.026 ^{cv}	4.50±0.055 ^{bv}	3.81±0.012 ^{cv}
	4	3.88±0.025 ^{ex}	3.90±0.010 ^{ew}	4.87±0.015 ^{avx}	3.98±0.015 ^{dw}	4.72±0.006 ^{bw}	4.01±0.010 ^{cw}
	7	4.10±0.015 ^{fy}	4.13±0.015 ^{ex}	4.99±0.006 ^{axy}	4.20±0.006 ^{dx}	4.91±0.010 ^{bx}	4.26±0.012 ^{cx}
	10	4.19±0.006 ^{fy}	5.12±0.006 ^{by}	5.15±0.021 ^{ay}	4.36±0.006 ^{ey}	5.10±0.006 ^{cy}	4.42±0.006 ^{dy}
	17	4.45±0.020 ^{cz}	5.27±0.006 ^{az}	5.33±0.021 ^{az}	4.86±0.318 ^{bz}	5.20±0.010 ^{az}	4.61±0.010 ^{cz}
Total phenol content [mg GAE/L]	1	0.407±0.002 ^{fv}	0.750±0.003 ^{av}	0.525±0.003 ^{dv}	0.578±0.002 ^{cv}	0.687±0.003 ^{bv}	0.511±0.002 ^{ev}
	4	0.618±0.002 ^{fw}	0.760±0.002 ^{aw}	0.677±0.003 ^{ew}	0.700±0.002 ^{dw}	0.754±0.002 ^{bw}	0.713±0.001 ^{cw}
	7	0.783±0.002 ^{dx}	0.764±0.001 ^{fx}	0.765±0.003 ^{ex}	0.831±0.001 ^{bx}	0.890±0.002 ^{ax}	0.793±0.001 ^{cx}
	10	0.815±0.002 ^{ey}	0.771±0.002 ^{fy}	1.097±0.001 ^{ey}	1.124±0.003 ^{dy}	1.274±0.002 ^{ay}	1.143±0.002 ^{by}
	17	0.951±0.003 ^{ez}	0.781±0.003 ^{fz}	1.233±0.002 ^{dz}	1.307±0.002 ^{bz}	1.592±0.002 ^{az}	1.296±0.002 ^{cz}

a-f Means with the same letter in the same row are not significantly different at $p<0.05$;
v-z Means with the same letter in the same column are not significantly at $p<0.05$.
SD standard deviation

further studies are required to establish the most efective conditions to comply with specifications in terms of microbial load and other caracteristics of juice processed by ultrasound.

Natural juices with high increase content of vitamin C could have an important impact on human health and therefore, would be of interest (for consumers, producers and policymakers) to have more information about the methods for improving the content of these valuable antioxidant using modern methods like the ultrasound treatments.

Ultrasound treatment had a significant effect on grapes juice *p*H after all the storage time, compared with untreated juice (table 3). Values of *p*H juice ranged between 3.633 ± 0.006 to 3.447±0.006. During storage, *p*H values and total acidity decrease in all treatment variants. Our results regarding *p*H and total acidity of sonicated samples are in accordance with observations of sonicated kasturi lime juice [17] and apple juice [18].

Ultrasound treatments had significantly effect on the total soluble solids (°Bx) compared with the untreated and pasteurized samples, the highest decrease was observed in case of variant 80% amplitude for 18min, after 4, 10 and 17 storage days. For all variants of treatments, this parameter decreased significantly during storage. The values of total solids content of juice (°Bx) ranged between 24.40 ± 0.000 for pasteurized variant and 11.13 ± 0.112 for variant A80%,18min (table 3). It has been reported that sonication treatment cause a significant decrease in °Brix also of kasturi lime juice [17].

During storage, tartaric acid content increased significantly. A very strong increasement was observed after each storage days in case of samples treated by ultrasound 18 min at both amplitude (A60% and A80%). After 10 and 17 storage days, the pasteurized juices showed a similar increase**.** This increase of tartaric acid content could indicate a start of fermentation none recommended in our case.

As shown in table 3, a significant increase ($p<0.05$) was observed in total phenolic content in the entire grape juice sample sonicated at both amplitude and at both time, also at untreated grape juice. The highest increase was observed in the case of variant A80% 18 min; this increase was in range from 0.687 mg GAE/L up to 1.592 mg GAE/L. The exception is the pasteurized grape juices (in this case, the increase was not significant). The increase of the total phenolic content was found also in the apple juice [18]. The possible reason for significant increases in these phenolic phytonutrients might be attributed to the enhanced disruption of cell wall due to cavitations as a result of rapid change in pressures of the liquid by shear forces exerted during sonication which might lead to the release of some chemically bound polyphenolic phytonutrients and ultimately increased their availability in the juice. Creation of hydroxyl radicals by bubble implosion during sonication to the aromatic ring of phenolic compounds might also be a cause of their improvement in the apple juice. It has already been reported that increase in antioxidant capacity of phenolic compounds might be attributed to the addition of second hydroxyl group in different positions of these compounds chemical structure [17, 18].

Conclusions

Results of this study show a significant difference between the total vitamin C content of the grapes juices treated by different methods, minimally processed

technology (pasteurization / ultrasound used for improve the product shelf life).

Ultrasound treatments, particullary at 80% amplitude for 36 min. had a significant effect on vitamin C content during the storage (ending up with values of 64.498 ± 4.022 mg L-AA/L after 17 storage days). However, the results presented arise from working with a few of biological samples. Also, it is necessary to continue this research with extended experiments.

References

1.AGUILAR, K., GARVIN, A., IBARZ, A., AUGUSTO, P.E.D., Ultrasonics Sonochemistry **37**, no. 7, 2017, p. 375.

2.VINATORU, M., Ultrasonics Sonochemistry, **8**, no. 3, 2001, p. 303-313.

3.VILKHU, K., MAWSON, R., SIMONS, L., BATES, D., Innovative Food Science and Emerging Technologies, **9**, no. 2, 2008, p. 161–169.

4.CHEOK, C.Y., CHIN, N.L., YUSOF, Y.A., TALIB, R.A., LAW, C.L., Industrial crops and Products, **50**, 2013, p. 1-7.

5.DAMSA, F.,WOINAROSCHY, A., OLTEANU, GH., BADARAU, C.L., Rev. Chim. (Bucharest), **67**, no. 4, 2016, p. 665-669.

6.TIWARI, B.K., O'DONNELL, C.P., PATRAS, A., CULLEN, P.J., Journal of Agricultural and Food Chemistry **56**, no. 21, 2008, p. 10071–10077.

7.CAMIRE, M.E., KUBOW, S., DONNELLY, D.J., Critical Reviews in Food Science Nutrition **49**, no. 10, 2009, p. 821- 840.

8.CAHILL, L., COREY, P.N., EI-SOHEMY, A., American Journal Epidemiology **170**, no. 4, 2009, p. 464-471.

9.SCHLEICHER, R.L., CARROLL, M.D., FORD, E.S., LACHER, D.A., American Journal Clinic Nutrition **90**, no. 5, 2009, p.1252-1263.

10.*** EC, Commission directive 2008/1001 EC of 28 October 2008, Official Journal of the European Union. http://eurlex. europa. eulLEXUriServ/LexUriServ.do?uri =OJ:L: 2008:285: 0009:0012: EN:PDF.

11.MCLENNAN, W., PODGER, A., Australian Bureau of Statistics, 1998. http://www.ausstats.abs.gov.au/ausstats/subscriber.nsf/O/CA.

12.BADARAU, C.L., TICAN, A., STEFAN, M., CHIRU, N., Scientific Papers. Series A. Agronomy **60**, 2017 p. 199.

13.ABID, M., JABBAR, S., WU, T., HASHIM, M.M., HU, B., LEI, S., ZHANG, X., ZENG, X., Ultrasonics Sonochemistry **20**, no. 5, 2013, p. 1182-1187.

14.CHENG, L.H., SOH, C.Y., LIEW, S.C., TEH, F.F., Food Chemistry **104**, no. 4, 2007, p. 1398.

15.KORN, M., PRIM, P.M., DE SOUSA, C.S., Microchemical Journal **73**, no. 3, 2002, p. 273-277.

16.SANCHEZ-MATA, M.C., CAMARA-HURTADO, M., DIEZ-MARCHEZ, C., TORIJA-ISASA, M.E., European Food Research and Technology **210**, 2000, p. 220-225.

17.BHAT, R., KAMARUDDIN, L., MIN-TZE, N.S.B.C, KARIM, A.A., Ultrasonics Sonochemistry **18**, no. 6, 2011, p. 1297.

18.ABID, M., JABBAR, S., WU, T., HASHIM, M.M., HU, B.H., LEI, S., ZHANG, X., ZENG, X., Ultrasonics Sonochemistry **20**, , p.1184.

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Article

An Overview of Effects Induced by Pasteurization and High-Power Ultrasound Treatment on the Quality of Red Grape Juice

Alina Margean [1], Mirabela Ioana Lupu [1,*], Ersilia Alexa [2], Vasile Padureanu [1], Cristina Maria Canja [1], Ileana Cocan [2], Monica Negrea [2], Gavrila Calefariu [3] and Mariana-Atena Poiana [2]

1 Food and Tourism Faculty, Transilvania University of Brasov, Castelului 148, 500014 Brasov, Romania; alinamargean@yahoo.com (A.M.); padu@unitbv.ro (V.P.); canja.c@unitbv.ro (C.M.C.)
2 Faculty of Food Engineering, Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Calea Aradului 119, 300641 Timisoara, Romania; alexa.ersilia@yahoo.ro (E.A.); ileanacocan@usab-tm.ro (I.C.); negrea_monica2000@yahoo.com (M.N.); atenapoiana@yahoo.com (M.-A.P.)
3 Faculty of Technological Engineering and Industrial Management, Transilvania University of Brasov, Universitatii 1, 500068 Brasov, Romania; gcalefariu@unitbv.ro
* Correspondence: lupu.mirabela@unitbv.ro; Tel.: +40-766670809

Academic Editors: Diego A. Moreno and Débora Villaño
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Abstract: In juice processing, ultrasound treatment has been tested as a potential alternative to conventional thermal methods to inactivate microorganisms and to enhance the nutritional status of juice. In this study, the impact of pasteurization and high-power ultrasound treatment on the quality of red grape juice was investigated in terms of the content of bioactive compounds such as phenolic compounds and L-ascorbic acid as well as regarding the microbiological and physicochemical properties. The grape juice was subjected to pasteurization (80 °C, 2 min) as well as to ultrasound treatment with an amplitude of 50 and 70% for 5 and 10 min. The results indicated the same level of total phenolic content for pasteurized and sonicated samples for 10 min with an amplitude of 70%, while the highest level of L-ascorbic acid was recorded for sonicated samples with an amplitude of 70% for 10 min. pH of sonicated samples decreased with amplitude and treatment time while total soluble solids and titratable acidity increased with amplitude and time. Moreover, the results indicated the usefulness of juice sonication to enhance the inactivation of microorganisms. Thus, the high-power ultrasound treatment might represent a viable technique to replace the conventional thermal treatment in grape juice processing.

Keywords: red grape juice; pasteurization; high-power ultrasound treatment; phenolic compounds; microorganisms

1. Introduction

Grapes are one of the largest fruit crops harvested around the world. However, grape juice is not consumed worldwide in large amounts because of its higher sugar content or higher acidity, but it can be mixed with apple or other juices [1–3]. The last decades were characterized by numerous studies related to the chemical constituents of grapes, especially phenolic compounds that are secondary plant metabolites with free radical scavenging activity [4,5]. The main phenols in grape belong either to the flavonoid family or the non-flavonoid one. Among the flavonoid family, anthocyanins, flavanols, flavonols and dihydroflavonols are considered to play an important role in the pigmentation and

flavor of grapes berries, while non-flavonoid phenolic compounds such as resveratrol have a role in the powerful biological activities of grape [6,7].

Many research studies have suggested that antioxidants might have positive effects on human health due to their effects against oxidative stress. Thus, it was considered that a diet based on a high daily intake of antioxidant dietary supplements from fruits and vegetables might result in a lower incidence of atherosclerosis, Alzheimer's disease, cancer, ocular disease, diabetes, rheumatoid arthritis and motor neuron disease [8].

In order to reduce microbial grow and to extend their shelf-life, fruit juices are usually processed by pasteurization. Besides the fact that this thermal procedure influences the quality of the products, it also has the disadvantage of denaturing the bioactive compounds due to ionization, hydrolysis and oxidation reactions [5,9]. Considering consumers' need for high quality, flavor and taste of fruit juices, producers are continuously looking for alternatives to the conventional thermal methods. Moreover, the consumers demand for nutritious and safe food products has resulted in increasing interest in non-thermal preservation techniques.

Recently, emerging technologies like ultrasound, microwave and pulsed electric field irradiation have been tested in the food industry to develop various effective food processing applications [10]. Ultrasounds are sound waves of frequencies higher than 16 kHz (higher than usually detected by the human ear). When transmitted through a liquid, solid or gas with elastic properties, the sound waves are moving at a speed depending on the wavelength and the type of material. When acting on a liquid medium, the fundamental effect of ultrasounds is to add to the hydrostatic pressure (P_h) an acoustic pressure (P_a) dependent on time (t), wave frequency (f) and the maximum amplitude of wave P_A. The relationship among the above factors is illustrated by the Equation (1) [10]:

$$P_a = P_A \times \sin(2 \times \pi \times f \times t) \quad (1)$$

The propagation of ultrasound waves in a medium generates physical and chemical effects which can be exploited for improving the efficiency of various food processing operations. Thus, the ultrasound technology was explored to enhance the food quality by reducing the process time, energy and increasing shelf life [11,12]. It has been reported that ultrasound energy can be used to increase extraction yields by disrupting cell tissues [12,13].

In terms of their applications, two approaches are usually used for ultrasound. Low-power ultrasound (LPU), at frequencies above 100 kHz, is a non-invasive technique and might be applied to monitor food processes and to evaluate the physico-chemical food properties, while high-power ultrasound (HPU), with frequencies between 18–100 kHz, causes physical, mechanical and chemical effects and might offer insight into the long term stability of fruit juices [14]. Most often, the power ultrasound range is used in the extraction of biologically active compounds from biological matrices, such as in the extraction of polyphenols from plant and waste food materials. Most of the commercial ultrasonic probes or cleaning baths work at low ultrasound frequencies (20–40 kHz) [15]. Low frequencies have been observed to generate large cavitation bubbles in extraction solvents which implode violently, generating high shear whiles creating microjets that ensure higher cellular degradation, increased solvent penetration and higher extraction rates [15,16]. In juice processing, HPU treatment might be used not only for controlling spoilage microorganisms, but also as an efficient and environmentally friendly treatment for enhancing the nutritional status of juices in terms of increasing the bioactive compounds [7,17]. It was reported that ultrasound was applied in extraction of plant materials because of increased sugar content, total acid content, phenolics content as well as color density of grape juice [2]. Moreover, the ultrasound treatment can influence the quality parameters of cranberry juice and nectar in terms of aromatic profile and sensory properties [18], the stabilization of cantaloupe melon juice [19] or the preservation of monomeric anthocyanins along with a significant microbial load reduction during storage [20].

Therefore, our study represents an attempt to identity alternative fruit processing techniques in order to reduce the microbial load and to improve the nutritional quality of the obtained products.

Moreover, this research reflects the possibility to replace the conventional thermal treatment with HPU treatment as a potential tool for setting up a more efficient and sustainable technology for juice processing.

In line with the above-mentioned considerations, the goal of this research was to investigate the impact of pasteurization and HPU treatment on the quality of red grape juice. In this purpose, the content of bioactive compounds such as phenolic compounds and L-ascorbic acid as well as the physicochemical attributes and the microbiological properties of pasteurized grape juice samples were compared with those of the juice samples subjected to HPU treatments at different parameters such as amplitudes of 50% (A50%) and 70% (A70%) for 5 and 10 min.

2. Results and Discussion

The effects of pasteurization and high-power ultrasound (HPU) treatments on the quality of red grape juice are discussed in terms of bioactive compounds such as total phenolic (TP) content, L-ascorbic acid (L-AsAc) or vitamin C content and the profile of polyphenolic compounds. Also, the changes in physicochemical parameters such as pH, total soluble solids (TSS) and titratable acidity (TA) as well as the microbiological quality expressed by total plate counts (TPC) and *Enterobacteriaceae* count (ENT) were investigated in response to the applied treatments. All mentioned parameters were investigated for untreated red grape juice, as a control sample (C), as well as for red grape juice samples subjected to pasteurization at 80 °C for 2 min (P), respectively to sonication with an amplitudes of 50 and 70% for 5 and 10 min, as follows: HPU (A50%, 5 min), HPU (A50%, 10 min), HPU (A70%, 5 min) and HPU (A70%, 5 min). Some authors also found that ultrasound processing parameters like amplitude and time plays a decisive role in both extraction and retaining of bioactive compounds and in determining the effects on the quality attributes [21].

To provide an overview of the effects induced by pasteurization and HPU treatments on investigated parameters, compared to untreated grape juice samples, the results obtained in this study were processed by a one-way ANOVA test. Based on the data obtained through statistical processing the significance of changes recorded in the investigated parameters in response to the applied treatments, reported with respect to the control grape juice sample can be determined. Also, the changes occurring in the investigated parameters as a result of HPU treatments were evaluated relative to pasteurization for assessing the possibility of replacing the conventional thermal treatment currently used in the grape juice processing, with HPU treatment, as a non-thermal food processing technique.

2.1. Effect of Operating Parameters of HPU Treatments on Temperature of Grape Juice Samples

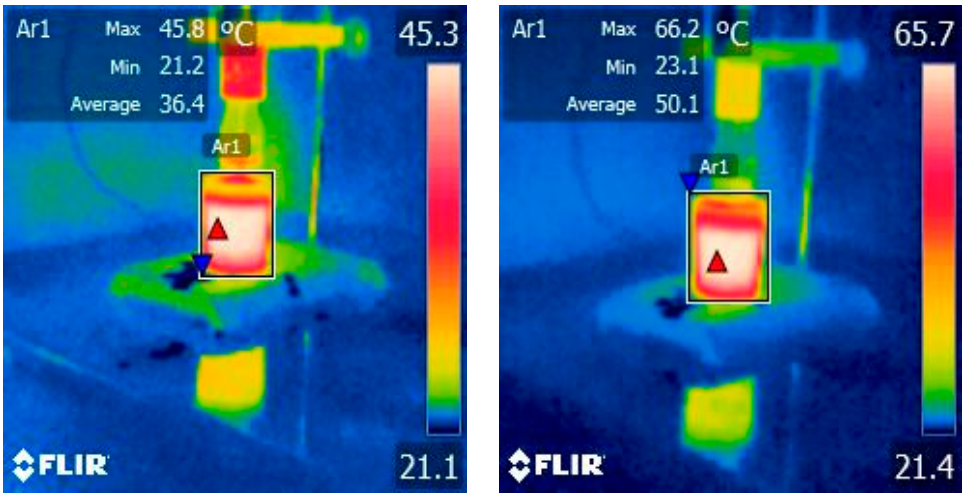
The operating parameters used during the HPU treatments can impact the temperature of the grape juice samples. The average temperature values measured every 2.5 min for ultrasound- treated juice samples are shown in Table 1 while in the Figures 1 and 2 the values measured by a forward-looking infrared (FLIR) camera at the end of sonication treatment with 50 and 70% amplitude can be seen.

During ultrasound treatment, additional attention was paid to measure the heat generated in the samples as an effect of the sonication. To account for the heat issue and the corresponding increase of the temperature, a thermovision camera was used to ensure the temperature of the sample did not exceed 60–66 °C. Temperatures above 70 °C in particular have been shown to lead to rapid polyphenol degradation, hence the need to select efficient extraction temperatures that ensure the stability of phenolic compounds [15,22,23]. It is also important to mention that the sensitivity of a sample to temperature-induced polyphenol degradation depends on the types of polyphenol compounds available in the extract or plant matrix, and their physicochemical and biochemical characteristics, as well as solvent-sample interactions [15].

Table 1. Changes in temperature of juice samples as a result of ultrasound treatments parameters.

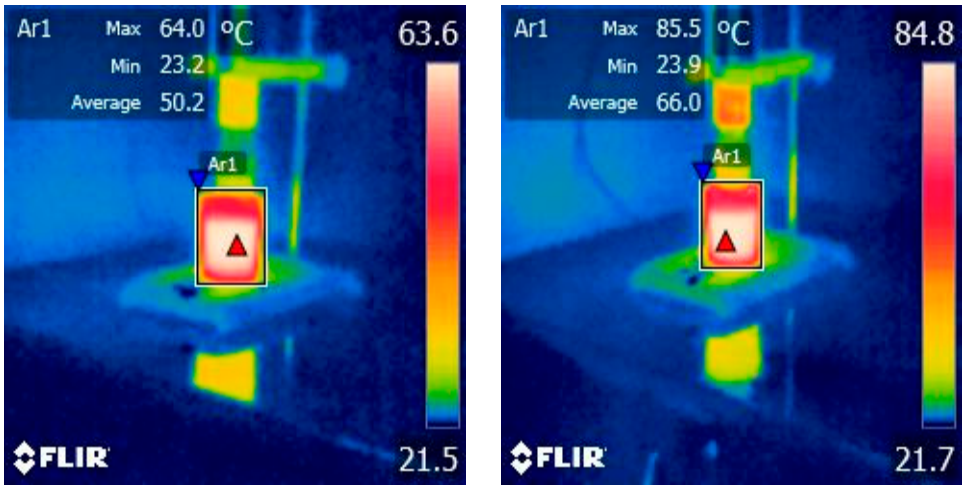
Time of Temperature Measuring (min)	T(°C)	
	HPU (A50%, 5 min)	HPU (A70%, 5 min)
0	18.9 ± 0.85	19.7 ± 0.70
2.5	25.7 ± 0.90	36.9 ± 1.00
5	36.4 ± 1.20	50.1 ± 0.70
Time of Temperature Measuring (min)	**T(°C)**	
	HPU (A50%, 10 min)	**HPU (A70%, 10 min)**
0	17.2 ± 0.85	19.9 ± 0.20
2.5	25.8 ± 0.70	34.9 ± 1.00
5	33.8 ± 1.30	51.4 ± 0.90
10	50.2 ± 1.20	66.0 ± 1.10

Results are expressed as the average value of three replicates ± standard deviation (SD).



(**a**) (**b**)

Figure 1. The temperature measured after 5 min of HPU treatment: (**a**) A50%; (**b**) A70%.



(**a**) (**b**)

Figure 2. The temperature measured after 10 min of HPU treatment: (**a**) A50%; (**b**) A70%.

Preliminary studies related to the temperature increase mention that at a power of 100 W and a frequency of 20 kHz, a treatment over 15 min in duration resulted in a temperature in the sample of about 50 °C [21].

The results obtained by Lieu and Le revealed that temperature and time had positive effects on yield of the treatment process [2]. We observed that the pattern of the average temperatures increasing was linear for both 50 and 70% amplitudes. When the higher amplitude was applied, i.e. 70%, the average temperatures increased by 13.7 °C for the same sonication treatment of 5 min and by 15.8 °C after 10 min.

2.2. Effects of Pasteurization and HPU Treatments on Total Phenolic (TP) Content, L-ascorbic Acid (L-Asac) Content and Polyphenolic Compounds Profile

In Table 2 the total phenolic (TP) and L-ascorbic acid (L-AsAc) contents of red grape juice samples after pasteurization and HPU treatments are presented. These results reveal decreases in TP content, with respect to the control sample, in response to pasteurization and ultrasound treatments.

Table 2. Effect of pasteurization and HPU treatments on the total phenolic (TP) and L-ascorbic acid (L-AsAc) content.

Parameter	Grape Juice Sample					
	C*	P**	HPU (A50%, 5 min)	HPU (A70%, 5 min)	HPU (A50%, 10 min)	HPU (A70%, 10 min)
TP (mg GAE/ 100 mL)	72.86 ± 0.02^{a}	70.64 ± 0.08^{b}	50.68 ± 0.05^{c}	54.56 ± 0.07^{d}	50.71 ± 0.09^{c}	69.88 ± 0.02^{e}
L-AsAc (mg/L)	454.4 ± 0.11^{a}	340.8 ± 0.09^{b}	227.2 ± 0.04^{c}	349.8 ± 0.05^{b}	458.4 ± 0.10^{a}	568 ± 0.08^{d}

* Control sample; ** pasteurized juice sample. One-way ANOVA test was used to compare the means differences among treatments; different superscripts in the same row indicate significant differences among the treatments (Tukey's test, $p < 0.05$). Results are expressed as the average value of three replicates ± standard deviation (SD).

The content of total phenols quantified as gallic acid equivalent (GAE) in treated red grape juice samples varies from 50.68 to 70.64 mg GAE/100 mL. Another study reported a level of TP content in red grape juices in the range 744–1177 mg GAE/L [24]. TP content among sonicated samples was significantly affected by the amplitude level ($p < 0.05$) and not impacted by the treatment time ($p > 0.05$).

In Figure 3 the changes of TP content in juice samples in response to the applied treatments are depicted. It can be noted that there were losses in the TP content of the grape juice samples in response to pasteurization compared to ultrasound treatment. Thus, Figure 3a shows the losses of TP content induced by pasteurization and HPU treatment with respect to the control sample while Figure 3b reveals the losses in juice sampled subjected to HPU treatment compared to the pasteurized sample.

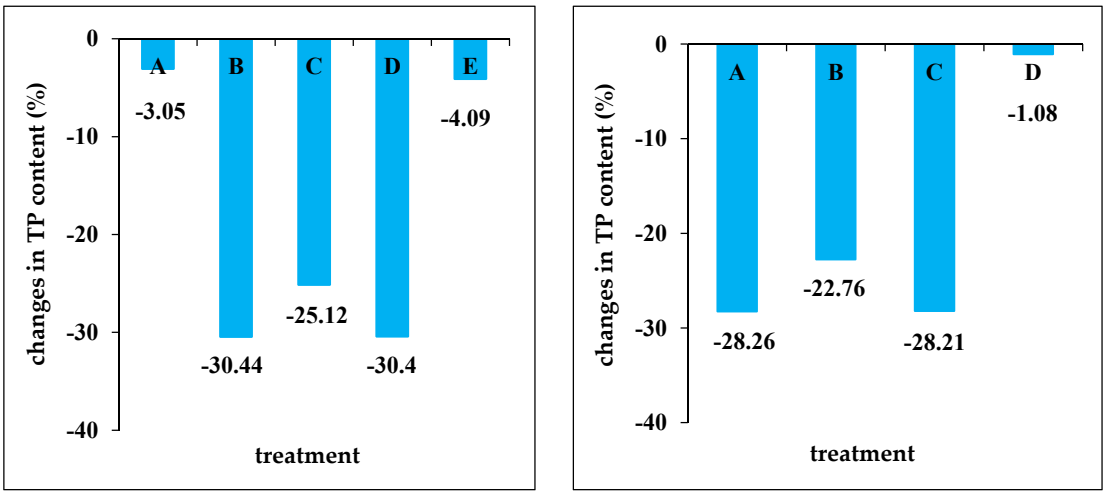
The first thing we could notice about the data from Figure 3a is that the pasteurization induced the lowest decreases in TP content, compared to the control sample (3.05%), while the HPU treatments (A50%, 5 min; A70%, 5 min and A50%, 10 min) led to the losses in the range 25–30%. It is worth noting that the HPU treatment (A70%, 10 min) has an effect close to that obtained through pasteurization, as regards the TP content losses (4.09%).

A closer look at Figure 3b reveals that that the losses of TP content induced by HPU treatments (A50%, 5 min; A70%, 5 min and A50%, 10 min) were in the range of about 22–28%, compared to the pasteurized sample. The HPU treatment (A70%, 10 min) induced the lowest losses in TP content, about 1% compared to the pasteurized sample. Therefore, by choosing an HPU treatment for 10 min with an amplitude of 70%, the retention of total phenolic compounds in grape juice sample could be improved.

The initial content of L-ascorbic acid in untreated red grape juice sample was 454.4 mg/L. Decreases in the L-ascorbic acid content in response to pasteurization and HPU treatment applied to red grape juice were recorded, indicating that ascorbic acid is highly thermosensitive. The L-ascorbic acid content in grape juice samples decreased by 25% in response to pasteurization and HPU treatment (A50%, 5 min) and by 53% by applying HPU treatment at a higher amplitude (A70%, 5 min).

A: P;
B: HPU (A50%, 5 min);
C: HPU (A70%, 5 min);
D: HPU (A50%, 10 min);
E: HPU (A70%, 10 min).

A: HPU (A50%, 5 min);
B: HPU (A70%, 5 min);
C: HPU (A50%, 10 min);
D: HPU (A70%, 10 min).



(a) **(b)**

Figure 3. The changes in total phenolic (TP) content of juice samples in response to the applied treatments: (**a**) reported to the control sample (C); (**b**) reported to the pasteurized sample (P).

The degradation of L-ascorbic acid is probably attributable to the oxidation processes that occur during these treatments. The results were in agreement with other previous studies. Thus, data reported by Cao et al. [25] reveals an initial content of ascorbic acid in fresh bayberry juice of 22.82 mg/100 mL and a decrease of 25.16% in response to pasteurization. Adekunte et al. [26] also reported that the content of ascorbic acid of tomato juice decreased by 96.9 to 60.7% in response to ultrasound treatment of 24.4–61 μm for 2–10 min.

However, after HPU treatment (A70%, 10 min) an increase in L-AsAc content to 568 mg/L in comparison with the untreated grape juice was recorded. An increase of ascorbic acid was also detected by other authors at high amplitude [27–29]. An explanation could be the elimination of dissolved oxygen, essential for ascorbic acid degradation, during the cavitation produced by the sonication treatment [28] which prevents ascorbic acid breakdown.

Figure 4 provides information regarding the changes recorded in L-AsAc content in juice samples in response to the applied treatments. It can be observed that by exposing the grape juice to pasteurization, compared to ultrasound treatments, both losses and increases in the content of L-AsAc were recorded. The effect of P and HPU treatment on L-AsAc content reported to the control sample is shown in the Figure 4a. In addition, the changes in L-AsAc content recorded in juice samples subjected to HPU treatment versus the pasteurized sample are presented in Figure 4b.

Our data reveal that pasteurization and the HPU treatments (A50%, 5 min; A70%, 5 min) induced losses in L-AsAc content as follows: 25, 50 and 23.02% with respect to the control sample. Contrary to this finding, by applying HPU treatments (A50%, 10 min; A70%, 10 min) increases in L-AsAc content of 0.88, or 25%, were recorded compared to the control sample (Figure 4a). As regards the changes in L-AsAc content induced by HPU treatment with regards to the pasteurized sample, the data depicted in Figure 4b prove that only by HPU treatment (A50%, 5 min) were losses observed. By applying the other HPU treatments (A70%, 5 min; A50%, 10 min; A70%, 10 min) increases in L-AsAc content, compared to the pasteurized sample, were recorded as follows: 2.64, 34.51 and 66.67%,. These data

highlight that the HPU treatment (A70%, 10 min) was the most effective among the investigated treatments to ensure a high content of vitamin C in grape juice samples.

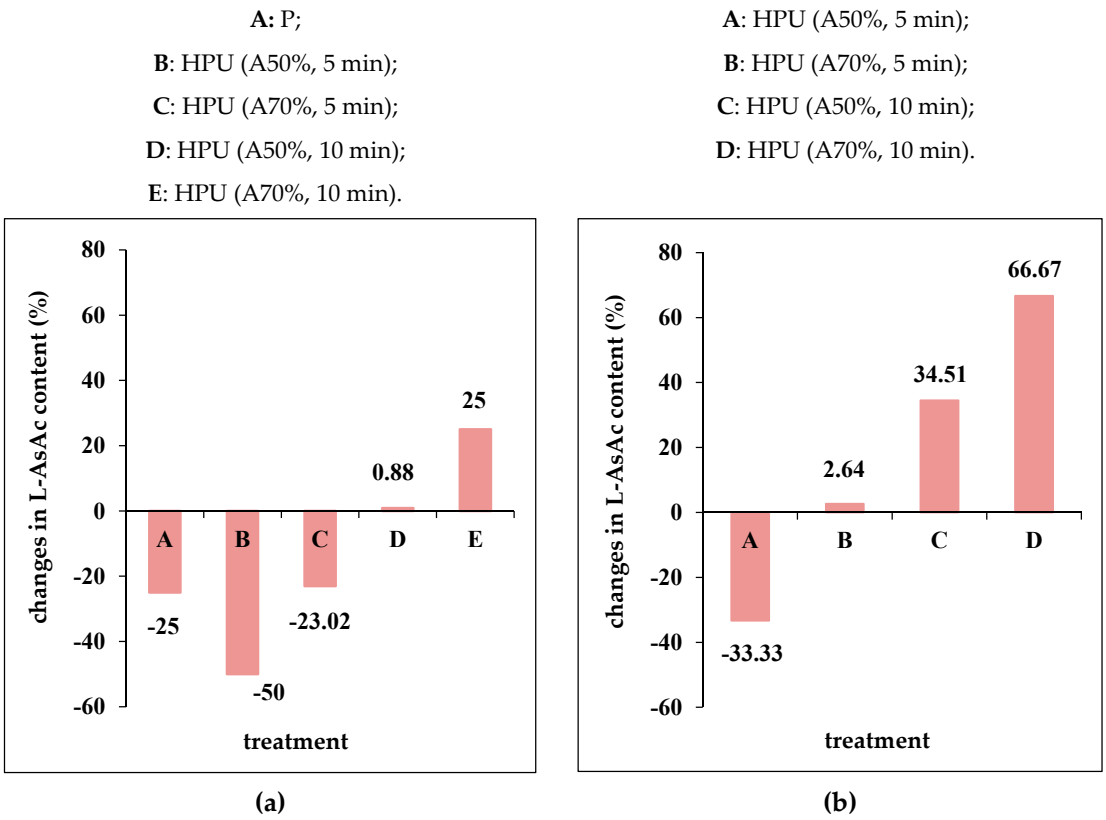


Figure 4. The changes in L-ascorbic acid (L-AsAc) content of juice samples in response to the applied treatments: (**a**) reported to the control sample (C); (**b**) reported to the pasteurized sample (P).

Polyphenols play an important role among red grape components as they determine the flavor and color of the juice [30,31] and have several beneficial properties for human health [30,32]. The evolution of individual phenolic compounds of red grape juice is shown in Table 3. Among phenolic compounds, the most known is resveratrol. Significantly higher amounts of resveratrol were observed in the grape juice samples exposed to ultrasound treatment, especially at A70%, 5 min (8.54 mg/L). Resveratrol was not detected in the juice sample subjected to pasteurization that suggests that during this thermal treatment it was destroyed. An increase of resveratrol in the ultrasound processing of grape juice was also observed by Hasan et al. [33]. A slight increase in caffeic acid has been reported in the clear apple juice treated with ultra-high pressure homogenization which is also a non-thermal technique [34] and also in a study on apple juice treatment by ultrasound at 70% amplitude and 25 kHz frequency [35]. An increase in gallic acid content from 2.15 mg/L recorded in the control sample to 2.16 mg/L in pasteurized samples and to 31.3 mg/L in a juice sample subjected to HPU treatment (A70%, 5 min) can be noted The highest level of protocatechuic acid (5.13 mg/L), epicatechin (76.80 mg/L), *p*-coumaric acid (1.20 mg/L), rutin (63.31 mg/L), rosmarinic acid (25.93 mg/L), quercetin (5.62 mg/L) and kaempferol (4.59 mg/L) were also detected in the grape juice samples subjected to HPU treatment (A70%, 5 min). The phenolic compounds can be found both in soluble form in the vacuole or bound to the other compounds such as cellulose, hemicellulose, pectin and lignin traces of the cell wall [28]. The increase of polyphenolic compounds in sonicated juice samples might be ascribed to the increase in the extraction efficacy by ultrasounds treatment causing disruption of cell walls and ultimately the liberation of bound polyphenolic compounds [27,36]. Also, the ultrasound treatment might enhance the release of phenolic compounds from the cell walls, due to the collapse through cavitation process in the surroundings of colloidal particles found in juice [28].

Table 3. Effect of pasteurization and HPU treatments on the polyphenolic compounds profile of grape juice samples.

Polyphenolic Compounds (mg/L)	Grape Juice Sample					
	C*	P**	HPU (A50%, 5 min)	HPU (A70%, 5 min)	HPU (A50%, 10 min)	HPU (A70%, 10 min)
Gallic acid	2.15 ± 0.03^{a}	2.16 ± 0.05^{a}	15.23 ± 0.04^{b}	31.30 ± 0.07^{c}	12.82 ± 0.11^{b}	19.49 ± 0.09^{c}
Protocatechuic acid	n.d.	2.08 ± 0.35^{a}	n.d.	5.13 ± 0.05 ^{b}	0.79 ± 0.44^{c}	n.d.
Caffeic acid	0.93 ± 0.21^{a}	2.62 ± 0.37^{a}	1.30 ± 0.52^{a}	14.31 ± 0.06^{ab}	4.41 ± 0.08^{ab}	23.27 ± 0.04^{ab}
Epicatechin	3.70 ± 0.16^{a}	10.70 ± 0.33^{a}	5.45 ± 0.15^{a}	76.80 ± 0.07^{ab}	n.d.	35.31 ± 0.54^{ab}
p-cumaric acid	0.28 ± 0.03^{a}	0.1 ± 0.11^{a}	0.04 ± 0.04^{a}	1.20 ± 0.04^{a}	1.13 ± 0.05^{a}	n.d.
Ferulic acid	0.27 ± 0,01^{a}	0.32 ± 0.03^{a}	n.d.	n.d.	n.d.	0.81 ± 0.12^{b}
Rutin	2.09 ± 0.11^{a}	2.51 ± 0.06^{a}	9.61 ± 0.1^{a}	63.31 ± 0.02^{b}	n.d.	39.02 ± 0.1^{ab}
Rosmarinic acid	n.d.	n.d.	1.19 ± 0.06^{a}	25.93 ± 0.21^{b}	0.39 ± 0.08^{a}	4.31 ± 0.05^{b}
Resveratrol	0.21 ± 0.12^{a}	n.d.	0.65 ± 0.13^{ab}	8.54 ± 0.14^{b}	0.80 ± 0.06^{ab}	2.48 ± 0.04^{b}
Quercetin	0.38 ± 0.07^{a}	n.d.	n.d.	5.62 ± 0.02^{b}	0.31 ± 0.08^{a}	0.71 ± 0.03^{ab}
Kaempferol	0.83 ± 0.05^{a}	0.92 ± 0.06^{a}	n.d.	4.59 ± 0.12^{b}	n.d.	n.d.

* Control sample; ** pasteurized juice sample; n.d. - not detected. One-way ANOVA test was used to compare the means differences among treatments; different superscripts in the same row indicate significant differences among the treatments (Tukey's test, $p < 0.05$). Results are expressed as the average value of three replicates ± standard deviation (SD).

We can appreciate that the overall polyphenolic profile of the red grape juice samples is given by the contribution of a multitude of individual polyphenolic compounds. It is well known that red grapes are recognized for the significant amount of anthocyanins in their composition (300–7500 mg/kg fresh weight) and less for the content of monomeric flavanols such as epicatechin (30–175 mg/kg fresh weight) or flavonols as quercetin or kaempferol (15–40 mg/kg fresh weight) [37]. Antocyanins, the most relevant polyphenolic compounds in red grapes, with flavonoid-like glycoside structures, show similar behavior under heating and their degradation depends on the temperature and pH [38]. At pH = 1 all the glycosidic bonds are susceptible to hydrolysis, while at a pH values in the range 2–4, specific for grape juice, aglycone-sugar bond is the most labile of the glycosidic bonds [38]. This means that under our working conditions the anthocyanins are less susceptible to destruction compared to flavonoids, which might justify the tendency of total phenolic content to increase, compared to the investigated individual polyphenolic compounds. In addition, the increase of total polyphenols content can also be attributed to hydroxycinnamic acids whose content can increase with the amplitude, respectively with the temperature.

The increase of HPU treatment time from 5 to 10 min, for a constant amplitude of 50 and 70%, leads to a significant increase in the temperature of the process, as was shown in Figures 1 and 2. The HPU treatment (A70%, 5 min) produces a heating from 23.1 up to 66.2 °C with an average value of 50.1 °C, while for HPU (A70%, 10 min) the temperature increases from 23.9 up to 85.5 °C with an average value of 66 °C. At a higher temperature, exceeding 60 °C, especially over extended periods, some phenolic compounds suffer oxidative degradation. It is also important to mention that the sensitivity of a sample to temperature-induced polyphenol degradation depends on the types of available polyphenol compounds [15]. Sharma et al. [39], highlighted that after heating, a decrease in total flavonoids was observed, which indicates that some flavonoids were probably destroyed, however, the total phenolics were increased [39]. In most fruits and vegetables, flavonoids contain C-glycoside bonds and exist as dimers and oligomers. Thermal food processing methods such as heating or boiling result in the formation of monomers by the hydrolysis of C-glycosides bonds [39]. Ross et al. [40], have shown that catechin and epicatechin content decreased with increasing heating temperature, but the content of some hydroxycinnamic acids increased.

According to the reported results it can be observed that both the ultrasound processing time and amplitude are influential factors for preserving the content of active principles. Our data reveal that HPU treatment with an amplitude of 70% for 10 min led to an increase in the TP and L-AsAc content, compared to 5 min, while HPU treatment (A70%, 5 min) might favor an increase of the levels of flavonoids such as rutin, quercetin, epicatechin and kaempferol as well as resveratrol, a natural

stilbene found in grape juice. At the same time, some hydroxycinnamic acids such as ferulic and caffeic acid are found in higher concentrations after exposing the grape juice to HPU treatment (A70%, 10 min).

2.3. Effects of Pasteurization and HPU Treatments on Ph, Total Soluble Solids (TSS) and Titratable Acidity (TA)

Physicochemical parameters such as pH, total soluble solids (TSS) and titratable acidity (TA) of untreated and pasteurized grape juice along with grape juice treated for 5 and 10 min with different ultrasound amplitudes (A50% and A70%) are shown in Table 4.

Table 4. Effects of ultrasound and pasteurization treatments on the pH, total soluble solids (TSS) and titratable acidity (TA).

Parameter	Grape Juice Sample					
	C*	P**	HPU (A50%, 5 min)	HPU (A70%, 5 min)	HPU (A50%, 10 min)	HPU (A70%, 10 min)
pH	3.62 ± 0.01^{a}	3.59 ± 0.01^{b}	3.52 ± 0.01^{c}	3.51 ± 0.01^{c}	3.50 ± 0.01^{d}	3.50 ± 0.01^{d}
TSS (°Brix)	30.18 ± 0.13^{a}	30.73 ± 0.09^{a}	26.47 ± 0.09^{bc}	27.73 ± 0.09^{b}	$27.13 \pm 0{,}05^{abc}$	29.67 ± 0.09^{ab}
TA (g of tartaric acid /L)	3.75 ± 0.07^{a}	4.23 ± 0.08^{b}	$3.79 \pm 0{,}03^{a}$	3.97 ± 0.12^{abc}	3.94 ± 0.16^{ab}	4.18 ± 0.11^{bc}

* Control sample; ** pasteurized juice sample. One-way ANOVA test was used to compare the means differences among treatments; different superscripts in the same row indicate significant differences among the treatments (Tukey's test, $p < 0.05$). Results are expressed as the average value of three replicates ± standard deviation (SD).

The results suggested that HPU treatment causes changes in samples as the pH decreased with increasing amplitude and treatment time. However, the pH value among sonicated samples was found to be significantly influenced by the treatment time ($p < 0.001$) and not influenced by the amplitude level ($p > 0.05$). Also, the pH values recorded for all juice samples subjected to HPU treatments were significantly different than those recorded for C and P samples ($p < 0.001$).

TSS of samples treated with different amplitudes and treatment times indicate a decrease compared with pasteurized juice. For sonicated samples, results suggested that TSS increased with amplitude and time, the value of sample sonicated with amplitude 70% for 10 min being 1.71% lower than for pasteurized juice. Although HPU treatment induced slight changes in samples, statistically the TSS content among sonicated samples was significantly influenced by amplitude level ($p < 0.001$) and not influenced by treatment time ($p > 0.05$). Also, all HPU samples were significantly different than the P sample ($p < 0.001$).

A higher value of TA was observed for pasteurized samples. For sonicated samples, results suggested that TA increased with amplitude and time, the value of sample sonicated with an amplitude of 70% for 10 min being 1.18% lower than the value recorded for the pasteurized juice sample. TA among sonicated samples was significantly influenced by both amplitude level and treatment time ($p < 0.05$).

As mentioned earlier, it is clear from the data that the different assayed combinations of ultrasound frequency and exposure time exert slight effects on the pH, TSS and TA of grape juice. Sugars are the main components of the fruit juices, being an important quality attribute and influencing consumers to accept or reject a product.

As regards the total soluble solids, around 80% are constituted by sugars [21]. The results indicated that pasteurization and sonication treatments had different effects on the total soluble solids of the grape juice. Our results related to TSS are similar to the results of sonicated sweet lime [21] and kastuni juice [41] and in contrast with the ones of sonicated orange juice and carrot [21]. However, Gao and Rupasinghe [42] obtained similar results as of both lower TSS of the sonicated sample compared with pasteurized one for an apple-carrot ratio of 60:40 and 90:10 and lower pH for an apple-carrot ratio of 90:10. Other authors found no significant changes in pH, TSS and TA due to the sonication treatment of grape juice using an ultrasonic treatment of 25 kHz at a constant temperature [27], or any changes [43] using an ultrasonic treatment of 28 kHz, respectively.

Regarding the TA, our results are not similar with the results observed by different authors in grape mash [2], apple-carrot juice [42] and grapes [44], which suggests that ultrasound treatment augmented the tartaric acid extraction. This may be attributed to the fact that we used high power ultrasound at a fixed frequency of 20 kHz.

2.4. Microbiological Quality

The application of sonication treatments has been recognized as a potential innovative technology that can meet Food and Drug Administration's (FDA's) requirements for achieving a 5-log reduction in the contaminant microorganisms (or pathogens) associated with fruit juices [45,46]. The microbiological quality of the grape juice samples was assessed on the basis of two indicators, namely the total plate count (TPC) and the *Enterobacteriaceae* count (ENT).

The total plate count (TPC) is used as a reliable indicator for the bacterial populations of a food sample. It is also called the aerobic colony count, the standard plate count, the mesophilic count or the aerobic plate count. TPC provides information about the total microbial load in a food sample. TPC quantifies the aerobic, mesophillic organisms that grow under aerobic conditions at a moderate temperature in the range 20–45 °C. The TPC includes all pathogens and non-pathogens and is used to assess the hygiene status of a food product [47]. The *Enterobacteriaceae* count (ENT) is considered by food producers as an indicator of hygiene practices, being useful to monitor the effectiveness of implemented preventive measures. The indicator microorganisms are commonly used to measure the quality of the practices used in order to ensure a proper processing of food products. The *Enterobacteriaceae* or *Escherichia coli* are used for assessing the enteric contamination of a food [48].

In Table 5 a reduction in the microbial load after pasteurization and sonication treatments of red grape juice can be observed. The control sample showed values of 4.53 log colony forming units (CFU)/mL for TPC and 1.51 log CFU/mL for ENT. The microorganisms were reduced in the pasteurized juice sample (TPC was 2.53 log CFU/mL and ENT 0.73 log CFU/mL) as well as in all samples exposed to HPU treatments. ENT and TPC of the sonicated samples were significantly influenced by the treatment time and the amplitude level ($p < 0.001$). As the data from Table 5 reveal, TPC and ENT recorded a decrease versus the control sample by increasing the amplitude level from 50 to 70% as well as by increasing the sonication time from 5 to 10 min. The lowest bacterial counts (TPC: 1.13 log CFU/mL and ENT: 0.53 log CFU/mL) were recorded after exposing the red grape juice sample to HPU treatment (A70%, 10 min). Similar results have been reported by other authors [41,45,49], revealing that the microbial load was lowered by increasing the ultrasound processing time. This indicates that the microbial cells might be resistant to sonication treatment and the cell destruction occurs only as the sonication treatment time is increased to a longer duration. Cell disruption may be caused by several factors such as combined physical and chemical mechanisms that occur during cavitation (caused by the changes in pressure), the formation of free radicals and hydrogen peroxide [49,50], leading to a thinning of microbial cell membranes, and the localized mild heating that occurs during sonication treatments [45,49].

Table 5. Impact of pasteurization and HPU treatments on the total plate count (TPC) and the *Enterobacteriaceae* count (ENT) of grape juice.

Parameter	Grape Juice Sample					
	C*	P**	HPU (A50%, 5 min)	HPU (A70%, 5 min)	HPU (A50%, 10 min)	HPU (A70%, 10 min)
TPC (log CFU/mL)	4.53 ± 0.01^{a}	2.53 ± 0.07^{b}	2.18 ± 0.03^{cd}	1.42 ± 0.02^{cd}	1.63 ± 0.02^{c}	1.13 ± 0.04^{c}
ENT (log CFU/mL)	1.51 ± 0.01^{a}	0.73 ± 0.04^{ab}	1.45 ± 0.05^{ab}	0.69 ± 0.03^{ab}	1.27 ± 0.02^{acd}	0.53 ± 0.0^{acd}

* Control sample; ** pasteurized juice sample. One-way ANOVA test was used to compare the means differences among treatments; different superscripts in the same row indicate significant differences among the treatments (Tukey's test, $p < 0.05$). Results are expressed as the average value of three replicates ± standard deviation (SD).

3. Materials and Methods

3.1. Grape Juice Preparation

Fresh red grapes (Merlot, from the Pietroasa Development Research Station for Viticulture and Winery, Buzau County, Romania) were split from bunches and crushed with a crusher-destemmer (Enoventa Technologies Enologiche, Piazzola sul Brenta, Italy) followed by the pressing process with a hydraulic press machine (L.U.C.M.E. Elettromeccanica, Verona, Italy). Only fruits without external injuries were used. Juice extraction and filtration were performed in a cold room at a temperature of 10 ± 1 °C. The obtained red grape juice was further subjected to pasteurization and HPU treatments. As a control sample was used the red grape juice before applying of any treatments. The control sample as well as the pasteurized and HPU treated juice samples were kept in sterilized, airtight container (bottles) and were stored at 4 °C until further analysis. All sample preparations and treatments were carried out in triplicate [45].

3.2. Pasteurization Process

Pasteurization of the grape juice samples was carried out on a hotplate with a magnetic mixer (IKA RTC Basic, Ika-Werke GmbH & Co. KG, Janke & Kunkel, Staufen, Germany) where the samples (150 mL) were placed in glass containers covered with aluminium foil over hot water bath at a temperature of 80 °C for 2 min. This temperature was chosen based on the previously reported results [18].

3.3. Ultrasound Equipment and Processing

The ultrasound treatments were conducted using a 750 W ultrasonic processor for small and medium volume applications (VCX 750, Sonics & Materials, Inc., Newtown, CT, USA) with a 1/2" (13 mm) probe used for ultrasound at a constant frequency of 20 kHz. A sample of 150 mL was placed in a glass beaker and the ultrasound probe was fixed at 45 mm depth in the juice sample. The amplitude was set at 50% (A50%) and 70% (A70%) and for each of these amplitudes, the grape juice samples were exposed to ultrasounds for 5 and 10 min. The main variables that are influencing the sonication are the intensity and the frequency of the waves. The amplitude of vibration of the ultrasonic source is proportional with the intensity of sonication that is the power dissipated per unit of surface area (W/cm^2) of the sonotrode. Thus, an augmentation of the amplitude induces an increasing of the sonochemical effects in the treated liquids. In order to identify the best extraction configuration, the amplitude may vary over a wide range [10]. Thus, depending on the amplitude and duration, four ultrasounds treatments were applied to the grape juice samples, as follows: HPU (A50%, 5 min), HPU (A50%, 10 min), HPU (A70%, 5 min) and HPU (A70%, 5 min). These combinations of amplitude and time of the applied HPU treatments were established according to the results of other previous studies, where a reduction of microbial count was obtained [28,49].

3.4. pH, Total Soluble Solids (TSS) and Titratable Acidity (TA)

The pH was measured using the electrochemical method [51] with a potentiometer (Consort C1010, Consort, Turnhout, Belgium). Titratable acidity (TA) and total soluble solids (TSS) of red grape juice samples were determined following OIV reference methods [52]. Total soluble solids (°Brix) were measured with an ABBE refractometer (ORT 1RS, KERN & SOHN GmbH, Balingen, Germany). Titratable acidity tests were performed on aliquots of 50 mL of the sample, 30 mL of boiled distilled water and 1 mL of bromothymol blue solution placed into a 250 mL beaker and titrated with standardized 0.1 mol/L NaOH (Sigma-Aldrich, Dublin, Ireland) until the same color was obtained as in the preliminary test (end-point color determination). The results were expressed as g of tartaric acid/L of juice.

3.5. L-ascorbic Acid Content (L-AsAc)

L-ascorbic acid (L-AsAc) or vitamin C determination of investigated grape juice samples was carried out by the 2,6-dichloroindophenol titrimetric method [53]. For this purpose, 10 mL of each sample was diluted with 10 mL oxalic acid 2% (Sigma-Aldrich), then, the mixture was filtered through Whatman filter paper and the clear extract was used for analysis. Further, 10 mL of the clear extract was taken and 1 mL hydrochloric acid 1N (ReAgent Chemicals, Runcorn, UK) was added. The obtained mixture was titrated with 2,6-Dichloroindophenol sodium salt (Sigma-Aldrich). A control sample titration was also performed. The results were expressed as mg/L of juice.

3.6. Determination of Total Phenolic (TP) Content and Polyphenolic Compounds Profile

The total phenolic content was determined by Folin-Ciocalteu assay [54]. For this purpose, 0.5 mL sample was treated with 1.25 mL reagent Folin-Ciocalteu (Merck, Darmstadt, Germany) diluted 1:10 with water. The sample was incubated for 5 min at room temperature and then 1 mL Na_2CO_3 60 g/L was added. After 30 min of incubation at 50 °C the absorption of samples was measured at 750 nm using a UV-VIS spectrophotometer (Specord 205, Analytic Jena, Jena, Germany). The calibration curve was prepared using gallic acid as standard in the concentration range 5-250 μg/mL. The results were expressed in mg gallic acid equivalents (GAE)/100 mL of juice.

The polyphenolic compounds profile was determinate by high performance liquid chromatography coupled with mass spectrometry (LC-MS) according to the method described by Abdel-Hameed et al. [55]. Thus, the main polyphenols from red grape juice samples were determined by LC-MS using SPD-10A UV (Shimadzu, Kyoto, Japan) and LC-MS 2010 detectors, and an EC 150/2 NUCLEODUR C18 Gravity SB 150 × 2 mm × 5 μm column (Macherey-Nagel, Düren, Germany). Chromatographic conditions were as follows: mobile phases A: water with formic acid at pH-3, B: acetonitrile with formic acid at pH-3, gradient program: 0.01–20 min 5%B, 20.01–50 min 5–40%B, 5–55 min, 40–95%B, 55–60 min 95%B. Mobile phase 0.2 mL/min, temperature 20 °C. The monitoring wavelength was 280 nm and 320. Calibration curves were performed between 20–50 μg/mL. Results were expressed in mg/L.

3.7. Microbiological Analysis

Total plate counts (TPC) and *Enterobacteriaceae* count (ENT) were carried out to evaluate the microbiological quality of the samples. The analyses were performed in triplicate according to the methodology established by the American Public Health Association. The samples were collected aseptically in a sterile vessel, immediately after processing. For sample preparation, mixing was performed using a stirrer to ensure a uniform distribution of the microorganisms in the mass product, followed by five dilutions consecutively (10–5). As dilution liquid was used sterile distilled water. TPC determination was performed according to SR EN ISO 4833 [56] using Plate Count Agar (PCA) medium and the detection of *Enterobacteriaceae* was performed according to ISO 21528-1,2, [57] using VRBG medium (agar with purple red ball glucose). The PCA medium allows the growth of all aerobic germs and the other two culture media have selective character, containing inhibitory factors for the development of other microorganisms, other than *Enterobacteriaceae*, respectively yeasts and molds. After sowing of two Petri plates from each consecutive serial dilution, they were incubated for a different period of time, namely: 72 h for TPC at 30 °C and 37 °C and 24 h in the case of *Enterobacteriaceae* at 37 °C [58]. The results were expressed as log colony forming units per milliliter of juice (log CFU/mL).

3.8. Data Analysis

All values were obtained from three independent experiments and each red grape juice sample was analyzed in triplicate. All obtained results are presented as average values followed by the standard deviation (SD) of three replicates. The one-way analysis of variance (ANOVA) test was used to analyze the data and differences among means obtained in response to the applied treatments

were compared by a Tukey test with a level of significance of $p < 0.05$. The statistical data analysis was performed using the JASP (Version 0.11.1, 2019) computer software (JASP Team, University of Amsterdam, Amsterdam, The Netherlandss).

4. Conclusions

The obtained results reveal that compared to pasteurization, the HPU treatment significantly improved the individual polyphenolic compounds and the L-AsAc content, without any significant effect on pH, TA and TSS. Also, important decreases in microbial counts without affecting the investigated bioactive compounds and physicochemical parameters of grape juice samples were induced by HPU treatment, compared to pasteurization. The TPC and ENT analysis results of the sonicated samples were significantly influenced by treatment time and amplitude level. The quality of red grape juice is influenced by choosing appropriate ultrasound treatment parameters. The ultrasound treatments with an amplitude of 70% were more efficient than those with A50% in terms of all investigated parameters. As for the processing time, our results reveal that while HPU treatment (A70%, 5 min) is recommended for enhancing the level of flavonoids such as rutin, quercetin, epicatechin, kaempferol as well as resveratrol, the HPU treatment (A70%, 10 min) was the most efficient in the reduction of the microbial load and obtaining the highest content of L-AsAc and TP together with a high level of some hydroxycinnamic acids as ferulic and caffeic acid. The data reported in this study suggest that HPU treatment might be used as an alternative emerging technique to successfully replace the pasteurization for improving the quality of the red grape juice. Nevertheless, we could go further in the experiments to establish the most adequate operating conditions of HPU treatment to be in agreement with national and international requirements in terms of microbial load for extending the self-life of grape juice.

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References

1. Kashyap, D.R.; Vohra, P.K.; Chopra, S.; Tewari, R. Applications of pectinases in the commercial sector: A review. *Bioresour. Technol.* **2001**, *77*, 215–227. [CrossRef]
2. Lieu, L.N.; Le, V.V.M. Application of ultrasound in grape mash treatment in juice processing. *Ultrason. Sonochem.* **2010**, *17*, 273–279. [CrossRef] [PubMed]
3. Spinelli, F.R.; Dutra, S.V.; Carnieli, G.; Leonardelli, S.; Drehmer, A.P.; Vanderlinde, R. Detection of addition of apple juice in purple grape juice. *Food Control* **2016**, *69*, 1–4. [CrossRef]
4. Martín, J.; Kuskoski, E.M.; Navas, M.J.; Asuero, A.G. Antioxidant Capacity of Anthocyanin Pigments. In *Flavonoids—From Biosynthesis to Human Health*; Justino, G.C., Ed.; IntechOpen: London, UK, 2017; pp. 205–255.
5. Ignat, I.; Volf, I.; Popa, V.I. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chem.* **2011**, *126*, 1821–1835. [CrossRef]
6. Moreno-Arribas, M.V.; Polo, M.C. Phenolic Compounds. In *Wine Chemistry and Biochemistry*; Moreno-Arribas, M.V., Polo, M.C., Eds.; Springer: New York, NY, USA, 2009; p. 437.
7. Nadeem, M.; Ubaid, N.; Qureshi, M.T.; Munir, M.; Mehmood, A. Effect of ultrasound and chemical treatment on total phenol, flavonoids and antioxidant properties on carrot-grape juice blend during storage. *Ultrason. Sonochem.* **2018**, *45*, 1–6. [CrossRef]

8. Hajhashemi, V.; Vaseghi, G.; Pourfarzam, M.; Abdollahi, A. Are antioxidants helpful for disease prevention? *Res. Pharm. Sci.* **2010**, *5*, 1–8.
9. Paniwnyk, L. Applications of ultrasound in processing of liquid foods: A review. *Ultrason. Sonochem.* **2017**, *38*, 794–806. [CrossRef]
10. Clodoveo, M.L.; Dipalmo, T.; Rizzello, C.G.; Corbo, F.; Crupi, P. Emerging technology to develop novel red winemaking practices: An overview. *Innov. Food Sci. Emerg. Technol.* **2016**, *38*, 41–56. [CrossRef]
11. Altemimi, A.; Choudhary, R.; Watson, D.G.; Lightfoot, D.A. Effects of ultrasonic treatments on the polyphenol and antioxidant content of spinach extracts. *Ultrason. Sonochem.* **2015**, *24*, 247–255. [CrossRef]
12. Bakht, A.; Geesi, M.H.; Riadi, Y.; Imran, M.; Ali, I.; Ahsan, M.J.; Ajmal, N. Ultrasound-assisted extraction of some branded tea: Optimization based on polyphenol content, antioxidant potential and thermodynamic study. *Saudij. Biol. Sci.* **2019**, *26*, 1043–1052. [CrossRef]
13. Vinatoru, M. An overview of the ultrasonically assisted extraction of bioactive principles from herbs. *Ultrason. Sonochem.* **2001**, *8*, 303–313. [CrossRef]
14. Dolatowski, Z.J.; Stadnik, J.; Stasiak, D. Applications of ultrasound in food technology. *Acta Sci. Pol. Technol. Aliment.* **2007**, *6*, 88–99.
15. Dzah, C.S.; Duan, Y.; Zhang, H.; Wen, C.; Zhang, J.; Chen, G.; Ma, H. The effects of ultrasound assisted extraction on yield, antioxidant, anticancer and antimicrobial activity of polyphenol extracts: A review. *Food Biosci.* **2020**, 100547. [CrossRef]
16. Wen, C.; Zhang, J.; Zhang, H.; Dzah, C.S.; Zandile, M.; Duan, Y.; Ma, H.; Luo, X. Advances in ultrasound assisted extraction of bioactive compounds from cash, crops—A review. *Ultrason. Sonochem.* **2018**, *48*, 538–549. [CrossRef]
17. Luo, H.; Schmid, F.; Grbin, P.R.; Jiranek, V. Viability of common wine spoilage organisms after exposure to high power ultrasonics. *Ultrason. Sonochem.* **2012**, *19*, 415–420. [CrossRef]
18. Jambrak, A.R.; Šimunek, M.; Petrovic, M.; Bedic, H.; Herceg, Z.; Juretic, H. Aromatic profile and sensory characterisation of ultrasound treated cranberry juice and nectar. *Ultrason. Sonochem.* **2017**, *38*, 783–793. [CrossRef]
19. Fonteles, T.V.; Costa, M.G.M.; Tiberio de Jesus, A.L.; Alcântara de Miranda, M.R.; Fernandes, F.A.N.; Rodrigues, S. Power ultrasound processing of cantaloupe melon juice: Effects on quality parameters. *Food Res. Int.* **2012**, *48*, 41–48. [CrossRef]
20. Perez-Grijalva, B.; Herrera-Sotero, M.; Mora-Escobedo, R.; Zebadua-Garcia, J.C.; Silva-Hernandez, E.; Oliart-Ros, R.; Perez-Cruz, C.; Guzman-Geronimo, R. Effect of microwaves and ultrasound on bioactive compounds and microbiological quality of blackberry juice. *LWT-Food Sci. Technol.* **2018**, *87*, 47–53. [CrossRef]
21. Khandpur, P.; Gogate, P.R. Effect of novel ultrasound based processing on the nutrition quality of different fruit and vegetable juices. *Ultrason. Sonochem.* **2015**, *27*, 125–136. [CrossRef]
22. Havlikova, L.; Mikova, K. Heat stability of anthocyanins. *Z. Lebensm. Unters. Forch.* **1985**, *181*, 427–432. [CrossRef]
23. Mojzer, E.B.; Hrnčič, M.K.; Škerget, M.; Knez, Ž.; Bren, U. Polyphenols: Extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. *Molecules* **2016**, *21*, 901. [CrossRef] [PubMed]
24. Moreno-Montoro, M.; Olalla-Herrera, M.; Gimenez-Martinez, R.; Navarro-Alarcon, M.; Rufian-Henares, J.A. Phenolic compounds and antioxidant activity of Spanish commercial grape juices. *J. Food Compos. Anal.* **2015**, *38*, 19–26. [CrossRef]
25. Cao, X.; Cai, C.; Wang, Y.; Zheng, X. Effects of ultrasound processing on physicochemical parameters, antioxidants, and color quality of bayberry juice. *J. Food Qual.* **2019**, *1*, 1–12. [CrossRef]
26. Adekunte, A.O.; Tiwari, B.K.; Cullen, P.J.; Scannell, A.G.M.; Donnell, C.P.O. Effect of sonication on colour, ascorbic acid and yeast inactivation in tomato juice. *Food Chem.* **2010**, *122*, 500–507. [CrossRef]
27. Abid, M.; Jabbar, S.; Wu, T.; Hashim, M.M.; Hu, B.; Lei, S.; Zhang, X.; Zeng, X. Effect of ultrasound on different quality parameters of apple juice. *Ultrason. Sonochem.* **2013**, *20*, 1182–1187. [CrossRef] [PubMed]
28. Cruz-Cansino, N.d.S.; Ramírez-Moreno, E.; León-Rivera, J.E.; Delgado-Olivares, L.; Alanis-Garcia, E.; Ariza-Ortega, J.A.; Manriquez-Torres, J.d.J.; Jaramillo-Bustor, D.P. Shelf life, physicochemical, microbiological and antioxidant properties of purple cactus pear (*Opuntia ficus indica*) juice after thermoultrasound treatment. *Ultrason. Sonochem.* **2015**, *27*, 277–286. [PubMed]
29. Cheng, L.H.; Soh, C.Y.; Liew, S.C.; Teh, F.F. Effects of sonication and carbonation on guava juice quality. *Food Chem.* **2007**, *104*, 1396–1401. [CrossRef]

30. Heinmaa, L.; Moor, U.; Põldma, P.; Raudsepp, P.; Kidmose, U.; Lo Scalzo, R. Content of health-beneficial compounds and sensory properties of organic apple juice as affected by processing technology. *LWT—Food Sci. Technol.* **2017**, *85*(Part B), 372–379. [CrossRef]
31. Nicolas, J.J.; Richard-Forget, F.C.; Goupy, P.M.; Amiot, M.-J.; Aubert, S.Y. Enzimatic browning reaction in apple and apple production, Critical Rewiews. *Food Sci. Nutr.* **1994**, *34*, 109–157.
32. Onakpoya, I.J.; Spencer, E.A.; Thompson, M.J.; Heneghan, C.J. The effect of chlorogenic acid on blood pressure: A systematic review and meta-analysis of randomized clinical trials. *J. Hum. Hypertens.* **2015**, *2*, 77–81. [CrossRef]
33. Hasan, M.M.; Yun, H.-K.; Kwak, E.-J.; Baek, K.-H. Preparation of resveratrol-enriched grape juice from ultrasonication treated grape fruits. *Ultrason. Sonochem.* **2014**, *21*, 729–734. [CrossRef] [PubMed]
34. Suárez-Jacobo, A.; Rüfer, C.E.; Gervilla, R.; Guamis, B.; Roig-Sagués, A.X.; Saldo, J. Influence of ultra-high pressure homogenisation on antioxidant capacity, polyphenol and vitamin content of clear apple juice. *Food Chem.* **2011**, *127*, 447–454. [CrossRef] [PubMed]
35. Abid, M.; Jabbar, S.; Wu, T.; Hashim, M.M.; Hu, B.; Lei, S.; Zeng, X. Sonication enhances polyphenolic compounds, sugars, carotenoids and mineral elements of apple juice. *Ultrason. Sonochem.* **2014**, *21*, 93–97. [CrossRef] [PubMed]
36. Kataoka, H. New trends in sample preparation for analysis of plant-derived medicines. *Curr. Org. Chem.* **2010**, *14*, 1698–1713. [CrossRef]
37. Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jimenez, L. Polyphenols: Food sources and bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727–747. [CrossRef] [PubMed]
38. Adams, J.B. Thermal degradation of anthocyanins with particular reference to the 3-glycosides of cyanidin. I. In acidified aqueous solution at 100 °C. *J. Sci. Food Agric.* **1973**, *24*, 747–762. [CrossRef]
39. Sharma, K.; Ko, E.Y.; Assefa, A.D.; Ha, S.; Nile, S.H.; Lee, E.T.; Park, S.W. Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. *J. Food Drug Anal.* **2015**, *23*, 243–252. [CrossRef]
40. Ross, C.F.; Hoye, C., Jr.; Fernandez-Plotka, V.C. Influence of heating on the polyphenolic content and antioxidant activity of grape seed flour. *J. Food. Sci.* **2011**, *76*, 884–890. [CrossRef]
41. Bhat, R.; Shuaidda, N.; Min-Tze, L.; Karim, A.A. Sonication improves kasturi lime (*Citrus microcarpa*) juice quality. *Ultrason. Sonochem.* **2011**, *18*, 1295–1300. [CrossRef]
42. Gao, J.; Rupasinghe, H.P.V. Nutritional, physicochemical and microbial quality of ultrasound-treated apple-carrot juice blends. *Food Nutr. Sci.* **2012**, *3*, 212–218. [CrossRef]
43. Aadil, M.R.; Zeng, X.-A.; Han, Z.; Sun, D.-W. Effects of ultrasound treatments on quality of grapefruit juice. *Food Chem.* **2013**, *141*, 3201–3206. [CrossRef] [PubMed]
44. Palma, M.; Barroso, C.G. Ultrasound-assisted extraction and determination of tartaric and malic acids from grapes and winemaking by-products. *Anal. Chim. Acta* **2002**, *458*, 119–130. [CrossRef]
45. Bhat, S.; Sharma, H.K. Combined effect of blanching and sonication on quality parameters of bottle gourd (*Lagenaria siceraria*) juice. *Ultrason. Sonochem.* **2016**, *33*, 182–189. [CrossRef] [PubMed]
46. Salleh-Mack, S.Z.; Roberts, J.S. Ultrasound pasteurization: The effects of temperature, soluble solids, organic acids and pH on the inactivation of *Escherichia coli* ATCC 25922. *Ultrason. Sonochem.* **2007**, *14*, 323–329. [CrossRef] [PubMed]
47. Brackett, R.E. Microbial Quality. In *Food Science and Technology, Postharvest Handling*; Shewfelt, R.L., Prussia, S.E., Eds.; Academic Press: New York, NY, USA, 1993; pp. 125–148.
48. Cordier, J.-L. Enterobacteriaceae. In *Emerging Foodborne Pathogens*; In Woodhead Publishing Series in Food Science, Technology and Nutrition; Motarjemi, Y., Adams, M., Eds.; Woodhead Publishing: Cambridge, UK, 2006; pp. 450–475.
49. Zafra-Rojas, Q.Y.; Cruz-Cansino, N.; Ramírez-Moreno, E.; Delgado-Olivares, L.; Villanueva-Sánchez, J.; Alanís-García, E. Effects of ultrasound treatment in purple cactus pear (*Opuntia ficus-indica*) juice. *Ultrason. Sonochem.* **2013**, *20*, 1283–1288. [CrossRef]
50. Oyane, I.; Takeda, T.; Oda, Y.; Sakata, T.; Furuta, M.; Okitsu, K.; Maeda, Y.; Nishimura, R. Comparison between the effects of ultrasound and γ-rays on the inactivation of *Saccharomyces cerevisiae*: Analyses of cell membrane permeability and DNA or RNA synthesis by flow cytometry. *Ultrason. Sonochem.* **2009**, *16*, 532–536. [CrossRef]

51. Webster, D. pH—principles and measurement. In *Encyclopedia of Food Sciences and Nutrition*, 2nd ed.; Caballero, B., Ed.; Academic Press: New York, NY, USA, 2003; pp. 4501–4507.
52. OIV. Methods of analysis of wines and musts. In *Compendium of international methods of wine and must analysis*; OIV: Paris, France, 2019; Volume 1, pp. 62–83, 366–368.
53. AOAC. Vitamin C (ascorbic acid) in vitamin preparations and juices. In *Official Methods of Analysis*, 15th ed.; Helrich, K., Ed.; AOAC, Inc: Arlington, VA, USA, 2000; p. 1058.
54. Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Meth. Enzymol.* **1999**, *299*, 152–178.
55. Abdel-Hameed, E.S.; Bazaid, S.A.; Salman, M.S. Characterization of the phytochemical constituents of Taif rose and its antioxidant and anticancer activities. *Biomed. Res. Int.* **2013**, 345465. [CrossRef]
56. International Organization for Standardization. *Microbiology of the food chain—Horizontal method for the enumeration of microorganisms—Part 1: Colony count at 30 degrees C by the pour plate technique (ISO Standard No. 4833-1: 2013)*; International Organization for Standardization: Geneva, Switzerland, 2013.
57. International Organization for Standardization. *Microbiology of the food chain—Horizontal method for the detection and enumeration of Enterobacteriaceae—Part 1: Detection of Enterobacteriaceae; Part 2: Colony-count technique (ISO Standard No. 21528-1,2: 2017)*; International Organization for Standardization: Geneva, Switzerland, 2017.
58. Lupu, M.; Puchianu, G.; Padureanu, V.; Canja, C.; Maier, A. The influence of ultrasound and pasteurisation on microbiological properties of grape juice. In Proceedings of the 18th International Multidisciplinary Scientific GeoConference SGEM, Albena, Bulgaria, 30 June–9 July 2018; pp. 7234–7728.

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Optimization of A Procedure to Improve the Extraction Rate of Biologically Active Compounds in Red Grape Must Using High-Power Ultrasound

Alina Maier [1], Vasile Padureanu [1,*], Mirabela Ioana Lupu [1], Cristina Maria Canja [1], Carmen Badarau [1], Cristina Padureanu [1], Ersilia Alexa [2] and Mariana-Atena Poiana [2,*]

[1] Faculty of Food and Tourism, Transilvania University of Brasov, Castelului 148, 500014 Brasov, Romania
[2] Faculty of Food Engineering, University of Life Sciences "King Mihai I" from Timisoara, Calea Aradului 119, 300645 Timisoara, Romania
* Correspondence: padu@unitbv.ro (V.P.); marianapoiana@usab-tm.ro (M.-A.P.); Tel.: +40-745-080-149 (V.P.); +40-726-239-838 (M.-A.P.)

Abstract: The primary focus in the production of quality red wine is the extraction of grape components, which can be achieved in a variety of ways. This work investigates the extraction yield of biologically active compounds from crushed Merlot grapes, as a result of ultrasound treatment applied before maceration, and optimizes the process parameters of a laboratory scale using response surface methodology (RSM) within a central composite design (CCD) model. The two factors whose response was studied were amplitude (A) % and treatment time (t), while the dependent variables were the total phenolic compounds (TPC), monomeric anthocyanins (MA), and antioxidant activity expressed as ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. The results showed that the application of high-power ultrasound treatment to crushed grapes for a few minutes increased both the extraction rate of bioactive compounds and the antioxidant activity by a maximum of 12 times for the TPC, 14 times for the MA, 3.6 times for the FRAP value, and 18.77% for the DPPH. The optimized solution had an amplitude of 90% and a treatment time of 4 min and 24 s. The validation experiments yielded errors between—8.70% and 3.14%, confirming the proposed model. Thus, the RSM model is recommended as a tool to optimize a procedure for enhancing both the extraction rate of the bioactive compounds from grapes and the antioxidant properties of grape must. Our results demonstrate the ultimate benefits of using ultrasonic treatment on crushed grapes at the beginning of the winemaking process, as a highly effective technique for improving the extraction of high-value bioactive chemicals, with significant application potential.

Keywords: red grapes; bioactive compounds; antioxidant activity; ultrasound; extraction optimization procedure

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1. Introduction

Wine is a cultural emblem, whose function has evolved over time from being a vital source of sustenance to becoming a social and cultural accompaniment to food. The production of wine grapes of 33.8 million tons and the volume of wine produced globally in 2022, which was between 257.5 and 262.3 million hectoliters of wine, with a midpoint estimate of 259.9 million hectoliters, is anticipated to be comparable to the level seen in 2021. The worldwide output level can be regarded as somewhat below average for the fourth year in a row [1].

To satisfy the varied sensory preferences of consumers, it is essential to be able to manufacture several red wine types, and wine producers are continually changing their winemaking techniques to this end. When making high-quality red wine, the extraction of grape components, which can be conducted in a variety of ways, is the major focus [2].

Due to their numerous positive health benefits, including antioxidant properties, neuroprotective action, anti-inflammation, antiaging antimicrobial activity, and lowering arterial blood pressure, phenolic compounds have attracted a lot of interest by the scientific community and the general public in recent years [3–7].

Moreover, customers place a high value on the sensory quality of red wine's color, preferring it to be a deep red. The primary pigments responsible for the young wines' vivid red color are called anthocyanins, the main polyphenolics in red grapes [8]. The initial monomeric anthocyanin profile is an element that affects the ultimate color of the wine and, additionally, might have anti-cancer and anti-glycemic properties [9].

Because of the inherent drawbacks to conventional extraction techniques, the effective extraction of these chemicals from natural sources continues to be a significant issue. The rising need for novel technical solutions that can deliver the highest extraction yields, guarantee the stability of the target compounds, and also satisfy environmental requirements, has been sparked by the growing interest in natural antioxidants [10].

Due to its eco-friendly nature, non-toxic behavior, and low energy usage, ultrasound (US) may be regarded as a sustainable, green, and innovative technology [11,12]. There are several uses for it in food technology, including analysis, preservation, and homogenization, extraction, filtering, and drying. In order to achieve these objectives, ultrasound principally employs acoustic waves, which cause mechanical and chemical reactions. These reactions are fundamentally different from those produced by conventional methods. The main goals of this technology are to speed up processing, save energy, and improve the quality, safety, and shelf life of food products [12,13]. To sum up, this technique is a green alternative for this industry, as it does not use toxic solvents. Additionally, it supports economic sustainability through its use in efficient extraction procedures [14].

Ultrasound is divided into three categories: high frequency–low power US (between 1 and 10 MHz), intermediate frequency–medium power US (between 100 kHz and 1 MHz), and low frequency–high power US (between 20 and 100 kHz). Strong physical effects are primarily produced at a US frequency of about 20 kHz by fragmentary transitory cavitation, promoting bioactive chemical release from plant matrices during cell disruption [15–17]. The effect of low frequency may be related, not only to cavitation bubble size, but also to its effect on mass transfer resistance [13]. High-power US treatment on food matrices can cause physical, chemical, and biological changes, and a variety of uses have been documented [18,19]. Numerous studies have demonstrated that high-power ultrasound is becoming more prevalent in the enological sector due to its ability to enhance a wine's organoleptic qualities, shorten processing times, and provide better microbial control in wines [20–22]. Furthermore, the International Organization of Vine and Wine has included ultrasonic treatment among the methods that can be used during winemaking [23].

Currently, adopting all the techniques designed to make color extraction and fragrance dissolution easier, throughout the winemaking process, is desired. Through the application of ultrasonic technology, the potential for the continuous extraction of qualitatively significant substances from the exocarp is explored. This is conducted by taking advantage of the phenomena caused by the transmission of ultrasound in a solid–liquid environment, such as grape must [13].

However, taking into account the fact that most research was carried out at the laboratory scale, and that the grape skins' total phenolic content varies according to the cultivar, soil type, climate, region of origin, and cultivation techniques, we consider that it is vital to optimize the factors involved in this form of extraction in order to build a workable ultrasound-assisted large-scale procedure. The extraction efficiency using high-power US is significantly influenced by a variety of factors, such as the temperature of the samples, the power of the ultrasonic device, the process frequency, the treatment's intensity, as well as the shape and size of the ultrasonic reactor [24].

Thanks to its reliable design, the statistical method known as response surface methodology (RSM) is currently widely utilized for optimization. The RSM is a collection of mathematical and statistical methods that are used to investigate the relationship between

process input variables and outputs (responses). The methodology can be used for process optimization in a variety of circumstances and is relatively affordable, practical, and effective [25].

The objective of this work was to investigate the extraction yield of biologically active compounds from crushed grapes, as a result of an ultrasonic treatment applied during the first stages of the winemaking process, before maceration. In addition, the aim was to identify the optimized process parameters in terms of amplitude (A) % and treatment time (t) at the laboratory scale, as well as to develop a procedure that improves both the extraction rate of bioactive compounds from grapes and the antioxidant properties of grape must. To this end, we first evaluated the high-value phytochemicals, such as the total phenolic compounds (TPC), which are generally associated with the organoleptic properties of finished wines and (with) their stability. We also quantified the monomeric anthocyanins (MA), which are responsible for the red color of red wines. In addition, the antioxidant activity was assessed, from all the possible biological functions performed by *V. vinifera* compounds, both the ferric reducing antioxidant power (FRAP) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assays are probably the most important regarding human health.

2. Materials and Methods

2.1. Grapes

The Merlot variety employed for the experimental research is the second most planted grape variety in the world, well-known for producing high-quality wines [26]. The red grapes, vintage 2019, were hand-harvested (approximately 100 kg) from a vineyard in Prahova, Romania (Pietroasa-Istrita Viticulture and Winemaking Research and Development Station). The grapes were harvested at technological maturation when they reached 29.8° Brix, then quickly transported to the laboratory for processing. Only healthy, selected bunches of grapes were used for the experimental research.

2.2. Ultrasonic Equipment

The sonication of all the experimental tests was performed using a probe-type ultrasonic device (Sonics VCX-750, Sonics and Materials Inc., Newtown, CT, USA). The amplitude can be set as a percentage, between 10% and 100%. The device produces an ultrasonic power of 750 W and frequency of 20 kHz. The most typical frequencies utilized in the extraction procedures were between 20 and 100 kHz.

2.3. Optimization Design

The experimental design was developed with Design Expert® software version 13 (Stat-Ease, Inc., Minneapolis, MN, USA, 2022), to optimize the number of experiments and operating conditions.

Based on the results of the single factor experiment, an optimization design was created. For these experiments, the RSM was used within a central composite design (CCD) model. The two factors whose response was studied were amplitude (A) % and treatment time (t). The high and low levels of each variable were determined by preliminary research that took place before carrying out of these experiments. The limits of the instrument were also taken into account when choosing the ranges for all the variables. The intensity and frequency of the waves were two of the most important operating parameters that affect the performance of ultrasound technology in extracting bioactive chemicals. The results of some studies indicated that the sonochemical effects in the treated fluids increase as the amplitude increases. Moreover, the amplitude varies over a wide range, from 20% to 90% [27], to determine the ideal extraction configuration, while other studies used treatment durations ranging from 2 to 10 min [28,29].

Furthermore, according to our preliminary research, there were no significant differences in the extraction of biologically active compounds between the control sample and the ultrasound-treated samples, when ultrasound was applied at low amplitude levels

(from 30% to 50%) for a maximum treatment time of 5 min. The extraction temperature during ultrasound extraction was shown to be closely related to the extraction time, as it was found that longer extraction times resulted in higher extraction temperatures. Therefore, the extraction time selected should be within the appropriate range. However, it was found that a maximum extraction time of 5 min was necessary because high temperatures promote the oxidation and destruction of phenolic compounds.

Taking into account the aforementioned factors, the levels of the independent variables, such as percent amplitude (A) and sonication time (t), were selected at 50%, 70%, and 90% for the ultrasound time of 3, 4, and 5 min (Table 1), respectively. The dependent variables were the TPC, MA, and antioxidant activity, expressed as the FRAP and DPPH free radical scavenging activity.

Table 1. Ultrasonic extraction test treatment options.

Independent Variables				Dependent Variables
Probe Diameter (mm)	**Frequency (kHz)**	**Amplitude (A) %**	**Treatment Time (t) min**	
13	20	50	3	TPC
		70	4	MA
		90	5	FRAP
				DPPH

To choose the best statistical model, such as linear, quadratic, and cubic models, the experimental data were examined based on the second-degree polynomial equation (Equation (1)):

$$Y_k = \alpha_0 + \sum_{i=1}^{n} \alpha_i X_I + \sum_{i=1}^{n} IX_i + \sum_{i<j}^{n} \alpha_{ij} X_i X_j \quad (1)$$

where Y_k is the dependent variable, α_0, α_i, α_{ii}, and α_{ij} are the regression coefficients of the model, which are constants, and X_i and X_j are the independent variables of the model. Response surface plots were generated using the expected values from the fitted model. To illustrate the influence of the independent variables on each response, two- and three-dimensional contour plots were generated for each response.

2.4. Sample Preparation

For the laboratory tests, randomly collected grape samples (100 g) were destemmed, hand-crushed, and then treated using a probe-type ultrasonic device. The control sample (C) that was not subjected to ultrasound treatment was evaluated separately. The samples were treated in a 100 mL Pyrex glass beaker fitted with a counter-current water-cooling jacket. The water used for cooling was kept at a constant temperature of 19 °C. The positioning of the acoustic amplifier in the container was standardized at a distance of 20 mm from the bottom. At the end of the ultrasonic treatment, the samples were vacuum filtered (Nalgene Rapid-Flow Filter Units, 0.45 μm PES membrane). The filtrate was collected in a vial and stored in a refrigerator at 4 °C for further use. All extraction experiments were conducted in this manner.

2.5. Total Polyphenolic Content and Anthocyanins Determination

The total polyphenolic content (TPC) was determined by using the Folin–Ciocalteu spectrophotometric method, with gallic acid as a reference and expressed as micrograms of gallic acid equivalents per milliliter (μg GAE/mL) [30]. A 0.5 mL sample was treated with 1.25 mL of Folin–Ciocalteu reagent (Merck, Darmstadt, Germany), previously diluted 1:10 (*v*/*v*) with distilled water. After incubation for 5 min at room temperature, 1 mL Na_2CO_3 60 g/L was added. A UV–VIS spectrophotometer (Specord 205, Analytik Jena Inc., Jena, Germany) was used to detect sample absorbance at 750 nm after 30 min of incubation at

50 °C. The calibration curve was established using gallic acid as standard, at concentrations ranging from 5 to 250 μg GAE/mL.

The monomeric anthocyanins (MA) were determined by the pH differential method [31]. Briefly, two dilutions of the same sample were prepared by adding 1 mL of wine to 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). After incubation for 15 min at room temperature, the absorbance was measured at 520 and 700 nm against deionized water. The results were expressed in milligrams of cyanidin-3-glucoside equivalents per liter (mg CGE/L). The total anthocyanin content of the samples was calculated using a molar absorption coefficient of 26,900 L/mol × cm and a molecular weight of 449.2 g/mol.

2.6. Antioxidant Activity

The antioxidant activity of the investigated samples was evaluated by FRAP and DPPH assays.

2.6.1. FRAP Assay

The protocol for the FRAP test was based on the method of Benzie and Strain [32]. For this purpose, 300 mM acetate buffer (pH = 3.6), 10 mM of 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM $FeCl_3 \times 6H_2O$ solution were used as stock solutions. The working solution was prepared by mixing 100 mL acetate buffer, 10 mL TPTZ solution, and 10 mL $FeCl_3 \times 6H_2O$ solution, which was then heated to 37 °C before use. Prior to analysis, the wine samples were diluted 1:50 (*v*/*v*) with distilled water, then a 0.5 mL aliquot of diluted samples was allowed to react with 2.5 mL of working solution for 30 min at 37 °C. The absorbance of the mixture was read at 593 nm against a blank sample obtained under the same working conditions. The results were expressed in μmol of Fe^{2+} equivalents per milliliter of grape must (μmol Fe^{2+}/mL).

2.6.2. DPPH Assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of the samples was measured following the procedure described by Kalita et al. [33], with some minor adjustments. For this purpose, 20 μL of each investigated sample was mixed in a microplate with 20 μL of distilled water, after which the resulting mixture was combined with 200 μL of a 120 mg/L DPPH radical solution prepared in 96% (*v*/*v*) ethanol. The microplate was left in the dark for 30 min, and the absorbance value (A sample) at 515 nm was measured against a blank of 96% (*v*/*v*) ethanol, using a plate reader (Tecan SunRiseTM, software MagellanTM, Tecan Group Ltd., Männedorf, Switzerland). The control was prepared under the same working conditions, containing 20 μL of 96% (*v*/*v*) ethanol instead of the investigated sample. The absorbance of the control (A control) was read against 96% (*v*/*v*) ethanol.

The DPPH scavenging activity (%) was calculated according to Equation (2), as follows:

$$\text{DPPH scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100 \quad (2)$$

2.7. Total Soluble Content and Acidity

The total soluble solids (TSS, ° Brix) and the titratable acidity (TA, g/L of tartaric acid) were analyzed according to the standard procedure of Council Regulation EEC 2676/90 [34].

2.8. Statistical Analysis

All the measurements were performed in triplicate and the results are presented as mean ± standard deviation (SD). The Design Expert® software version 13 (Stat-Ease, Inc., Minneapolis, USA, 2022) was used for the statistical analysis. Significant differences between the samples were assessed using a one-way ANOVA, with post-hoc analysis using the HSD Tukey test.

The Pearson correlation coefficient between the total phenolic content, monomeric anthocyanins, and antioxidant activity was calculated using JASP software, version 0.17.1 (JASP Team, Amsterdam, The Netherlands, 2023).

3. Results and Discussion

The effects of an ultrasonic pre-treatment to improve the extraction rate of polyphenols and, subsequently, to increase the antioxidant activity of several samples, with three replicates each, all from red grapes, the Merlot variety, vintage 2019, were followed in the laboratory. The experimental design provided for the randomized treatment of 13 samples with 3 repetitions each, with 5 central points, as shown in Table 2.

Table 2. Design of the central composite experimental model and results.

Std Order	Run Order	Factors		Responses			
		A (%)	B (min)	TPC (μg GAE/mL)	MA (mg CGE/L)	FRAP (μmol Fe^{2+}/mL)	DPPH Scavenging Activity (%)
2	1	90	3	1396.06 ± 5.23	223.46 ± 2.32	41.78 ± 2.25	74.5 ± 0.2
9	2	70	4	947.92 ± 3.79	167.05 ± 1.46	27.52 ± 1.21	73.3 ± 0.6
1	3	50	3	124.5 ± 1.01	20.67 ± 0.72	15.02 ± 0.83	66.8 ± 0.5
6	4	90	4	1177.85 ± 4.87	214.97 ± 2.36	42.96 ± 1.98	75.9 ± 0.6
11	5	70	4	948.05 ± 4.56	169.7 ± 1.55	28.85 ± 1.09	73.2 ± 0.3
5	6	50	4	136.4 ± 1.08	25.49 ± 0.65	16.09 ± 0.78	68.2 ± 0.2
7	7	70	3	482.31 ± 3.82	108.37 ± 1.02	27.17 ± 0.94	72.1 ± 0.5
4	8	90	5	1172.1 ± 3.75	176.29 ± 2.45	48.32 ± 2.23	77.2 ± 1.0
12	9	70	4	949.23 ± 3.98	166.6 ± 1.47	28.02 ± 0.74	73.2 ± 0.9
10	10	70	4	951.19 ± 4.56	168.15 ± 1.62	29.15 ± 1.16	73.3 ± 0.8
3	11	50	5	148.3 ± 2.04	26.22 ± 1.23	18.68 ± 0.43	71.1 ± 0.6
13	12	70	4	950.24 ± 4.21	167.24 ± 1.74	27.97 ± 0.54	73.4 ± 0.5
8	13	70	5	1126.11 ± 2.01	137.74 ± 1.03	29.97 ± 0.81	73.5 ± 0.7

Amplitude (A); time (B); total polyphenol content (TPC); monomeric anthocyanins content (MA); antioxidant activity: FRAP and DPPH. The results are expressed as the mean value of the three replicates ± the standard deviation (SD).

In terms of the content of bioactive compounds, such as the TPC and MA, as well as the antioxidant activity measured by the FRAP and DDPH assays, the effects of the high-power ultrasonic treatment on extraction kinetics were explored. All the above parameters were studied in untreated red grapes must as a control sample (C) and on the samples subjected to ultrasound treatment at 50, 70, and 90% amplitude for 3, 4, and 5 min, respectively, as follows: SM50/3 (A: 50%, t: 3 min), SM50/4 (A: 50%; t: 4 min), SM50/5 (A: 50%; t: 5 min), SM70/3 (A: 70%; t: 3 min), SM70/4 (A: 70%; t: 4 min), SM70/5 (A: 70%; t: 5 min), SM90/3 (A: 90%; t: 3 min), SM90/4 (A: 90%; t: 4 min), and SM90/5 (A: 90%, t: 5 min).

Analytical determinations were performed immediately after crushing for the control sample and immediately after treatment for the ultrasound-treated samples.

Two factors, the amplitude level and the duration of the ultrasound treatment, were found to have an effect on the parameters studied, with single or interaction effects. The ANOVA analysis of variance performed on the analytical parameters, in relation to the different conditions of amplitude and treatment time, revealed significant differences in the total polyphenolic content, the monomeric anthocyanins, and the antioxidant activity. Using the RSM and ANOVA, the best extraction parameters were statistically examined.

The process of sonication results in the development of cavitation bubbles, which have the potential to violently collapse, releasing energy and increasing the temperature and pressure. These bubbles may burst close to a solid surface or interact chemically with molecules, harming cell structures and altering the selectivity or permeability of cell membranes [20].

To monitor the corresponding temperature rise, the temperature of the sonicated samples was measured at 1 min intervals, using a thermovision camera. The mean value of the untreated sample (C), and the mean values recorded by a forward-looking infrared (FLIR) camera for each sample when the sonication treatment ended are illustrated in Figure 1.

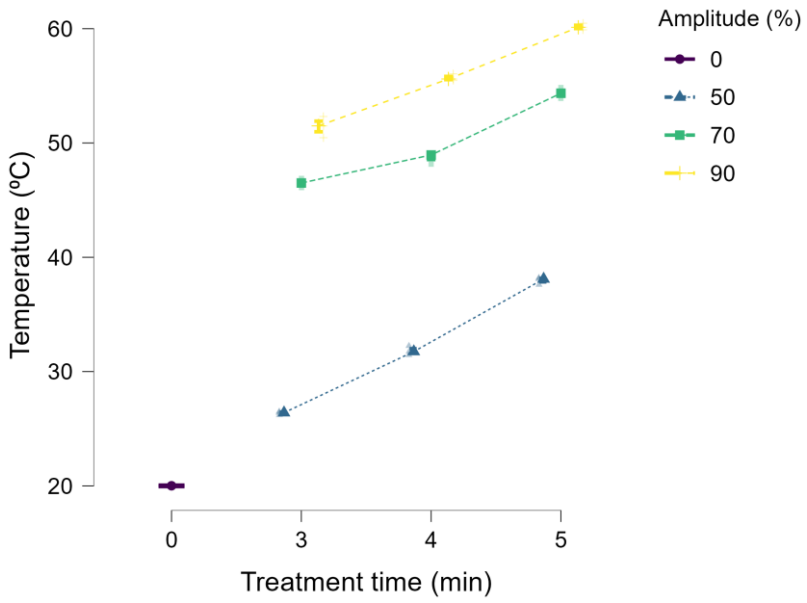


Figure 1. Changes in the temperature of the samples treated with US compared to the control sample (C).

An increase in the temperature of the samples following the ultrasound treatment was evident, compared to the reference sample, C (20 °C), correlated with the intensity and time of the treatment. The minimum and maximum average temperatures reached in the samples were 26.20 °C for SM50/3 and 60.17 °C for SM90/5, respectively. The results confirmed the fact that the increasing temperature of the must samples depends on the operating parameters used during the ultrasound treatments, as it was established that the device's real input power was transformed into heat that was released into the medium [26].

It can be observed that the increasing temperature in the sonicated samples was influenced to a higher measure by increasing the amplitude compared with the treatment time. Thereby, the temperature reached in SM70/5 (54.23 °C), treated with a lower amplitude but the maximum time (5 min), was close to that of SM90/3 (51.53 °C) treated with a higher amplitude but the minimum time (3 min).

Increased temperature often results in higher extraction yields. Temperature optimization can be performed to obtain the best yield of the target compounds without degradation, as this parameter can vary depending on the type of product [35].

It was also concluded that the intensity of the ultrasound was directly related to the amplitude of the transducer. Increasing the amplitude increased the ultrasound intensity and resulted in an increase in the sonochemical effects [36].

Using different levels of device power, intensity, frequency, amplitude, and treatment time, previous research on red grapes and wines of various types has yielded very encouraging findings on the ability of ultrasound to extract and improve the physicochemical quality of wines, as well as its ability to accelerate wine aging or control microorganisms [37–41].

In addition, other authors reported that the application of US produced interesting findings on the recovery of bioactive compounds from marc and lees, and the extraction of various aromatic compounds from wines [42–44].

Table 3 depicts the results for the physicochemical and analytical determinations of the untreated red grape must.

Table 3. Characteristics of the red grape must.

Sample	TSS (° Brix)	TA (g Tartaric Acid/L)	TPC (µg GAE/mL)	MA (mg CGE/L)	FRAP (µmol Fe^{2+}/mL)	DPPH Scavenging Activity (%)
C	29.9 ± 0.1	3.4 ± 0.03	114.42 ± 2.67	16.06 ± 0.9	13.43 ± 1.02	65.0 ± 0.4

Untreated red grape must (C); total soluble solids (TSS); total acidity (TA); total polyphenolic content (TCP); monomeric anthocyanins content (MA); antioxidant activity: FRAP and DPPH. The results are expressed as the mean value of the three replicates ± the standard deviation (SD).

3.1. Total Polyphenolic Content

The TPC extraction yields were observed to rise with any increase in temperature. This impact might primarily be ascribed to the cavitation phenomenon, which is preferred at lower temperatures, and which makes it easier to release extractable chemicals and enhances mass movement via diffusion or by rupturing plant cell walls [23,32,45].

The TPC for sonicated samples varied from 124.5 micrograms of gallic acid equivalents per milliliter (GAE/mL) to 1396.06 µg GAE/mL, as shown in Table 2. The lowest amount of TPC was found in the SM50/3 sample (run order three) using 50% amplitude for 3 min, while the higher TPC content was found for the SM90/3 sample (run order one) using 90% amplitude for 3 min. It can be seen that there was an increase, generally proportional with the treatment conditions, of 9% to 12 times for the total polyphenol content of the sonicated samples compared with untreated sample (C). These findings indicate that amplitude is a significant factor in the extraction of total phenols.

Previous studies have shown a higher extraction yield of TPC when high-power ultrasound was applied to grapes or during winemaking [27,46]. Other authors have reported that no degradation of the polyphenols was observed after the sonication of red wine [29].

Moreover, the ultrasound treatment with an amplitude of 90% for 3 min determined the highest extraction of TPC in the sample SM90/3, while lower values were recorded when applied at the same amplitude of 90% for 4 min and 5 min, by 15.63% in the sample SM90/4 and 16.04% in the sample SM90/5, respectively. The possible explanation for this decrease might be the high temperatures reached in the immediate vicinity of the ultrasonic probe, as well as the average temperatures of 55.71 °C and 60.17 °C reached in samples SM90/4 and SM90/5, respectively, that could lead to the degradation of phenolic compounds. Other research has shown that polyphenol can be protected against thermal breakdown processes at temperatures as high as 50 degrees Celsius [47].

Despite their benefits, many phenolic compounds quickly hydrolyze and oxidize at higher temperatures, particularly when extracted for extended periods of time [48,49]. At extraction temperatures over 60 °C, Akowuah, Mariam, and Chin demonstrated that the total polyphenol content of the extracts reduced as a result of oxidative degradation [50]. The vulnerability of phenolic compounds to high temperatures was demonstrated, as early as three decades ago, by Havlikova and Mikova [51]. They demonstrated that high temperatures and prolonged extraction times enhanced the rate at which phenolic components oxidized and lowered the yield of the extracts. For instance, during maceration, the extraction of polyphenols is typically carried out between 20 and 50 °C, never over this range [52].

Furthermore, it is important to remember that the sensitivity of a sample to temperature-induced polyphenol degradation is influenced by the types of polyphenolic compounds present in the plant extract or matrix, as well as their physicochemical and biochemical properties [47].

However, additional variables such as plant species, geographical origin, or cultivars may also have an impact on the quantity of phenolic chemicals recovered using an ultrasound-assisted approach [10].

The optimal extraction parameters for the TPC were statistically investigated using the RSM and ANOVA (Table 4).

Table 4. ANOVA and coefficient table for the quadratic model. Response 1: total phenolic compounds.

Source	F-Value	*p*-Value	R^2	Adjusted R^2	Coefficients
Model	**13.40**	**0.0018**	**0.9054**	**0.8378**	
Intercept					921.98
A	60.33	0.0001			556.13
B	1.07	0.3361			73.94
AB	0.4989	0.5028			61.94
A^2	3.47	0.1049			−196.5
B^2	0.2193	0.6538			−49.42
Lack of fit	36229.38	<0.0001			

Amplitude (A); time (B).

As Table 4 shows, the amplitude term is significant for the enhanced extraction of polyphenols. The model F-value of 13.40 means that the model is significant. Due to the noise, there is only a 0.18% chance that such a large F-value could occur. Moreover, *p*-values less than 0.0500 indicate that the model terms are significant. In this case, amplitude is a significant model term. Values greater than 0.1000 indicate that the model terms are not significant. A high R^2 value (0.9054) indicates a good level of model fit. The lack of fit F-value of 36,229.38 indicates that the lack of fit is significant. There is only a 0.01% chance that such a large lack of fit F-value could occur due to noise.

The final equation in terms of the actual factors for the TPC is shown in Equation (3). The equation can be used to make predictions about the response for given levels of each factor.

$$Y_{TPC} = -5385.266 + 108.970 \times A + 686.076 \times B - 3.097 \times A \times B - 0.491 \times A^2 - 49.418 \times B^2 \quad (3)$$

where A is the amplitude and B represents the time.

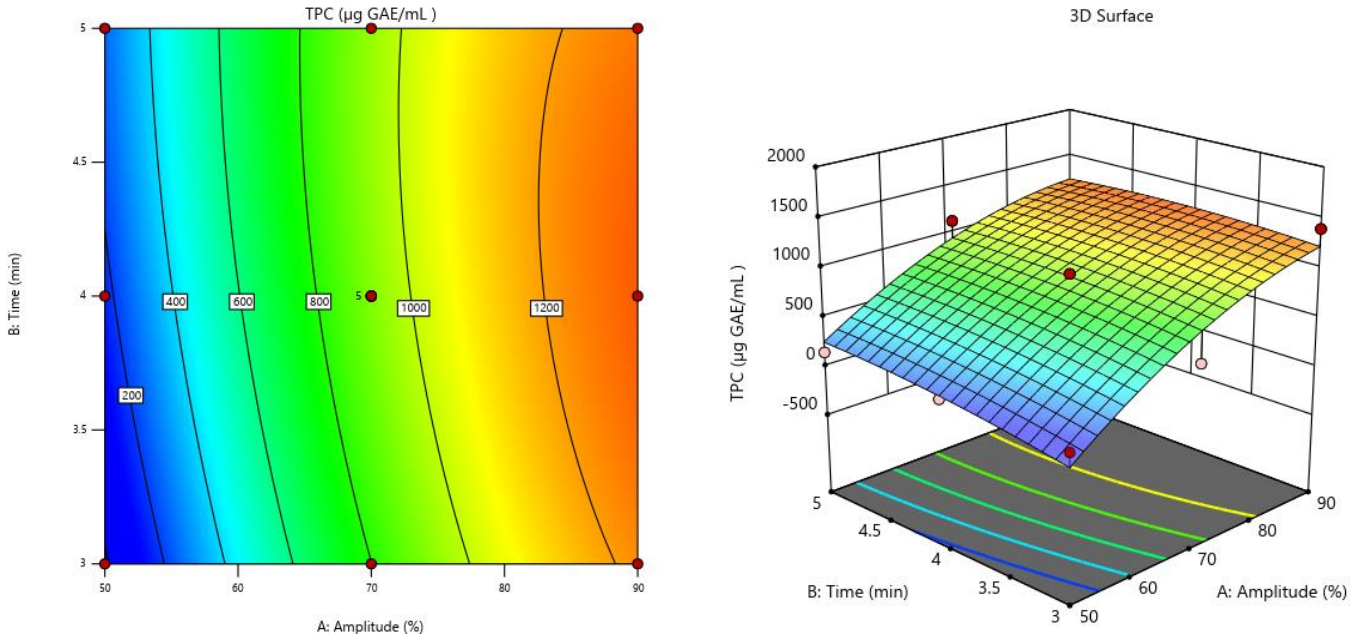
The influence of the process parameters A and B on the extraction rate of the TPC in samples treated with US is shown in Figure 2.

Figure 2 clearly illustrates that the amplitude of ultrasonic waves has a greater impact on the outcomes than the time of the treatment. A higher treatment time with smaller amplitudes (50%) has a detrimental effect, and less TPC is extracted. Higher amplitude values (90%), on the other hand, greatly improve the TPC extraction yield.

The potential of ultrasonic waves to have a detrimental effect on biomass, aiding the extraction of cell contents, has been ascribed to ultrasound assisted extraction, having an improving influence on extracting phenolics from plants. Using ultrasound and different operating conditions, previous studies have shown increased yields of phenolic extracts as plant bioactive compounds, when applied to blueberry pomace, mango peels, or coffee beans [53–55].

In addition, it was observed that heat breakdown of the polyphenols may take place at high temperatures. A reduction in the extraction yield owing to temperature might be attributed to phenolic components degrading at high temperatures [56].

In the described situation, the optimal circumstances would be a higher amplitude, and a reduced duration of the treatment.



(**a**) (**b**)

Figure 2. Influence of the process parameters on the TPC: (**a**) amplitude (A) and time (B) as contour graph; (**b**) amplitude (A) and time (B) as 3D surface.

3.2. Monomeric Anthocyanins

Anthocyanins are a major family of phenolic compounds found in red wine. The treatment tests showed a strong interaction of the cavitation phenomena with the cells of the exocarp, with an increase in the concentration of anthocyanins. Our results were consistent with others that include ultrasound-assisted extraction on the list of new technologies that have been developed with the goal of reducing extraction times and enhancing yields [57].

The MA content for the sonicated samples varied from 20.67 mg cyanidin 3-glucoside equivalents (CGE)/L to 223.46 mg CGE/L, as shown in Table 2. Similar to the TPC, the lowest amount of MA was found for the SM50/3 sample (run order three) using 50% amplitude for 3 min, while the higher MA content was found for the SM90/3 sample (run order one) using 90% amplitude for 3 min. It can be observed that there was an increase, generally proportional with the treatment conditions, of 28.7% to about 14 times for the MA of the sonicated samples compared with untreated sample (C).

Regarding the extraction of MA, US treatment with A 90% for 3 min led to the highest extraction (sample SM90/3), while slightly lower values of 3.8% (sample SM90/4) and significant lower values of 21.11% (sample SM90/5) were recorded when we used an amplitude of 90% for 4 min and 90% for 5 min, respectively. It is observed that the extraction of monomeric anthocyanins increases by a maximum value and then start to decrease, probably also due to the influence of the final temperature reached in the treated samples.

The anthocyanin content of grape juice was negatively impacted by higher amplitude levels and longer treatment times, as other authors have shown [28]. Moreover, it was observed that anthocyanins are susceptible to degradation due to a variety of variables such as pH, temperature, oxygen, water activity, and co-pigments [58].

Other researchers revealed that the temperature has a substantial impact on anthocyanin extraction, with maximal extraction occurring at roughly 30 to 35 °C. However, temperature increases enhance extraction by increasing the solubility of anthocyanins and the diffusion coefficient. However, temperatures over a particular point resulted in a reduction in anthocyanin output, e.g., at temperatures above 45 °C, anthocyanins decrease dramatically [59].

As it can be noticed, for the highest extraction of both the TPC and MA ultrasound amplitude and treatment time are the same: 90% amplitude for 3 min.

The difference in behavior between the total phenolics and anthocyanins might be explained by anthocyanins' greater vulnerability to high temperatures [56].

According to Table 5, model terms for the monomeric anthocyanins A, A^2, and B^2 are significant since their *p*-values are less than 0.0500, as shown. If the value is higher than 0.1000, the model terms are not considered relevant. An F-value this large might be caused by noise just 0.01% of the time, but the model's F-value of 44.35 suggests that the model is important. The lack of fit is implied to be significant by the lack of fit F-value of 396.80.

Table 5. ANOVA and coefficient table for the quadratic model. Response 2: monomeric anthocyanins.

Source	F-Value	*p*-Value	R^2	Adjusted R^2	Coefficients
Model	44.35	<0.0001	0.9694	0.9475	
Intercept					162.77
A	190.42	<0.0001			90.39
B	0.0972	0.7644			2.04
AB	2.70	0.1444			–13.18
A^2	9.71	0.0169			–30.08
B^2	7.97	0.0257			–27.25
Lack of fit	396.80	<0.0001			

Amplitude (A); time (B).

The adjusted R^2 of 0.9475 and the predicted R^2 of 0.8878 are in fair agreement (i.e., the difference is less than 0.2), indicating that the model makes accurate predictions. Equation (4) displays the final equation in terms of the actual factors for monomeric anthocyanins. For specific levels of each element, predictions regarding the response can be made using the equation.

$$Y_{MA} = -1134.49 + 17.683 \times A + 262.123 \times B - 0.659 \times A \times B - 0.075 \times A^2 - 27.254 \times B^2 \quad (4)$$

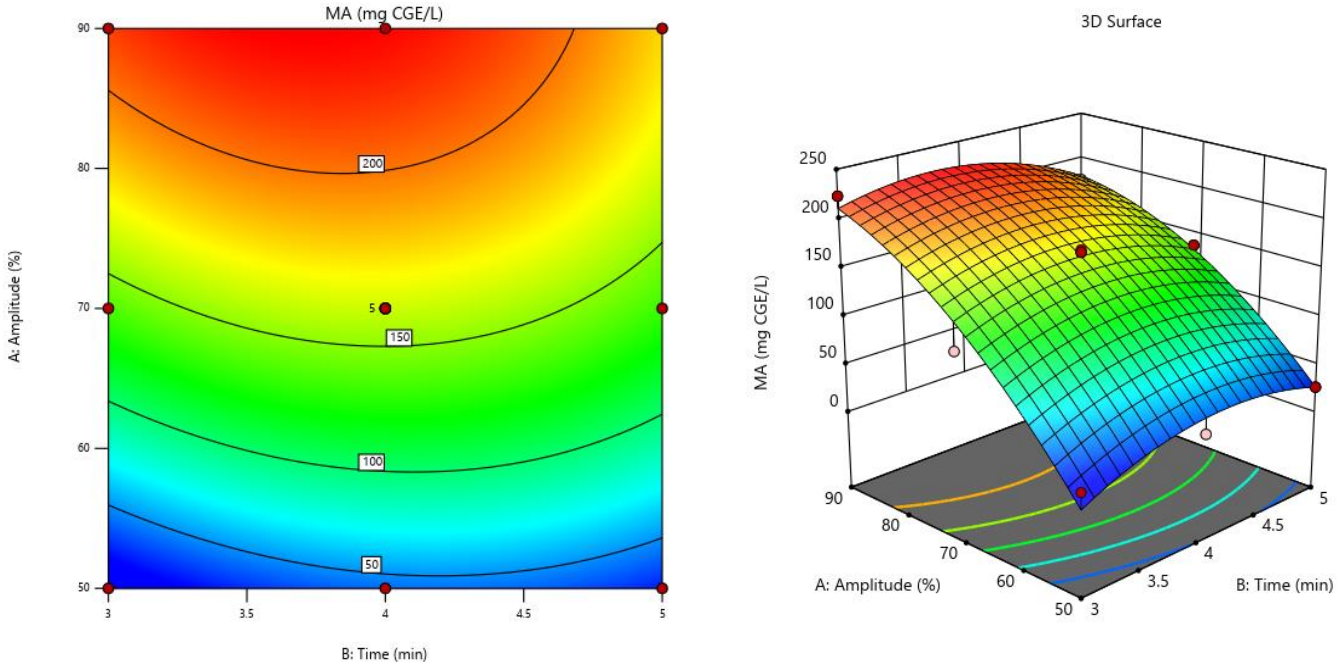
where A is the amplitude and B represents the time.

The effect of the process parameters A and B on the extraction rate of the MA in samples that underwent US treatment is shown in Figure 3.

According to Figure 3, which compares the effects of the treatment duration and ultrasonic wave amplitude on the extraction of the monomeric anthocyanins from red grape exocarp, the extraction yield of the MA increases with both up to a certain point before starting to decline. However, as the treatment time increases over 3 min using the ultrasound wave amplitude at the maximum level (90%), the extraction rate of the MA decreases. Excessive cavitation occurs at high amplitudes, raising the temperature around the probe, creating more hydroxyl radicals, and resulting in excessive degradation [60]. Moreover, as a smaller amplitude may not deliver enough energy to break the cell wall, the lowest extraction yields of the MA were observed in the samples where the minimum amplitude level was used (A = 50%).

Using ultrasound and different working conditions, previous studies have also shown increased yields for anthocyanin extractions from blueberry marc, mulberry wine residues, or purple sweet potatoes [61–63].

Similar to the TPC, the optimal circumstances for the MA enhanced extraction would be a higher amplitude, and a reduced duration of the treatment.



(**a**) (**b**)

Figure 3. Influence of the process parameters on the MA: (**a**) amplitude (A) and time (B) as contour graph; (**b**) amplitude (A) and time (B) as 3D surface.

3.3. Antioxidant Activity

The antioxidant properties of the phenolic compounds from grapes have been extensively researched, including the scavenging of free radicals, the suppression of lipid oxidation, the decrease of hydroperoxide production, and so on. These properties are especially significant in terms of bioactivity. Moreover, antioxidants can stop the oxidation process by preferentially interacting with an oxidizing agent, rather than the desired target molecules or cells [64].

In addition to the FRAP assay, the DPPH scavenging activity was evaluated to further support our research findings. Both the DPPH and FRAP assays could be used to determine the antioxidant activity, as both showed high reproducibility [65].

The maximum antioxidant activity, 48.32 μmol Fe^{2+}/mL and 77.2%, as measured by the FRAP and DDPH assays, respectively, was observed in the sonicated samples of run order 8, acquired at 90% amplitude for 5 min (sample SM90/5) (Table 2). The lowest activity, 15.02 μmol Fe^{2+}/mL or 66.8% (run order 3) was observed at 50% amplitude for 3 min (sample SM50/3).

It was noted that the greatest extraction of antioxidants required the same ultrasonic amplitude (90%) as for the TPC and MA, but the maximum time was 5 min, not 3 min as for the TPC and MA. Moreover, the fact that antioxidants also include non-phenolic chemicals, which may require a higher temperature for extraction, may help to explain a slightly higher extraction temperature of 60.17 °C for the maximum value of antioxidant compounds. Previous studies that claimed antioxidants required a higher temperature for extraction are consistent with this conclusion [25]. Moreover, the results might suggest that the antioxidant activity of the samples was significantly influenced by the TPC levels.

It can be seen that the antioxidant activity of the sonicated samples increases in proportion to the treatment conditions, from 11.84% to about 3.6 times as measured by the FRAP and from 2.77 to 18.77% as measured by the DPPH free radical scavenging assay, compared to the untreated sample (C).

According to the mechanism of action exhibited by antioxidant compounds, the methods used to measure antioxidant activity evaluate the ability of antioxidant species to scavenge free radicals or the ability of antioxidants to reduce an oxidant compound [65].

FRAP values quantify the ability of the antioxidant compounds contained in red grape must samples to donate electrons and, thus, reduce the oxidized species. A relevant indicator of a compound possessing potential antioxidant activity may be its reducing power [66].

It can be observed that the presence of a significant amount of phenolic compounds in red grape must, as a result of US treatment, led to high FRAP values.

The DPPH radical scavenging activity reflects the hydrogen-donating capacity of the samples, thanks to the presence of bioactive substances. When the DPPH radical scavenging activity of the samples was examined, the same conclusion was reached as in the FRAP evaluation, namely that the antioxidant potential was higher after treatment with ultrasound.

A closer examination of the results obtained from the FRAP and DPPH tests revealed that the increase in the electron-donating capacity or reducing power of the samples in response to US treatment was greater than that recorded for their free radical scavenging capacity.

The study performed by Rajurkar and Hande [67] revealed that the phenolic compounds exhibited a much higher correlation with the reducing power than with the DPPH free radical scavenging activity. Thus, it is estimated that the phenolic compounds extracted from red grapes provide antioxidant protection directly through the mechanism of reducing the oxidized intermediates in the chain reaction.

Our results suggest that the FRAP and DPPH assays could be used to distinguish the dominant mechanism, by which polyphenolic compounds from grape must act as antioxidants.

Other results showed that the antioxidant activity of phenolic compounds may have a concentration saturation limit, beyond which the activity cannot be increased by concentration. However, some results showed that there are factors other than concentration that influence the antioxidant properties of phenolic compounds, as the relationship between the phenolic compounds and the antioxidant activity was inconsistent [8,68]. Furthermore, other research showed a significant relationship between the polyphenolic compounds assessed in the various samples and the outcomes of the antioxidant activity tests [69].

As their p-values are less than 0.0500, Table 6 shows that the model parameters A, B, and A^2 are significant for the antioxidant activity measured by both the FRAP and DPPH assays. Values greater than 0.1000 indicate that the model terms are not decisive. The model significance is indicated by the model F-value of 330.16 and 58.81, respectively. The F-values for lack of fit, 2.40 and 102.78, respectively, indicate that the lack of fit is not significant.

Table 6. ANOVA and coefficient table for the quadratic model. Response 3: antioxidant activity.

Source	F-Value		*p*-Value		R^2		Adjusted R^2		Coefficients	
	FRAP	**DPPH**	**FRAP**	**DPPH**	**FRAP**	**DPPH**	**FRAP**	**DPPH**	**FRAP**	**DPPH**
Model	330.16	58.81	<0.0001	<0.0001	0.9958	0.9767	0.9928	0.9601		
Intercept									28.14	73.17
A	1587.16	246.65	<0.0001	<0.0001					13.88	3.58
B	33.68	37.65	0.0004	0.0005					2.17	1.40
AB	2.85	2.05	0.1354	0.1954					0.7200	–0.4000
A^2	12.04	6.08	0.0104	0.0431					1.78	–0.8293
B^2	2.59	0.0556	0.1515	0.8203					0.8266	–0.0793
Lack of fit	2.40	102.78	0.2085	0.0003						

Amplitude (A); time (B).

The predicted R^2 (0.9727 FRAP and 0.7829 DPPH) and the adjusted R^2 (0.9928 FRAP and 0.9601 DPPH) are in good agreement (i.e., the difference is less than 0.2), indicating that the model makes accurate predictions.

Equations (5) and (6) display the final equations in terms of the actual factors for the antioxidant activity as measured by the FRAP and DPPH assays. For specific levels of each element, the equation can be used to predict the response.

$$Y_{FRAP} = 16.030 - 0.074 \times A - 6.965 \times B + 0.036 \times A \times B + 0.004 \times A^2 + 0.826 \times B^2 \quad (5)$$

$$Y_{DPPH} = 37.996 + 0.549 \times A + 3.434 \times B - 0.020 \times A \times B - 0.002 \times A^2 - 0.079 \times B^2 \quad (6)$$

where A is the amplitude and B represents the time.

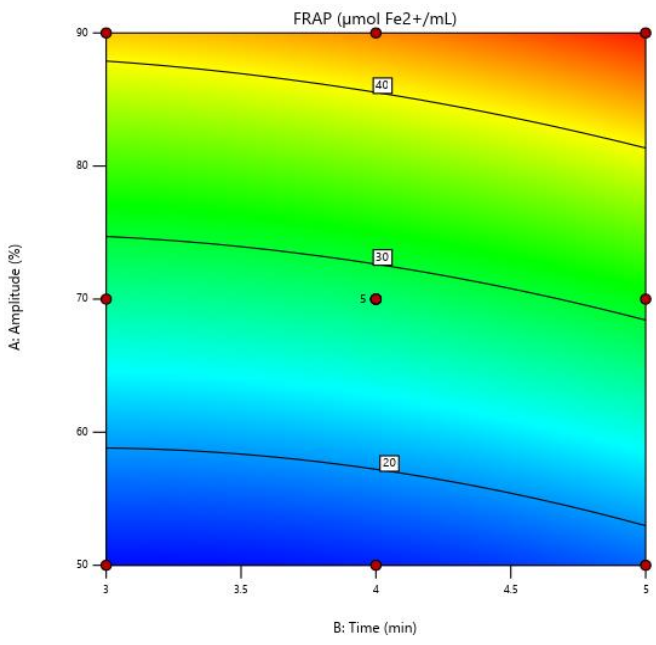
Figure 4 shows the kinetics of the antioxidant activity in samples treated with US, as well as the 3D response surfaces and contour plots.

From Figure 4, which compares the effects of the treatment time and ultrasonic wave amplitude on the antioxidant activity of the samples, it can be seen that both the FPAP and DPPH values increase with both factors, up to a maximum value of 48.32 µmol Fe^{2+}/mL and 77.2% in sample SM90/5.

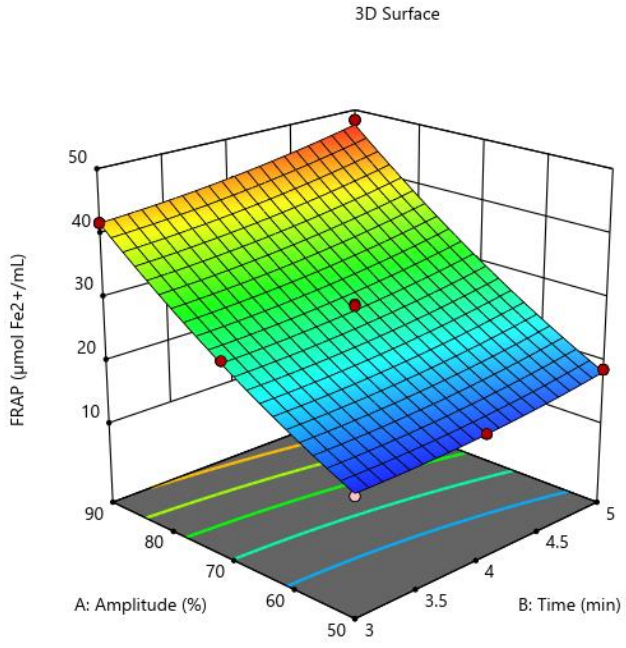
Other researchers came to the conclusion that the antioxidant activity of wines is more closely related to the type of individual phenolic compounds found in the wines than to the TPC. It has also been proposed that the antioxidant activity is mostly attributed to the flavan-3-ol fraction, rather than anthocyanins [70].

However, the grapes and wines showed a strong link between their phenolic composition and the antioxidant activity. The main substances involved in this bioactivity varied depending on the type of sample examined. The primary category, with a significant contribution to this feature, was identified as anthocyanins. Additionally, variations in the phenolic component quantities may account for the variations in the antioxidant efficacy between grape cultivars [71].

In the situation described, the optimal circumstances for antioxidant activity would be a higher amplitude and a longer duration of treatment compared to the TPC and MA.

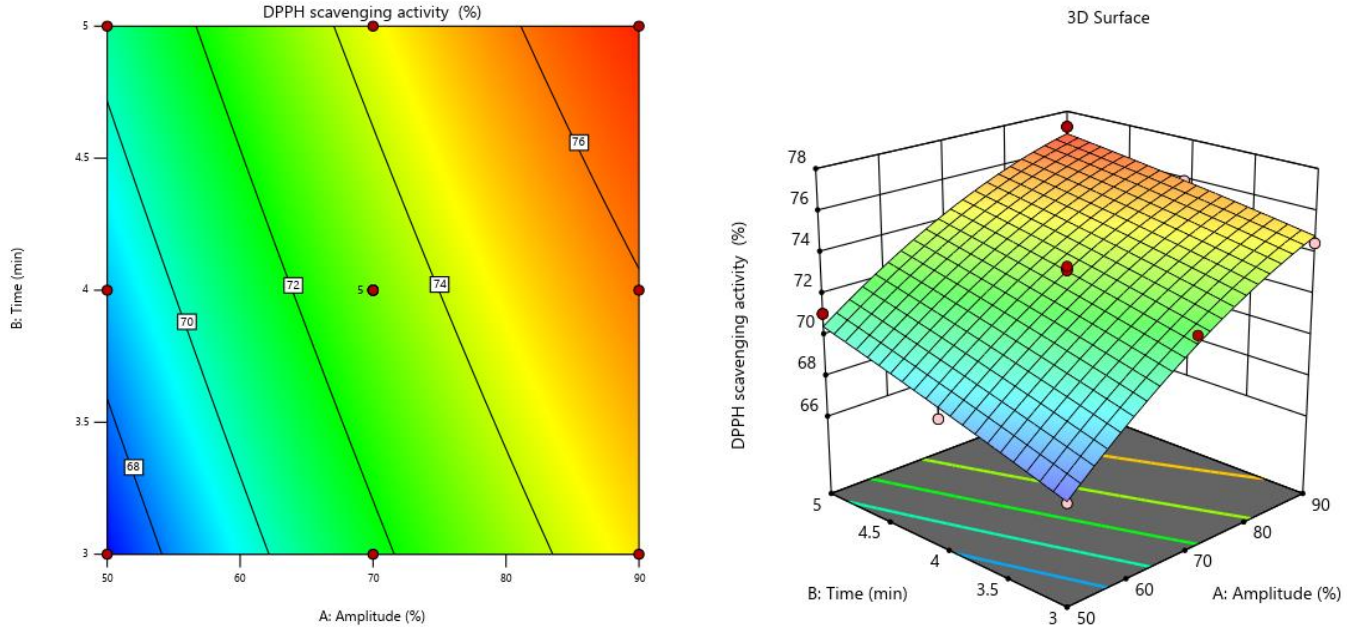


(**a**)



(**b**)

Figure 4. *Cont.*



(**c**) (**d**)

Figure 4. Influence of the process parameters on the antioxidant activity: (**a**) amplitude (A) and time (B) as contour graph for the FRAP values; (**b**) amplitude (A) and time (B) as the 3D surface for the FRAP values; (**c**) amplitude (A) and time (B) as the contour graph for the DPPH values; (**d**) amplitude (A) and time (B) as the 3D surface for the DPPH values.

3.4. Correlations between Total Phenolic Content, Monomeric Anthocyanins and Antioxidant Activity

The results of our study show that the higher the amplitude and the longer the duration of the ultrasound treatment, the higher the antioxidant activity, expressed both as the FRAP or DPPH free radical scavenging activity, as the maximum values for the antioxidant activity were found when an ultrasonic amplitude of 90% was applied for 5 min (sample SM90/5): 48.32 μmol Fe^{2+}/mL and 77.2%, respectively.

It can also be observed that the antioxidant activity of the sonicated samples, expressed by both the FRAP and DPPH tests, increased in proportion to the total polyphenol and monomeric anthocyanin content.

For the maximum values observed for the total polyphenol content (1396.06 μg GAE/mL) and the monomeric anthocyanins (223.4 mg CGE/L), when an ultrasonic amplitude of 90% was applied for 3 min (sample SM90/3), the antioxidant activity was 3.11 times higher when measured with the FRAP test and 14.62% higher when measured as DPPH radical scavenging activity (%) compared to the control sample (C). These improved values confirmed the greatest influence of phenolics and anthocyanins on the antioxidant activity.

These results are in agreement with those reported by some authors who have shown a positive correlation between the TPC and the antioxidant activity evaluated by DPPH [72], or who have linked the high antiradical power of the samples to their anthocyanin concentration [73], but are in contrast to others [74,75].

The current analysis shows a very good correlation ($p < 0.01$) between the TPC and antioxidant activity as the FRAP (Pearson's r = 0.917) and as the DPPH radical scavenging activity (%) (Pearson's r = 0.878). The same, very good, Pearson correlations were found between the MA and the antioxidant activity as the FRAP (Pearson's r = 0.928) and as the DPPH radical scavenging activity (%) (Pearson's r = 0.888).

Finally, our results showed that the antioxidant activity of the sonicated samples was influenced by the TPC and MA, although the samples with the highest TPC and MA did

not always show the highest values for antioxidant activity expressed either as the FRAP or as the free radical scavenging activity, DPPH %.

3.5. Optimization of the Experimental Model for Increasing the Extraction Rate of Biologically Active Compounds and the Antioxidant Activity in Grape Must

Aiming to maximize the extraction of biologically active compounds in grape must, as well as to enhance its antioxidant activity, we investigated the optimization function of the model made with the Design Expert® software version 13, (Stat-Ease, Inc., Minneapolis, USA, 2022). For numerical optimization, any combination of one or more objectives was optimized. The goals we applied to either factors or responses were as follows: amplitude and treatment time in range; TPC, MA, FRAP, and DPPH maximized.

The optimization function of the software gave us 20 found solutions, indicating the solution with the highest value of desirability, which was 0.918 (Figure 5), namely an amplitude of 90% and a treatment time of 4 min and 24 s, as the solution selected to maximize the extraction of the TPC, MA, and antioxidant activity as the FRAP and DPPH scavenging activity.

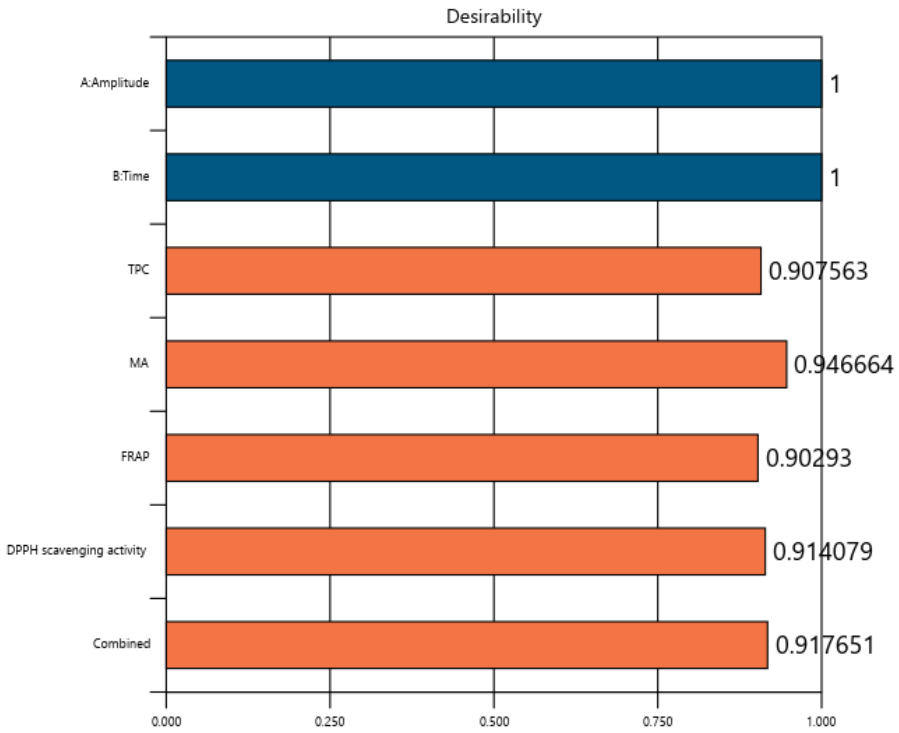


Figure 5. Graphical view of the optimal solution: the optimal factor settings are shown as red bars and the optimal response predictions are shown in blue.

To identify the factor settings that meet specified objectives, numerical optimization searches the design space using the models built during the analysis (Figure 6). Under certain conditions, the model can accurately predict the extraction rate of compounds.

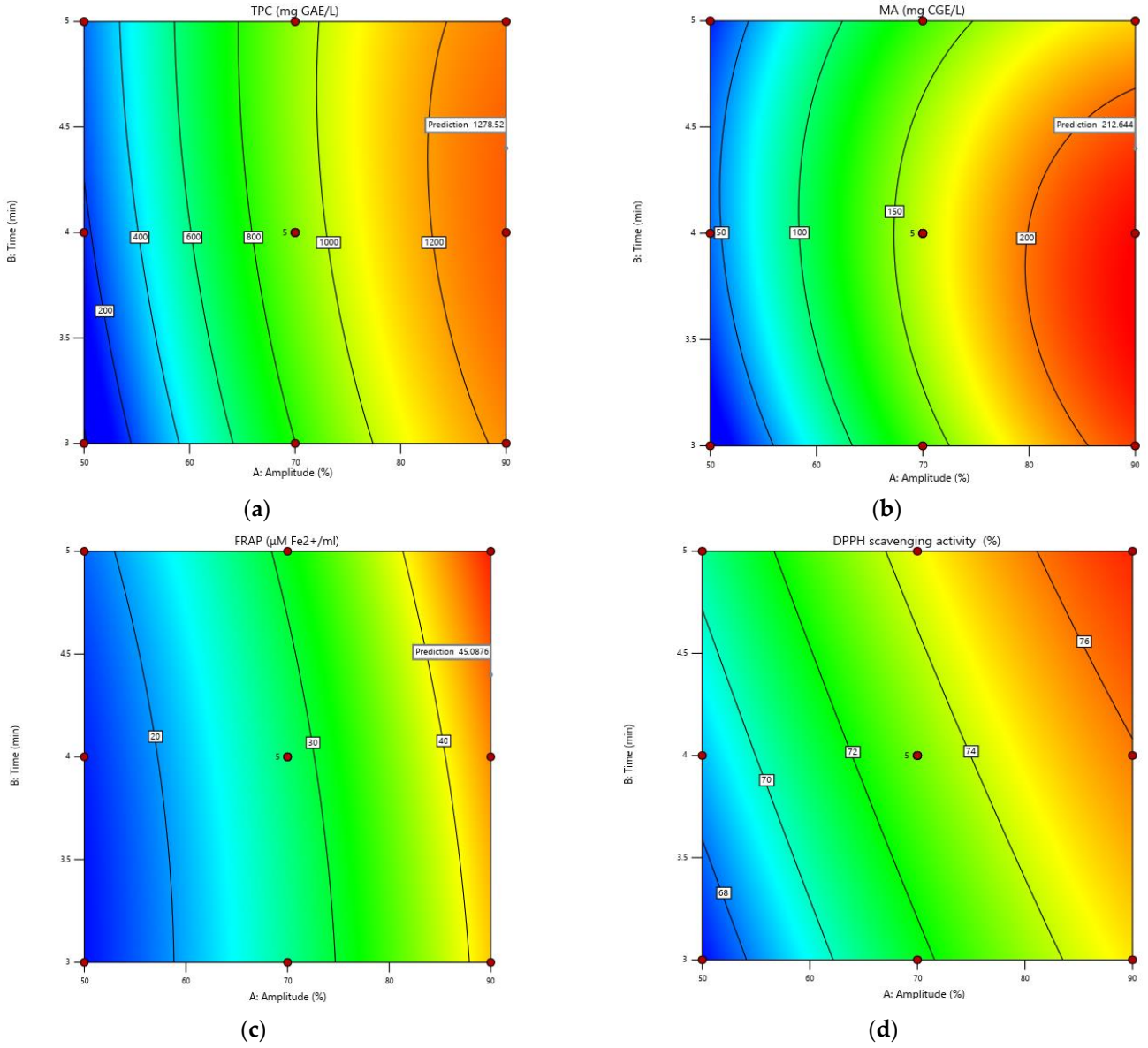


Figure 6. The numerical optimization plots for the optimal solution: (**a**) predicted value for the TPC; (**b**) predicted value for the MA; (**c**) predicted value for the FRAP; (**d**) predicted value for the DPPH.

Figure 7 shows the optimization graph for the optimal displayed solution, highlighting the point at which the response criteria is met.

3.6. Confirmation of the Model

The optimization analysis was used to guide the validation trials, which were conducted using the same methodology at the ideal selected amplitude—90% and treatment of time 4 min and 24 s. The predicted data were found to be TPC—1278.52 (µg GAE/mL), MA—212.64 (mg/L), FRAP—45.09 (µmol Fe^{2+}/mL) and DPPH—76.31%, as observed in Figures 6 and 7.

The observed response data and the predicted data from the modelled response were compared under the extraction conditions. The errors for the TPC, MA, FRAP, and DPPH were −8.70%, −0.99, 3.14 and 0.25%, respectively, as shown in Table 7, indicating the confirmation of the model.

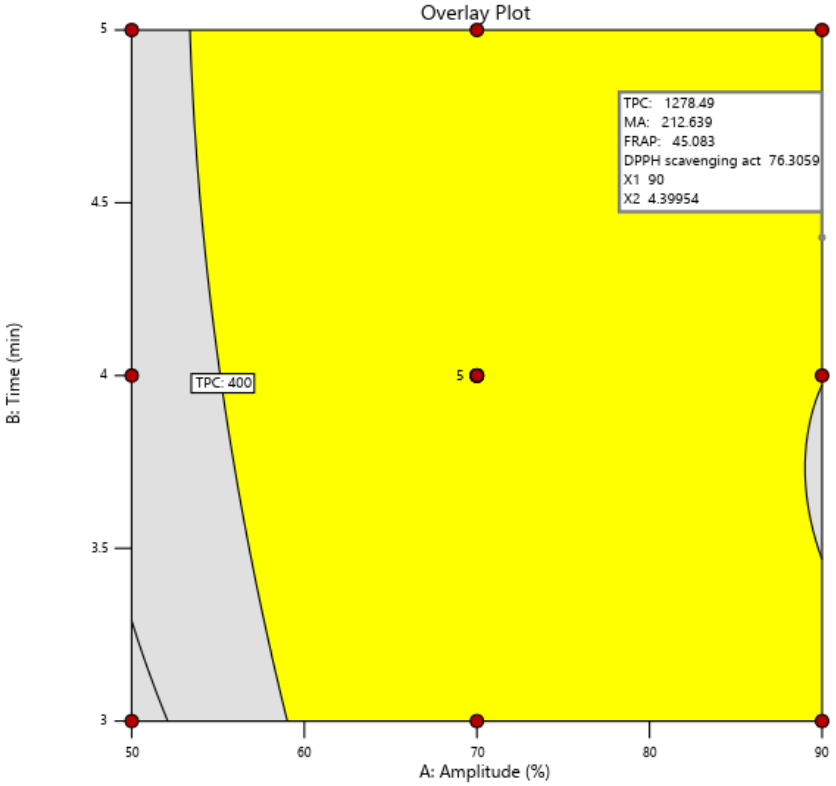


Figure 7. The overlay graph for the optimal solution: the bright yellow color defines the acceptable factor settings and grey color defines the unacceptable factor settings.

Table 7. Confirmation table of the model.

Response Data	TPC (μg GAE/mL)	MA (mg CGE/L)	FRAP (μmol Fe^{2+}/mL)	DPPH Scavenging Activity (%)
Observed	1176.16	210.56	46.55	76.5
Predicted	1278.52	212.64	45.09	76.31
Error (%)	–8.70	–0.99	3.14	0.25

Total polyphenol content (TPC); monomeric anthocyanins content (MA); antioxidant activity (FRAP).

The small differences between the predicted and observed values showed that the extraction optimization model obtained with the RSM in this study had a high degree of fit, and has the potential to be used even for large-scale extraction of phenolics and other antioxidant compounds from grapes using high-power ultrasound.

4. Conclusions

The acceleration of extraction kinetics using a physical technique can be important when the levels of physiologically active compounds in grapes are high. The use of ultrasound reduces the extraction times, while intensifying and almost maximizing the extraction yield and antioxidant activity. Overall, the use of ultrasound in winemaking opens up the possibility to optimize and better manage the vinification of red grapes. Due to the relatively short time required for ultrasound exposure, the application of ultrasound can be considered as a continuous pre-treatment for crushed red grapes prior to filling the winemaking tank. High-power ultrasound treatment of crushed grapes for a few minutes increases both the extraction rate of the bioactive compounds and the antioxidant activity by 12 times for the TPC, 14 times for the MA, 3.6 times for the FRAP and by 18.77% for the free radical scavenging activity, DPPH. However, a treatment time of 5 min and an amplitude of 90% led to a decrease in the TPC and MA, while the FRAP and DPPH values

increased with the same operating parameters. The optimal solution to develop a procedure that maximizes the TPC and MA extraction rate and the antioxidant activity during the first stage of winemaking seems to be an A of 90% and a t of 4 min and 24 s. The use of high-power ultrasound in the winemaking process for the treatment of crushed grapes has proved to be a very promising and effective technique for the extraction of high-value bioactive compounds, with great potential for commercial use. For this reason, considering the optimal solution found in this study, our further research on the impact of ultrasound treatment will include an in-depth investigation related to the content of the individual polyphenolic compounds of samples during the winemaking process, namely grape must, maceration, fermentation and bottling, with the aim of reducing the maceration period and better managing the whole process.

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References

1. OIV. World Wine Production Outlook—OIV First Estimates. no. November. International Organisation of Vine and Wine, 2022; p. 1. Available online: https://www.oiv.int/public/medias/8553/en-oiv-2021-world-wine-production-first-estimates-to-update.pdf (accessed on 14 January 2023).
2. Ntuli, R.G.; Saltman, Y.; Ponangi, R.; Jeffery, D.W.; Bindon, K.; Wilkinson, K.L. Impact of skin contact time, oak and tannin addition on the chemical composition, color stability and sensory profile of Merlot wines made from flash détente treatment. *Food Chem.* **2023**, *405*, 134849. [CrossRef]
3. Zhang, Q.; Wang, T. Effect of ultrasound irradiation on the evolution of color properties and major phenolic compounds in wine during storage. *Food Chem.* **2017**, *234*, 372–380. [CrossRef] [PubMed]
4. Huang, G.; Chen, S.; Dai, C.; Sun, L.; Sun, W.; Tang, Y.; Xiong, F.; He, R.; Ma, H. Effects of ultrasound on microbial growth and enzyme activity. *Ultrason. Sonochem.* **2017**, *37*, 144–149. [CrossRef]
5. Zhang, X.; Zuo, Z.; Yu, P.; Li, T.; Guang, M.; Chen, Z.; Wang, L. Rice peptide nanoparticle as a bifunctional food-grade Pickering stabilizer prepared by ultrasonication: Structural characteristics, antioxidant activity, and emulsifying properties. *Food Chem.* **2021**, *343*, 128545. [CrossRef]
6. Aekthammarat, D.; Tangsucharit, P.; Pannangpetch, P.; Sriwantana, T.; Sibmooh, N. Moringa oleifera leaf extract enhances endothelial nitric oxide production leading to relaxation of resistance artery and lowering of arterial blood pressure. *Biomed. Pharmacother.* **2020**, *130*, 110605. [CrossRef]
7. Brenes, A.; Viveros, A.; Chamorro, S.; Arija, I. Use of polyphenol-rich grape by-products in monogastric nutrition. A review. *Anim. Feed Sci. Technol.* **2016**, *211*, 1–17. [CrossRef]
8. Xia, E.Q.; Deng, G.F.; Guo, Y.J.; Bin Li, H. Biological activities of polyphenols from grapes. *Int. J. Mol. Sci.* **2010**, *11*, 622–646. [CrossRef] [PubMed]
9. Kumar, L.; Tian, B.; Harrison, R. Interactions of Vitis vinifera L. cv. Pinot Noir grape anthocyanins with seed proanthocyanidins and their effect on wine color and phenolic composition. *LWT* **2022**, *162*, 113428. [CrossRef]
10. Yusoff, I.M.; Mat Taher, Z.; Rahmat, Z.; Chua, L.S. A review of ultrasound-assisted extraction for plant bioactive compounds: Phenolics, flavonoids, thymols, saponins and proteins. *Food Res. Int.* **2022**, *157*, 111268. [CrossRef]
11. Chemat, F.; Rombaut, N.; Meullemiestre, A.; Turk, M.; Perino, S.; Fabiano-Tixier, A.S.; Abert-Vian, M. Review of Green Food Processing techniques. Preservation, transformation, and extraction. *Innov. Food Sci. Emerg. Technol.* **2017**, *41*, 357–377. [CrossRef]

12. Nie, V.; Chen, D.; Ye, J.; Lu, Y.; Dai, Z. Optimization and kinetic modeling of ultrasonic-assisted extraction of fucoxanthin from edible brown algae Sargassum fusiforme using green solvents. *Ultrason. Sonochem.* **2021**, *77*, 105671. [CrossRef] [PubMed]
13. Chemat, F.; Rombaut, N.; Sicaire, A.G.; Meullemiestre, A.; Fabiano-Tixier, A.S.; Abert-Vian, M. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrason. Sonochem.* **2017**, *34*, 540–560. [CrossRef] [PubMed]
14. Pallarésa, N.; Lorenzo, J.; Munekata, P.; Barba, F. Ultrasound Processing: A Sustainable Alternative. In *Sustainable Production Technology in Food*, 1st ed.; Elsevier: Amsterdam, The Netherlands, 2021; pp. 155–164.
15. Körzendörfer, A. Vibrations and ultrasound in food processing—Sources of vibrations, adverse effects, and beneficial applications—An overview. *J. Food Eng.* **2022**, *324*, 110875. [CrossRef]
16. Cui, Q.; Wang, L.; Wang, G.; Zhang, A.; Wang, X.; Jiang, L. Ultrasonication effects on physicochemical and emulsifying properties of Cyperus esculentus seed (tiger nut) proteins. *LWT* **2021**, *142*, 110979. [CrossRef]
17. Hadidi, M.; Ibarz, A.; Pagan, J. Optimisation and kinetic study of the ultrasonic-assisted extraction of total saponins from alfalfa (*Medicago sativa*) and its bioaccessibility using the response surface methodology. *Food Chem.* **2020**, *309*, 125786. [CrossRef] [PubMed]
18. Paniwnyk, L. Applications of ultrasound in processing of liquid foods: A review. *Ultrasonic Sonochem.* **2017**, *38*, 794–806. [CrossRef] [PubMed]
19. Chemat, M.; Khan, M.K. Applications of Ultrasound in Food Technology: Processing, Preservation and Extraction. *Ultrasonics Sonochem.* **2010**, *18*, 813–835. [CrossRef]
20. Simancas, R.O.; Díaz-Maroto, M.C.; Alañón Pardo, M.E.; Pérez Porras, P.; Bautista-Ortín, A.B.; Gómez-Plaza, E.; Pérez-Coello, M.S. Effect of power ultrasound treatment on free and glycosidically-bound volatile compounds and the sensorial profile of red wines. *Molecules* **2021**, *26*, 1193. [CrossRef]
21. Bautista-Ortin, A.B.; Jiménez-Martínez, M.D.; Jurado, R.; Iniesta, J.A.; Terrades, S.; Andrés, A.; Gómez-Plaza, E. Application of high-power ultrasounds during red wine vinification. *Int. J. Food Sci. Technol.* **2017**, *52*, 1314–1323. [CrossRef]
22. Morata, A. *Red Wine Technology*, 1st ed.; Academic Press: Cambridge, MA, USA, 2019; pp. 210–248.
23. International Organisation of Vine and Wine. International Code of Oenological Practices, 2022, no. 2022 Issue. Paris, France. Available online: https://www.oiv.int/public/medias/8672/code-2022-en.pdf (accessed on 10 December 2022).
24. Poojary, M.M.; Barba, F.J.; Aliakbarian, B.; Francesco Donsì, F.; Pataro, G.; Daniel, A.; Dias, D.A.; Juliano, P. Innovative alternative technologies to extract carotenoids from microalgae and seaweeds. *Mar. Drugs* **2016**, *14*, 1–34. [CrossRef]
25. Jamshaid, S.; Ahmed, D. Optimization of ultrasound-assisted extraction of valuable compounds from fruit of Melia azedarach with glycerol-choline chloride deep eutectic solvent. *Sustain. Chem. Pharm.* **2022**, *29*, 100827. [CrossRef]
26. Anić, M.; Osrečak, M.; Andabaka, Z.; Tomaz, I.; Večenaj, V.; Jelić, D.; Kozina, B.; Kontić, J.K.; Karoglan, M. The effect of leaf removal on canopy microclimate, vine performance and grape phenolic composition of Merlot (*Vitis vinifera* L.) grapes in the continental part of Croatia. *Sci. Hortic.* **2021**, *285*, 110161. [CrossRef]
27. Ferraretto, P.; Rolle, P.; Celotti, E. Applicazione degli ultrasuoni come tecnica innovativa per ottimizzare l'estrazione dei composti fenolici e favorire la lisi del lievito. *Riv. Internet Vitic. Enol.* **2011**, *9*, 1–10.
28. Tiwari, B.K.; Patras, A.; Brunton, N.; Cullen, P.J.; Donnell, C.P.O. Effect of ultrasound processing on anthocyanins and color of red grape juice. *Ultrason. Sonochem.* **2010**, *17*, 598–604. [CrossRef] [PubMed]
29. Natolino, A.; Celotti, E. Ultrasound treatment of red wine: Effect on polyphenols, mathematical modeling, and scale-up considerations. *LWT* **2022**, *154*, 112843. [CrossRef]
30. Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Meth Enzym.* **1999**, *299*, 152–178.
31. Lee, J.; Durst, R.W.; Wrolstad, R.E. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *J. AOAC Int.* **2005**, *88*, 1269–1278. [CrossRef] [PubMed]
32. Benzie, I.F.; Strain, J.J. Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal Biochem.* **1996**, *239*, 70–76. [CrossRef]
33. Kalita, D.; Jayanty, S.S. Comparison of Polyphenol Content and Antioxidant Capacity of Colored Potato Tubers, Pomegranate and Blueberries. *J. Food Process Technol.* **2014**, *5*, 358–364.
34. Commission Regulation (EEC) No 2676/90 of 17 September 1990 Determining Community Methods for the Analysis of Wines (OJ L 272 03.10.1990). Available online: http://data.europa.eu/eli/reg/1990/2676/oj (accessed on 20 October 2022).
35. Palma, M.; Barroso, C.G. Ultrasound-assisted extraction and determination of tartaric and malic acids from grapes and winemaking by-products. *Anal. Chim. Acta* **2002**, *458*, 119–130. [CrossRef]
36. Mason, T.J.; Lorimer, J.P. *Applied Sonochemistry: The Uses of Power Ultrasound in Chemistry and Processing*; Wiley-VCH: Weinheim, Germany, 2002; pp. 15–43.
37. Ferraretto, P.; Cacciola, V.; Ferran Batllo, I.; Celotti, E. Ultrasounds application in winemaking: Grape maceration and yeast lysis. *Ital. J. Food Sci.* **2013**, *25*, 160–168.
38. Zhang, Q.; Shen, Y.; Fan, X.H.; Martin, J.F.G. Preliminary study of the effect of ultrasound on physicochemical properties of red wine. *CyTA J. Food* **2015**, *14*, 55–64. [CrossRef]

39. Martin, J.F.G.; Sun, D.W. Ultrasound and electric fields as novel techniques for assisting the wine ageing process: The state-of-the-art research. *Trends Food Sci. Technol.* **2013**, *33*, 40–53. [CrossRef]
40. Ćurko, N.; Tomašević, M.; Jambrak, A.R. The effect of high-power ultrasound on phenolic composition, chromatic characteristics, and aroma compounds of red wines. *Croat. J. Food Sci. Technol.* **2017**, *9*, 136–144. [CrossRef]
41. Luo, H.; Schmid, F.; Grbin, P.R.; Jiranek, V. Viability of common wine spoilage organisms after exposure to high power ultrasonics. *Ultrason. Sonochem.* **2012**, *19*, 415–420. [CrossRef]
42. Bosiljkov, T.; Dujmić, F.; Bubalo, M.C.; Hribar, J.; Vidrih, R.; Brnčić, M.; Zlatic, E.; Redovniković, I.R.; Jokić, S. Natural deep eutectic solvents and ultrasound-assisted extraction: Green approaches for extraction of wine lees anthocyanins. *Food Bioprod. Process.* **2017**, *102*, 195–203. [CrossRef]
43. Drosou, C.; Kyriakopoulou, K.; Bimpilas, A.; Tsimogiannis, D.; Krokida, M. A comparative study on different extraction techniques to recover red grape pomace polyphenols from vinification byproducts. *Ind. Crops Prod.* **2015**, *75*, 141–149. [CrossRef]
44. Roman, T.; Tonidandel, L.; Nicolini, G.; Bellantuono, E.; Barp, L.; Larcher, R.; Celotti, E. Evidence of the possible interaction between ultrasound and thiol precursors. *Foods* **2020**, *9*, 104. [CrossRef]
45. Reche, C.; Rosselló, C.; Dalmau, E.; Eim, V.; Simal, S. Quantification of microstructural changes in artichoke by-products by image analysis after high-power ultrasound-assisted extraction of bioactive compounds. *LWT* **2022**, *171*, 114127. [CrossRef]
46. Pérez-Porras, P.; Bautista-Ortín, A.B.; Jurado, R.; Gómez-Plaza, E. Using high-power ultrasounds in red winemaking: Effect of operating conditions on wine physico-chemical and chromatic characteristics. *LWT* **2021**, *138*, 110645. [CrossRef]
47. Dzah, C.S.; Duan, Y.; Zhang, H.; Wen, C.; Zhang, J. The effects of ultrasound assisted extraction on yield, antioxidant, anticancer and antimicrobial activity of polyphenol extracts: A review. *Food Biosci.* **2020**, *35*, 100547. [CrossRef]
48. Bi, J.; Yang, Q.; Sun, J.; Chen, J.; Zhang, J. Study on ultrasonic extraction technology and oxidation resistance of total flavonoids from peanut hull. *Food Sci. Technol. Res.* **2011**, *17*, 187–198. [CrossRef]
49. Dai, J.; Mumper, R.J. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules* **2010**, *15*, 7313–7352. [CrossRef] [PubMed]
50. Akowuah, G.A.; Mariam, A.; Chin, J.H. The effect of extraction temperature on total phenols and antioxidant activity of Gynura procumbens leaf. *Pharmacogn. Mag.* **2009**, *5*, 81–85.
51. Havlikova, L.; Mikova, K. Heat Stability of Anthocyanins. *Z. Leb. Forsch.* **1985**, *181*, 427–432. [CrossRef]
52. Jackman, R.L.; Yada, R.Y.; Tung, M.A.; Speers, R.A. Anthocyanins as food colorants—A review. *J. Food Biochem.* **1987**, *11*, 201–247. [CrossRef]
53. Tao, Y.; Han, Y.; Liu, W.; Peng, L.; Wang, Y.; Kadam, S.; Show, P.L.; Ye, X. Parametric and phenomenological studies about ultrasound-enhanced biosorption of phenolics from fruit pomace extract by waste yeast. *Ultrason. Sonochem.* **2019**, *52*, 193–204. [CrossRef]
54. Martínez-Ramos, T.; Benedito-Fort, J.; Watson, N.J.; Ruiz-López, I.I.; Che-Galicia, G.; Corona-Jiménez, E. Effect of solvent composition and its interaction with ultrasonic energy on the ultrasound-assisted extraction of phenolic compounds from Mango peels (*Mangifera indica* L.). *Food Bioprod. Process.* **2020**, *122*, 41–54. [CrossRef]
55. Zainol, M.K.; Mohd Subri, I.; Zamri, A.I.; Mohd Zin, Z.; Fisal, A.; Mamat, H. Antioxidative properties and proximate analysis of spent coffee ground (SCG) extracted using ultrasonic-methanol assisted technique as a potential functional food ingredient. *Food Res.* **2020**, *4*, 636–644. [CrossRef]
56. Antony, A.; Farid, M. Effect of Temperatures on Polyphenols during Extraction. *Appl. Sci.* **2022**, *12*, 2107. [CrossRef]
57. Constantin, O.E.; Istrati, D.I. Extraction, Quantification and Characterization Techniques for Anthocyanin Compounds in Various Food Matrices—A Review. *Horticulturae* **2022**, *8*, 1084. [CrossRef]
58. Chung, C.; Rojanasasithara, T.; Mutilangi, W.; McClements, D.J. Stabilization of natural colors and nutraceuticals: Inhibition of anthocyanin degradation in model beverages using polyphenols. *Food Chem.* **2016**, *212*, 596–603. [CrossRef]
59. Cacace, J.E.; Mazza, G. Optimisation of extraction of anthocyanins from black currants with aqueous ethanol. *J. Food Sci.* **2006**, *68*, 240–248. [CrossRef]
60. Ying, Z.; Han, X.; Li, J. Ultrasound-assisted extraction of polysaccharides from mulberry leaves. *Food Chem.* **2011**, *127*, 1273–1279. [CrossRef]
61. Fu, X.; Wang, D.; Belwal, T.; Xie, J.; Xu, Y.; Li, L.; Zou, L.; Zhang, L.; Luo, Z. Natural deep eutectic solvent enhanced pulse-ultrasonication assisted extraction as a multi-stability protective and efficient green strategy to extract anthocyanin from blueberry pomace. *LWT* **2021**, *144*, 111220. [CrossRef]
62. Zhang, L.; Fan, G.; Khan, M.A.; Yan, Z.; Beta, T. Ultrasonic-assisted enzymatic extraction and identification of anthocyanin components from mulberry wine residues. *Food Chem.* **2020**, *323*, 126714. [CrossRef]
63. Huang, H.; Xu, Q.; Belwal, T.; Li, L.; Aalim, H.; Wu, Q.; Duan, Z.; Zhang, X.; Luo, Z. Ultrasonic impact on viscosity and extraction efficiency of polyethylene glycol: A greener approach for anthocyanins recovery from purple sweet potato. *Food Chem.* **2019**, *283*, 59–67. [CrossRef] [PubMed]
64. Vaquero, M.J.R.; Alberto, M.R.; Nadra, M.C.M. Antibacterial effect of phenolic compounds from different wines. *Food Control* **2007**, *18*, 93–101. [CrossRef]
65. Thaipong, K.; Boonprakob, U.; Crosby, K.; Cisneros-Zevallos, L.; Byrne, D.H. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J. Food Compos. Anal.* **2006**, *19*, 669–675. [CrossRef]

66. Vasyliev, G.S.; Vorobyova, V.I.; Linyucheva, O.V. Evaluation of reducing ability and antioxidant activity of fruit pomace extracts by spectrophotometric and electrochemical methods. *J. Anal. Methods Chem.* **2020**, *2020*, 1–16. [CrossRef]
67. Rajurkar, N.S.; Hande, S.M. Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. *Indian J. Pharm. Sci.* **2011**, *73*, 146–151. [CrossRef]
68. Dani, C.; Oliboni, L.S.; Vanderlinde, R.; Pra, D.; Dias, J.F.; Yoneama, M.L.; Bonatto, D.; Salvador, M.; Henriques, J.A.P. Antioxidant activity and phenolic and mineral content of rose grape juice. *J. Med. Food* **2009**, *12*, 188–192. [CrossRef] [PubMed]
69. Negro, C.; Aprile, A.; Luvis, A.I.; De Bellis, L.; Miceli, A. Antioxidant activity and polyphenols characterization of four monovarietal grape pomaces from salento (Apulia, italy). *Antioxidants* **2021**, *10*, 1406. [CrossRef] [PubMed]
70. Banc, R.; Socaciu, C.; Miere, D.; Filip, L.; Cozma, A.; Stanciu, O.; Loghin, F. Benefits of Wine Polyphenols on Human Health: A Review. *Bull. UASVM Food Sci. Technol.* **2014**, *71*, 79–87. [CrossRef]
71. Lingua, M.S.; Fabani, M.P.; Wunderlin, D.A.; Baroni, M.V. From grape to wine: Changes in phenolic composition and its influence on antioxidant activity. *Food Chem.* **2016**, *208*, 228–238. [CrossRef] [PubMed]
72. Fernández-Pachón, M.S.; Villaño, D.; García-Parrilla, M.C.; Troncoso, A.M. Antioxidant activity of wines and relation with their polyphenolic composition. *Anal. Chim. Acta* **2004**, *513*, 113–118. [CrossRef]
73. Gismondi, A.; Marco, G.; di Canuti, L.; Canini, A. Antiradical activity of phenolic metabolites extracted from grapes of white and red *Vitis vinifera* L. cultivars. *Vitis* **2017**, *56*, 19–26.
74. Rivero-Pérez, M.D.; Muñiz, P.; González-Sanjosé, M.L. Antioxidant Profile of Red Wines Evaluated by Total Antioxidant Capacity, Scavenger Activity, and Biomarkers of Oxidative Stress Methodologies. *J. Agric. Food Chem.* **2007**, *55*, 5476–5483. [CrossRef]
75. Banc, R.; Loghin, F.; Miere, D.; Ranga, F.; Socaciu, C. Phenolic composition and antioxidant activity of red, rosé and white wines originating from Romanian grape cultivars. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2020**, *48*, 716–734. [CrossRef]

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

Alina MAIER[1], Vasile PADUREANU[1*], Mirabela LUPU[1], Cristina CANJA[1], Geronimo Raducu BRANESCU[1], Cristina PADUREANU[1], Mariana-Atena POIANA[2*]

[1] Faculty of Food and Tourism, Transilvania University of Brasov, Castelului 148, 500014 Brasov, Romania
[2] Faculty of Food Engineering, University of Life Sciences "King Mihai I" from Timisoara, Calea Aradului 119, 300645 Timisoara
** Corresponding author: V. Padureanu e-mail: padu@unitbv.ro; M.A. Poiana e-mail: marianapoiana@usvt.ro*

RESEARCH ARTICLE

Abstract

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

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

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INTRODUCTION

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

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

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

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

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

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

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

MATERIALS AND METHODS

Materials

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

Ultrasonic equipment

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

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

Sample preparation

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

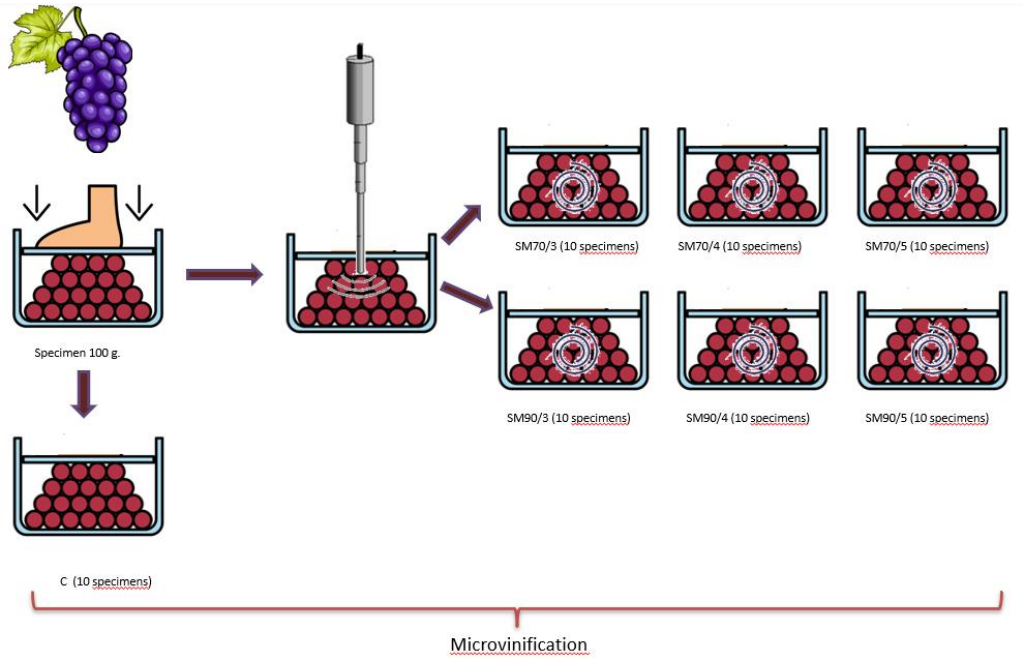

Figure 1. Sample and specimen preparation for laboratory testing. Sonicated samples (SM) at 70 and 90% amplitude for 3, 4, and 5 minutes: SM70/3 (A: 70%; t: 3 min), SM70/4 (A: 70%; t: 4 min), SM70/5 (A: 70%; t: 5 min), SM90/3 (A: 90%; t: 3 min), SM90/4 (A: 90%; t: 4 min), SM90/5 (A: 90%, t: 5 min).

Methods

Total polyphenolic content and anthocyanins determination

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

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

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

Chromatic characteristics

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

Ferric reducing antioxidant power (FRAP) assay

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

Statistical Analysis

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

RESULTS AND DISCUSSIONS

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

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

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

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

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

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

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

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

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

Effects of Ultrasound Treatment on Total Polyphenol Content

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

Effects of Ultrasound Treatment on Monomeric Anthocyanins

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

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

Effects of Ultrasound Treatment on Antioxidant Capacity

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

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

Effects of Ultrasound Treatment on Chromatic Characteristics

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

Evaluating the Effects of Ultrasound Treatment on Maceration Time

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

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

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

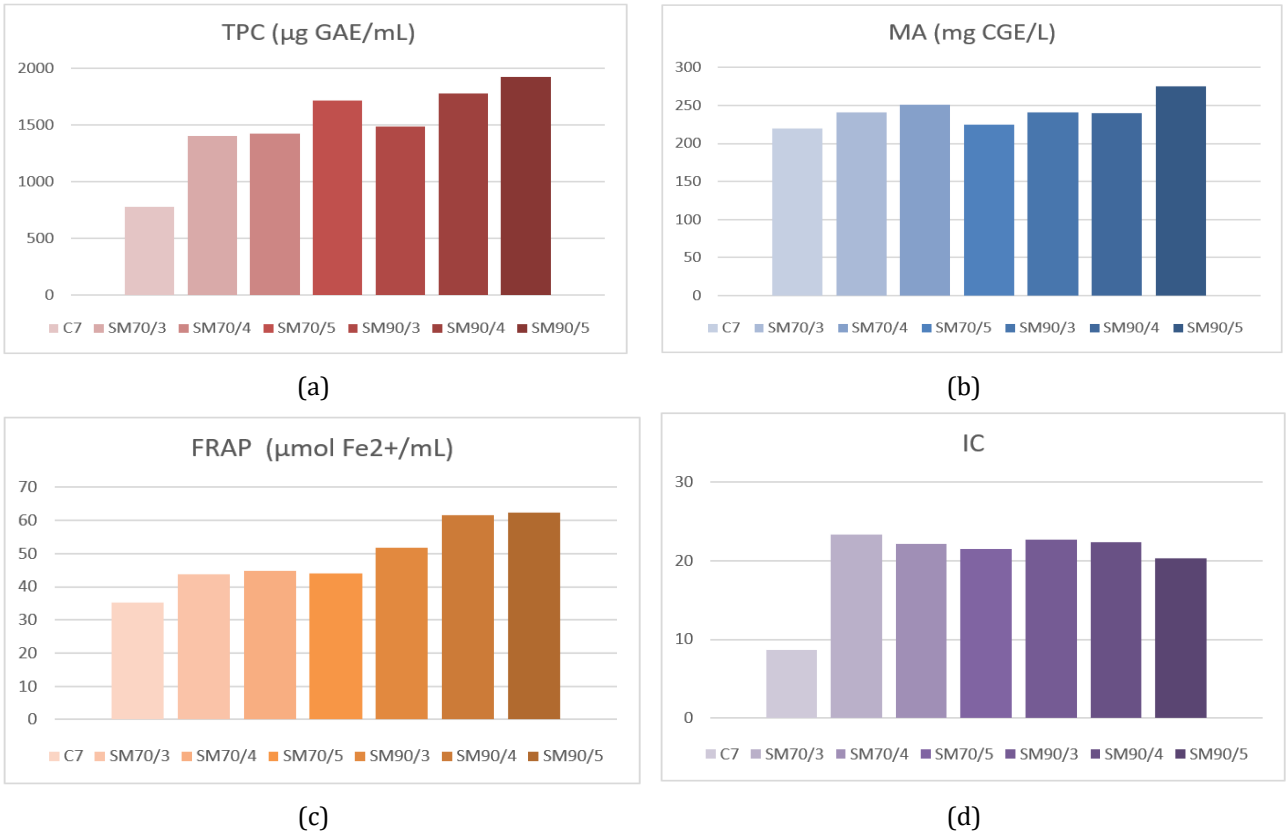

Figure 2. Comparison between the bioactive compounds and the chromatic characteristics of the sonicated samples after 3 days of maceration in comparison to the untreated sample after 7 days of maceration: (a) Total phenolic content; (b) Monomeric anthocyanins; (c) Ferric reducing antioxidant power (FRAP);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

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

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

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

Correlation between maceration time, amplitude level and ultrasonic treatment time

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

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

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

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

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

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

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

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

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

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

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

CONCLUSIONS

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

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

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Conflicts of Interest

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

REFERENCES

1. Castaldo L, Narváez A, Izzo L, Graziani G, Gaspari A, Di Minno G, Ritieni A. Red wine consumption and cardiovascular health. Molecules. 2019 Oct 8;24(19):3626.
2. Banc R, Socaciu C, Miere D, Filip L, Cozma A, Stanciu O, et al. Benefits of Wine Polyphenols on Human Health: A Review. Bulletin UASVM Food Science and Technology. 2014;71(2):79–87.
3. Gutiérrez-Escobar R, Aliaño-González MJ, Cantos-Villar E. Wine Polyphenol Content and Its Influence on Wine Quality and Properties: A Review. Molecules. 2021 Jan 30;26(3):718.
4. Nemzer B, Kalita D, Yashin AY, Yashin YI. Chemical Composition and Polyphenolic Compounds of Red Wines: Their Antioxidant Activities and Effects on Human Health—A Review. Beverages. 2021 Dec 28;8(1):1.
5. Buljeta I, Pichler A, Šimunović J, Kopjar M. Beneficial Effects of Red Wine Polyphenols on Human Health: Comprehensive Review. Curr Issues Mol Biol. 2023 Jan 17;45(2):782–98.
6. Nardini M. Phenolic Compounds in Food: Characterization and Health Benefits. Molecules. 2022 Jan 25;27(3):783.
7. Luzzini G, Slaghenaufi D, Pasetto F, Ugliano M. Influence of grape composition and origin, yeast strain and spontaneous fermentation on aroma profile of Corvina and Corvinone wines. LWT. 2021 May;143:111120.
8. Gómez-Plaza E, Osete-Alcaraz A, Jurado R, Iniesta JA, Bautista-Ortín AB. The application of high power ultrasounds for improving the phenolic extraction and color from grape-derived products. Acta Hortic. 2020 Mar;(1274):1–8.
9. Alencar NMM, Cazarin CBB, Corrêa LC, Maróstica Junior MR, Biasoto ACT, Behrens JH. Influence of maceration time on phenolic compounds and antioxidant activity of the Syrah must and wine. J Food Biochem. 2018 Apr;42(2) : e 12471.
10. Yusoff IM, Mat Taher Z, Rahmat Z, Chua LS. A review of ultrasound-assisted extraction for plant bioactive compounds: Phenolics, flavonoids, thymols, saponins and proteins. Food Research International. 2022;157(February):111268. Available from: https://doi.org/10.1016/j.foodres.2022.111268
11. Mukhtar K, Nabi BG, Arshad RN, Roobab U, Yaseen B, Ranjha MM, Aadil RM, Ibrahim SA. Potential impact of ultrasound, pulsed electric field, high-pressure processing and microfludization against thermal treatments preservation regarding sugarcane juice (Saccharum officinarum). Ultrason Sonochem. 2022 Nov;90:106194.

12. Aadil RM, Zeng XA, Wang MS, Liu ZW, Han Z, Zhang ZH, Hong J, Jabbar S. A A potential of ultrasound on minerals, micro-organisms, phenolic compounds and colouring pigments of grapefruit juice. Int J Food Sci Technol. 2015 May 22;50(5):1144–50.
13. Khan MK, Ghauri YM, Alvi T, Amin U, Khan MI, Nazir A, Saeed F, Aadil RM, Nadeem MT, Babu I, Maan AA. Microwave assisted drying and extraction technique; kinetic modelling, energy consumption and influence on antioxidant compounds of fenugreek leaves. Food Science and Technology. 2022;42.
14. Ahmadian S, Kenari RE, Amiri ZR, Sohbatzadeh F, Khodaparast MHH. Effect of ultrasound-assisted cold plasma pretreatment on cell wall polysaccharides distribution and extraction of phenolic compounds from hyssop (Hyssopus officinalis L.). Int J Biol Macromol. 2023 Apr;233:123557.
15. Heydari M, Carbone K, Gervasi F, Parandi E, Rouhi M, Rostami O, Abedi-Firoozjah R, Kolahdouz-Nasiri A, Garavand F, Mohammadi R. Cold Plasma-Assisted Extraction of Phytochemicals: A Review. Foods. 2023 Aug 24;12(17):3181.
16. Molino A, Mehariya S, Di Sanzo G, Larocca V, Martino M, Leone GP, et al. Recent developments in supercritical fluid extraction of bioactive compounds from microalgae: Role of key parameters, technological achievements and challenges. Journal of CO2 Utilization. 2020 Feb;36:196–209.
17. Zia S, Khan MR, Shabbir MA, Aslam Maan A, Khan MK, Nadeem M, Khalil AA, Din A, Aadil RM. An Inclusive Overview of Advanced Thermal and Nonthermal Extraction Techniques for Bioactive Compounds in Food and Food-related Matrices. Food Reviews International. 2022 Aug 18;38(6):1166–96.
18. Ranjha MM, Kanwal R, Shafique B, Arshad RN, Irfan S, Kieliszek M, Kowalczewski PŁ, Irfan M, Khalid MZ, Roobab U, Aadil RM. A Critical Review on Pulsed Electric Field: A Novel Technology for the Extraction of Phytoconstituents. Molecules. 2021 Aug 12;26(16):4893.
19. Noranizan M, Pean LF, Li JW, Aadil RM, Ahmad T, Rosli SZ, Yong YY, Kim DO. Impact of juice extraction method on the physicochemical, functional, and sensory properties of Sabah snake grass (Clinacanthus nutans) juice mix. Int Food Res J. 2020;27(5):835–45.
20. Sridhar A, Ponnuchamy M, Kumar PS, Kapoor A, Vo DVN, Prabhakar S. Techniques and modeling of polyphenol extraction from food: a review. Environ Chem Lett. 2021 Aug 17;19(4):3409–43.
21. Ranjha MM, Irfan S, Lorenzo JM, Shafique B, Kanwal R, Pateiro M, Arshad RN, Wang L, Nayik GA, Roobab U, Aadil RM. Sonication, a Potential Technique for Extraction of Phytoconstituents: A Systematic Review. Processes. 2021 Aug 14;9(8):1406.
22. Ali M, Manzoor MF, Goksen G, Aadil RM, Zeng XA, Iqbal MW, Lorenzo JM. High-intensity ultrasonication impact on the chlorothalonil fungicide and its reduction pathway in spinach juice. Ultrason Sonochem. 2023 Mar;94:106303.
23. Hussain M, Gantumur MA, Manzoor MF, Hussain K, Xu J, Aadil RM, Qayum A, Ahmad I, Zhong H, Guan R. Sustainable emerging high-intensity sonication processing to enhance the protein bioactivity and bioavailability: An updated review. Ultrason Sonochem. 2023 Jul; 97:106464.
24. Patist A, Bates D. Industrial Applications of High Power Ultrasonics. In: Ultrasound Technologies for Food and Bioprocessing Food Engineering Series. Springer, New York, NY; 2011. p. 599–616.
25. Zhang QA, Zheng H, Lin J, Nie G, Fan X, García-Martín JF. The state-of-the-art research of the application of ultrasound to winemaking: A critical review. Vol. 95, Ultrasonics Sonochemistry. Elsevier B.V.; 2023.
26. Gómez-Plaza E, Osete-Alcaraz A, Jurado R, Iniesta JA, Bautista-Ortín AB. The application of high power ultrasounds for improving the phenolic extraction and color from grape-derived products. Acta Hortic. 2020 Mar;(1274):1–8.
27. Plaza EG, Jurado R, Iniesta JA, Bautista-Ortín AB. High power ultrasounds: A powerful, non-thermal and green technique for improving the phenolic extraction from grapes to must during red wine vinification. InBIO Web of Conferences 2019 (Vol. 12, p. 02001). EDP Sciences.
28. Romero-Díez R, Matos M, Rodrigues L, Bronze MR, Rodríguez-Rojo S, Cocero MJ, Matias AA. Microwave and ultrasound pre-treatments to enhance anthocyanins extraction from different wine lees. Food Chem. 2019 Jan; 272:258–66.
29. Ferraretto P, Cacciola V, Ferran Batllo I, Celotti E. Ultrasounds application in winemaking: grape maceration and yeast lysisof. Ital J Food Sci. 2013;25:160–8.
30. OIV O. Compendium of international methods of wine and must analysis. International Organisation of Vine and Wine: Paris, France. 2009:154-96.

31. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Meth Enzymol. 1999;(299):152–78.
32. Lee J, Durst RW, Wrolstad RE. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. J AOAC Int. 2005;88(5):1269–78.
33. Benzie IF, Strain JJ. Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal Biochem. 1996;239(1):70–6.
34. Stat-Ease Inc. DESIGN-EXPERT® VERSION 12 SOFTWARE TRIAL. Minneapolis, MN 55413-2561; 2020.
35. JASP Team. JASP [Internet]. 2022. Available from: https://jasp-stats.org
36. Bautista-Ortín AB, Jiménez-Martínez MD, Jurado R, Iniesta JA, Terrades S, Andrés A, Gómez-Plaza E. Application of high-power ultrasounds during red wine vinification. Int J Food Sci Technol. 2017;1–10.
37. Setford PC, Jeffery DW, Grbin PR, Muhlack RA. Factors affecting extraction and evolution of phenolic compounds during red wine maceration and the role of process modelling. Trends in Food Science & Technology. 2017 Nov 1;69:106-17.
38. Maier A, Padureanu V, Lupu MI, Canja CM, Badarau C, Padureanu C, Alexa E, Poiana MA. Optimization of A Procedure to Improve the Extraction Rate of Biologically Active Compounds in Red Grape Must Using High-Power Ultrasound. Sustainability (Switzerland). 2023 Apr 1;15(8).
39. Natrella G, Noviello M, Trani A, Faccia M, Gambacorta G. The Effect of Ultrasound Treatment in Winemaking on the Volatile Compounds of Aglianico, Nero di Troia, and Primitivo Red Wines. Foods. 2023 Feb 1;12(3).
40. Lukić K, Brnčić M, Ćurko N, Tomašević M, Valinger D, Denoya GI, Barba FJ, Ganić KK.. Effects of high power ultrasound treatments on the phenolic, chromatic and aroma composition of young and aged red wine. Ultrason Sonochem. 2019 Dec; 59:104725.
41. Ranjha MM, Amjad S, Ashraf S, Khawar L, Safdar MN, Jabbar S, Nadeem M, Mahmood S, Murtaza MA. Extraction of Polyphenols from Apple and Pomegranate Peels Employing Different Extraction Techniques for the Development of Functional Date Bars. International Journal of Fruit Science. 2020 Sep 21;20(sup3):S1201–21.
42. Pérez-Porras P, Bautista-Ortín AB, Jurado R, Gómez-Plaza E. Using high-power ultrasounds in red winemaking: Effect of operating conditions on wine physico-chemical and chromatic characteristics. LWT. 2021 Mar 1;138.
43. Dalagnol LMG, Dal Magro L, Silveira VCC, Rodrigues E, Manfroi V, Rodrigues RC. Combination of ultrasound, enzymes and mechanical stirring: A new method to improve Vitis vinifera Cabernet Sauvignon must yield, quality and bioactive compounds. Food and Bioproducts Processing. 2017 Sep;105:197–204.
44. He F, Liang NN, Mu L, Pan QH, Wang J, Reeves MJ, et al. Anthocyanins and Their Variation in Red Wines I. Monomeric Anthocyanins and Their Color Expression. Molecules. 2012 Feb 7;17(2):1571–601.
45. He F, Liang NN, Mu L, Pan QH, Wang J, Reeves MJ, et al. Anthocyanins and Their Variation in Red Wines II. Anthocyanin Derived Pigments and Their Color Evolution. Molecules. 2012 Feb 7;17(2):1483–519.
46. Lingua MS, Fabani MP, Wunderlin DA, Baroni M V. From grape to wine: Changes in phenolic composition and its influence on antioxidant activity. Food Chem. 2016;208:228–38. Available from: http://dx.doi.org/10.1016/j.foodchem.2016.04.009
47. Banc R, Loghin F, Miere D, Ranga F, Socaciu C. Phenolic composition and antioxidant activity of red, rosé and white wines originating from Romanian grape cultivars. Not Bot Horti Agrobot Cluj Napoca. 2020;48(2):716–34.
48. Ivanova V, Vojnoski B, Stefova M. Effect of winemaking treatment and wine aging on phenolic content in Vranec wines. J Food Sci Technol. 2012 Apr 3;49(2):161–72.
49. Alencar NMM, Cazarin CBB, Corrêa LC, Maróstica Junior MR, Biasoto ACT, Behrens JH. Influence of maceration time on phenolic compounds and antioxidant activity of the Syrah must and wine. J Food Biochem. 2018 Apr;42(2):e12471.
50. Busse-Valverde N, Gómez-Plaza E, López-Roca JM, Gil-Muñoz R, Bautista-Ortín AB. The Extraction of Anthocyanins and Proanthocyanidins from Grapes to Wine during Fermentative Maceration Is Affected by the Enological Technique. J Agric Food Chem. 2011 May 25;59(10):5450–5.
51. González-Neves G, Gil G, Barreiro L. Influence of grape variety on the extraction of anthocyanins during the fermentation on skins. European Food Research and Technology. 2008 Apr 6;226(6):1349–55.
52. El Darra N, Grimi N, Maroun RG, Louka N, Vorobiev E. Pulsed electric field, ultrasound, and thermal pretreatments for better phenolic extraction during red fermentation. European Food Research and Technology. 2013 Jan 30;236(1):47–56.

53. Carrera C, Ruiz-Rodríguez A, Palma M, Barroso CG. Ultrasound assisted extraction of phenolic compounds from grapes. Anal Chim Acta. 2012;732:100–4. Available from: http://dx.doi.org/10.1016/j.aca.2011.11.032
54. Constantin OE, Istrati DI. Extraction , Quantification and Characterization Techniques for Anthocyanin Compounds in Various Food Matrices — A Review. 2022;
55. Mason TJ, Lorimer JP. Applied Sonochemistry: The Uses of Power Ultrasound in Chemistry and Processing. 1st Editio. Wiley VCH; 2002.

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

Alina MAIER[1], Vasile PĂDUREANU[1], Cristina CANJA[1], Mirabela Ioana LUPU[1], Geronimo Răducu BRĂNESCU[1], Cristina PĂDUREANU[1], Mariana-Atena POIANĂ[2]

[1]Transylvania University of Brasov, 29 Eroilor Blvd, Brasov, Romania
[2]University of Life Sciences "King Mihai I" from Timisoara,
119 Calea Aradului, Timisoara, Romania

Corresponding author emails: canja.c@unitbv.ro, marianapoiana@usvt.ro

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).

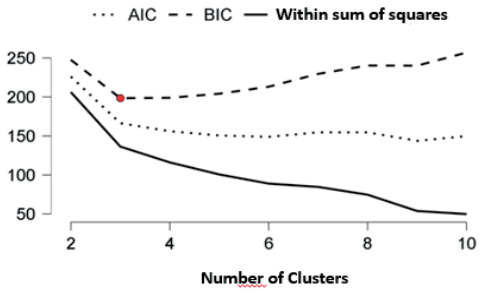

Figure 2. Elbow Diagram

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.

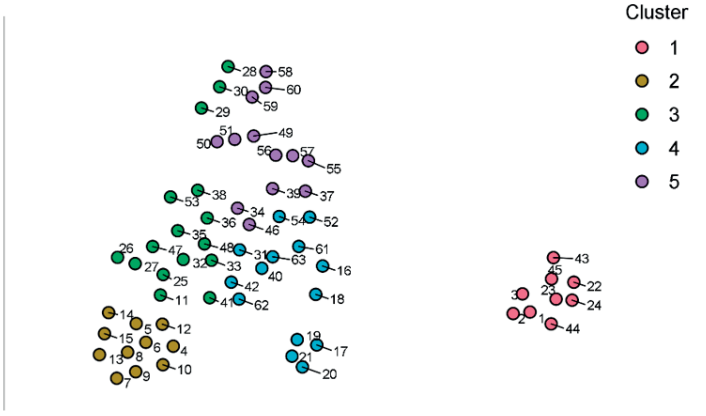

Figure 3. Two-dimensional t-SNE plot illustrating clustering of samples

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.

REFERENCES

Aadil, R. M., Zeng, X., Wang, M., Liu, Z., Han, Z., Zhang, Z., Hong, J., & Jabbar, S. (2015). A potential of ultrasound on minerals, micro-organisms, phenolic compounds and colouring pigments of grapefruit juice. *International Journal of Food Science & Technology*, *50*(5), 1144–1150. https://doi.org/10.1111/ijfs.12767

Ahmadian, S., Kenari, R. E., Amiri, Z. R., Sohbatzadeh, F., & Khodaparast, M. H. H. (2023). Effect of ultrasound-assisted cold plasma pretreatment on cell wall polysaccharides distribution and extraction of phenolic compounds from hyssop (Hyssopus officinalis L.). *International Journal of Biological Macromolecules*, *233*, 123557. https://doi.org/10.1016/j.ijbiomac.2023.123557

Alencar, N. M. M., Cazarin, C. B. B., Corrêa, L. C., Maróstica Junior, M. R., Biasoto, A. C. T., & Behrens, J. H. (2018). Influence of maceration time on phenolic compounds and antioxidant activity of the Syrah must and wine. *Journal of Food Biochemistry*, *42*(2), e12471. https://doi.org/10.1111/jfbc.12471

Ali, M., Manzoor, M. F., Goksen, G., Aadil, R. M., Zeng, X.-A., Iqbal, M. W., & Lorenzo, J. M. (2023). High-intensity ultrasonication impact on the chlorothalonil fungicide and its reduction pathway in spinach juice. *Ultrasonics Sonochemistry*, *94*, 106303. https://doi.org/10.1016/j.ultsonch.2023.106303

Banc, R., Loghin, F., Miere, D., Ranga, F., & Socaciu, C. (2020). Phenolic composition and antioxidant activity of red, rosé and white wines originating from Romanian grape cultivars. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *48*(2), 716–734. https://doi.org/10.15835/nbha48211848

Banc, R., Socaciu, C., Miere, D., Filip, L., Cozma, A., Stanciu, O., & Loghin, F. (2014). Bene f its of Wine Polyphenols on Human Health : A Review. *Bulletin UASVM Food Science and Technology*, *71(2)*, 79–87.

Bautista-Ortin, A. B., Jimenez-Martinez, M. D., Jurado, R., Iniesta, J. A., Terrades, S., Andres, A., & Plaza, G. E. (2017). Application of high-power ultrasounds during red wine vinification. *International Journal of Food Science and Technology*, 1–10.

Benzie, I. F., & Strain, J. J. . (1996). Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal Biochem.*, *239*(1), 70–76.

Buljeta, I., Pichler, A., Šimunović, J., & Kopjar, M. (2023). Beneficial Effects of Red Wine Polyphenols on Human Health: Comprehensive Review. *Current Issues in Molecular Biology*, *45*(2), 782–798. https://doi.org/10.3390/cimb45020052

Castaldo, Narváez, Izzo, Graziani, Gaspari, Minno, & Ritieni. (2019). Red Wine Consumption and Cardiovascular Health. *Molecules*, *24*(19), 3626. https://doi.org/10.3390/molecules24193626

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

Ferraretto, P., & Celotti, E. (2016). Preliminary study of the effects of ultrasound on red wine polyphenols. *CyTA - Journal of Food*, *March*.

Ferraretto, P., Rolle, P., & Celotti, E. (2011). Applicazione degli ultrasuoni come tecnica innovativa per ottimizzare l'estrazione dei composti fenolici e favorire la lisi del lievito. *Rivista Internet Di Viticoltura e Enologia*, *9/3*, 1–10.

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

Gómez-Plaza, E., Osete-Alcaraz, A., Jurado, R., Iniesta, J. A., & Bautista-Ortín, A. B. (2020). The application of high power ultrasounds for improving the phenolic extraction and color from grape-derived products. *Acta Horticulturae*, *1274*, 1–8. https://doi.org/10.17660/ActaHortic.2020.1274.1

Gutiérrez-Escobar, R., Aliaño-González, M. J., & Cantos-Villar, E. (2021). Wine Polyphenol Content and Its Influence on Wine Quality and Properties: A Review. *Molecules*, *26*(3), 718. https://doi.org/10.3390/molecules26030718

He, F., Liang, N.-N., Mu, L., Pan, Q.-H., Wang, J., Reeves, M. J., & Duan, C.-Q. (2012a). Anthocyanins and Their Variation in Red Wines I. Monomeric Anthocyanins and Their Color Expression. *Molecules*, *17*(2), 1571–1601. https://doi.org/10.3390/molecules17021571

He, F., Liang, N.-N., Mu, L., Pan, Q.-H., Wang, J., Reeves, M. J., & Duan, C.-Q. (2012b). Anthocyanins and Their Variation in Red Wines II. Anthocyanin Derived Pigments and Their Color Evolution. *Molecules*, *17*(2), 1483–1519. https://doi.org/10.3390/molecules17021483

Heydari, M., Carbone, K., Gervasi, F., Parandi, E., Rouhi, M., Rostami, O., Abedi-Firoozjah, R., Kolahdouz-Nasiri, A., Garavand, F., & Mohammadi, R. (2023). Cold Plasma-Assisted Extraction of Phytochemicals: A Review. *Foods*, *12*(17), 3181. https://doi.org/10.3390/foods12173181

Hussain, M., Gantumur, M.-A., Manzoor, M. F., Hussain, K., Xu, J., Aadil, R. M., Qayum, A., Ahmad, I., Zhong, H., & Guan, R. (2023). Sustainable emerging high-intensity sonication processing to enhance the protein bioactivity and

bioavailability: An updated review. *Ultrasonics Sonochemistry*, *97*, 106464. https://doi.org/10.1016/j.ultsonch.2023.106464

JASP Team. (2023). *JASP* (0.17.1). https://jasp-stats.org

KHAN, M. K. I., GHAURI, Y. M., ALVI, T., AMIN, U., KHAN, M. I., NAZIR, A., SAEED, F., AADIL, R. M., NADEEM, M. T., BABU, I., & MAAN, A. A. (2022). Microwave assisted drying and extraction technique; kinetic modelling, energy consumption and influence on antioxidant compounds of fenugreek leaves. *Food Science and Technology*, *42*. https://doi.org/10.1590/fst.56020

Khmelev, V. N., Sevodin, V. P., Shesternin, V. I., Kuzovnikov, Y. M., & Levin, S. V. (2015). Using of ultrasound in grape wine making process. *2015 16th International Conference of Young Specialists on Micro/Nanotechnologies and Electron Devices*, 218–220. https://doi.org/10.1109/EDM.2015.7184530

Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *Journal of AOAC International*, *88*(5), 1269–1278. https://doi.org/10.1093/jaoac/88.5.1269

Lingua, M. S., Fabani, M. P., Wunderlin, D. A., & Baroni, M. V. (2016). From grape to wine: Changes in phenolic composition and its influence on antioxidant activity. *Food Chemistry*, *208*, 228–238. https://doi.org/10.1016/j.foodchem.2016.04.009

Luzzini, G., Slaghenaufi, D., Pasetto, F., & Ugliano, M. (2021). Influence of grape composition and origin, yeast strain and spontaneous fermentation on aroma profile of Corvina and Corvinone wines. *LWT*, *143*, 111120. https://doi.org/10.1016/j.lwt.2021.111120

Maier, A., Padureanu, V., Lupu, M. I., Canja, C. M., Badarau, C., Padureanu, C., Alexa, E., & Poiana, M.-A. (2023). Optimization of A Procedure to Improve the Extraction Rate of Biologically Active Compounds in Red Grape Must Using High-Power Ultrasound. *Sustainability (Switzerland)*, *15*(8). https://doi.org/10.3390/su15086697

Margean, A., Lupu, M. I., Alexa, E., Padureanu, V., Canja, C. M., Cocan, I., Negrea, M., Calefariu, G., & Poiana, M.-A. (2020). An overview of effects induced by pasteurization and high-power ultrasound treatment on the quality of red grape juice. *Molecules*, *25*(7). https://doi.org/10.3390/molecules25071669

Mason, T. J., & Lorimer, J. P. (2002). *Applied Sonochemistry: The Uses of Power Ultrasound in Chemistry and Processing* (1st Editio). Wiley VCH,.

Molino, A., Mehariya, S., Di Sanzo, G., Larocca, V., Martino, M., Leone, G. P., Marino, T., Chianese, S., Balducchi, R., & Musmarra, D. (2020). Recent developments in supercritical fluid extraction of bioactive compounds from microalgae: Role of key parameters, technological achievements and challenges. *Journal of CO2 Utilization*, *36*, 196–209. https://doi.org/10.1016/j.jcou.2019.11.014

Mukhtar, K., Nabi, B. G., Arshad, R. N., Roobab, U., Yaseen, B., Ranjha, M. M. A. N., Aadil, R. M., & Ibrahim, S. A. (2022). Potential impact of ultrasound, pulsed electric field, high-pressure processing and microfludization against thermal treatments preservation regarding sugarcane juice (Saccharum officinarum). *Ultrasonics Sonochemistry*, *90*, 106194. https://doi.org/10.1016/j.ultsonch.2022.106194

Nardini, M. (2022). Phenolic Compounds in Food: Characterization and Health Benefits. *Molecules*, *27*(3), 783. https://doi.org/10.3390/molecules27030783

Natolino, A., & Celotti, E. (2022). Ultrasound treatment of red wine: Effect on polyphenols, mathematical modeling, and scale-up considerations. *Lwt*, *154*, 112843. https://doi.org/10.1016/j.lwt.2021.112843

Nemzer, B., Kalita, D., Yashin, A. Y., & Yashin, Y. I. (2021). Chemical Composition and Polyphenolic Compounds of Red Wines: Their Antioxidant Activities and Effects on Human Health—A Review. *Beverages*, *8*(1), 1. https://doi.org/10.3390/beverages8010001

Noranizan, M. ;, Pean, L. F. ;, Li, J. W. ;, Aadil, R. M., Ahmad, T. ;, Rosli, S. Z. ;, Yong, Y. Y. ;, & Kim, D.-O. (2020). Impact of juice extraction method on the physicochemical, functional, and sensory properties of Sabah snake grass (Clinacanthus nutans) juice mix. *International Food Research Journal*, *27(5)*, 835–845.

OIV (International organisation of vine and wine). (2021). *Compendium of international methods of wine and must analysis* (Vol. 1). OIV - 35 Rue de Monceau, 75008 Paris.

Patist, A., & Bates, D. (2011). Industrial Applications of High Power Ultrasonics. In *Ultrasound Technologies for Food and Bioprocessing. Food Engineering Series.* (pp. 599–616). Springer, New York, NY. https://doi.org/10.1007/978-1-4419-7472-3_24

Pérez-Porras, P., Bautista-Ortín, A. B., Jurado, R., & Gómez-Plaza, E. (2021). Using high-power ultrasounds in red winemaking: Effect of operating conditions on wine physico-chemical and chromatic characteristics. *LWT*, *138*. https://doi.org/10.1016/j.lwt.2020.110645

Plaza, E. G., Jurado, R., Iniesta, J. A., & Bautista-Ortin, A. B. (2019). High power ultrasounds: A powerful, non-thermal and green technique for improving the phenolic extraction from grapes to must during red wine vinification. *BIO Web Conf., 41st World Congress of Vine and Wine*, *12*. https://www.bio-conferences.org/articles/bioconf/full_html/2019/01/bioconf-oiv2018_02001/bioconf-oiv2018_02001.html

Ranjha, M. M. A. N., Irfan, S., Lorenzo, J. M., Shafique, B., Kanwal, R., Pateiro, M., Arshad, R. N., Wang, L., Nayik, G. A., Roobab, U., & Aadil, R. M. (2021). Sonication, a Potential Technique for Extraction of Phytoconstituents: A Systematic Review. *Processes*, *9*(8), 1406. https://doi.org/10.3390/pr9081406

Ranjha, M. M. A. N., Kanwal, R., Shafique, B., Arshad, R. N., Irfan, S., Kieliszek, M., Kowalczewski, P. Ł., Irfan, M., Khalid, M. Z., Roobab, U., & Aadil, R. M. (2021). A Critical Review on Pulsed Electric Field: A Novel Technology for the Extraction of Phytoconstituents. *Molecules*, *26*(16), 4893. https://doi.org/10.3390/molecules26164893

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Meth Enzymol*, *299*, 152–178.

Sridhar, A., Ponnuchamy, M., Kumar, P. S., Kapoor, A., Vo, D.-V. N., & Prabhakar, S. (2021). Techniques and modeling of polyphenol extraction from food: a review. *Environmental Chemistry Letters*, *19*(4), 3409–3443. https://doi.org/10.1007/s10311-021-01217-8

Stat-Ease Inc. (2020). *DESIGN-EXPERT® VERSION 12 SOFTWARE TRIAL*.

Yusoff, I. M., Mat Taher, Z., Rahmat, Z., & Chua, L. S. (2022). A review of ultrasound-assisted extraction for plant bioactive compounds: Phenolics, flavonoids, thymols, saponins and proteins. *Food Research International*, *157*(February), 111268. https://doi.org/10.1016/j.foodres.2022.111268

Zhang, Q. A., Zheng, H., Lin, J., Nie, G., Fan, X., & García-Martín, J. F. (2023). The state-of-the-art research of the application of ultrasound to winemaking: A critical review. In *Ultrasonics Sonochemistry* (Vol. 95). Elsevier B.V. https://doi.org/10.1016/j.ultsonch.2023.106384

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

Zhang, Q., & Wang, T. (2017b). Effect of ultrasound irradiation on the evolution of Effect of ultrasound irradiation on the evolution of color properties and major phenolic compounds in wine during storage. *Food Chemistry*, *234*, 372–380.

Zia, S., Khan, M. R., Shabbir, M. A., Aslam Maan, A., Khan, M. K. I., Nadeem, M., Khalil, A. A., Din, A., & Aadil, R. M. (2022). An Inclusive Overview of Advanced Thermal and Nonthermal Extraction Techniques for Bioactive Compounds in Food and Food-related Matrices. *Food Reviews International*, *38*(6), 1166–1196. https://doi.org/10.1080/87559129.2020.1772283

46. SIMPOZIJ AKTUALNI ZADACI MEHANIZACIJE POLJOPRIVREDE

Pregledni rad
Review paper

APPLICATION OF ULTRASOUND IN WINEMAKING PROCESS

Alina MĂRGEAN*, Vasile PĂDUREANU, Mirabela Ioana LUPU

Transilvania University of Brașov, Food and Tourism Faculty, Engineering and Management in Food and Tourism Department, 148 Castelului Street, 500014, Brașov, România
*E-mail of corresponding author: alinamargean@yahoo.com

SUMMARY

Ultrasound has been used as a successful tool in food industry but only in the last years its benefits started to be analyzed and implemented in processing, among which winemaking. Even though several studies have been performed regarding how ultrasound is influencing the extraction, fermentation or ageing, the subject is still of interest. Ultrasound offers the potential for improving existing processes and the developing of new process options. The demand for good quality wines has encouraged the use of ultrasound technology in winemaking process, consecutive to the research that shows ultrasound can play an important role in food technology in processing, preservation and extraction. This review summarizes the application of high and low intensity ultrasound in winemaking process in order to reveal whether this tool might be a feasible method for improving the quality in winemaking process.

Key words: ultrasound, winemaking, fermentation, maturation

INTRODUCTION

A product rich in phenolic compounds that have antioxidant properties, wine is an important component in many traditions. The crucial factors during winemaking include grape quality and variety, winemaking process and practices, wine storage (Clodoveo et al., 2016, Di Lorenzo et al., 2016).

Traditionally, red wine is made starting from crushing and destemming grapes, and the must remaining in contact with skins and seeds lead to starting the maceration. During this period, the extraction of the coloring and tannic components into the future wine takes place in the presence of alcohol produced during fermentation. When the macerating period ends, the fermented must is separated by skins and seeds using a press and the alcoholic

fermentation can continue until the parameters of the chosen wine are reached. Afterwards, spontaneously or through inoculation of selected lactic acid bacteria strains wine may undergo malolactic fermentation that confer a rounder and fuller mouthfeel to the wine. Then, the stage of wine ageing follows and consists in maturation in oak barrels (oxidative aging) and bottling (reductive aging). Also, clarification processes had to be carried out (Clodoveo et al., 2016).

Food industry became aware of the importance in implementing new technologies that study and monitor properties of food during processing as a reaction to consumers demand for higher quality and food safety products. Nowadays, the industry is searching for innovative techniques that might enhance or complement the conventional practices.

Ultrasound is such an emerging, efficient, environment friendly, green technology and the methods upon which ultrasound applications are based on include direct application to the product, coupling with the device and submerge in an ultrasonic bath (Chemat et al., 2011).

In winemaking, high frequency ultrasound can be used for monitoring and accelerating the fermentation processes. Another potential use of ultrasound in winemaking is for the improved extraction of coloring matter and the tannins from the initial processing of the fruit. The bacteria and yeast plays an important role in the winemaking process. High power ultrasound may be used to control wine spoilage organisms (Luo et al., 2012). Also, the ultrasound treatment on wine lees accelerates the protective colloids release (Cacciola et al., 2013). Ultrasound might be employed as a method to change the physicochemical properties of red wine including the electric conductivity, total phenolic compounds and chromatic characteristics and might be used to accelerate the ageing process of the wine with a longer effect on the evolution of both, color characteristics and phenolic compounds of wine during storage (Ferraretto et al., 2013, Martin and Sun, 2013, Ferraretto and Celotti, 2016, Zhang et al., 2016, Bautista-Ortin et al., 2017).

PRINCIPLE AND MECHANISM OF ULTRASOUND

Ultrasound is sound waves with frequencies higher than the upper audible limit of human hearing (16–20 kHz). In the food industry, the sound ranges employed can be divided into high frequency diagnostic ultrasound and low frequency ultrasound. High frequency diagnostic ultrasound (above 100 kHz) is a non-destructive technique and causes no physical or chemical alterations. They are successfully used for monitoring of food processes. The low frequency ultrasonic waves (18 – 100 kHz) are capable of altering material properties: physical disruption, acceleration of chemical reactions (Dolatowski et al., 2007). Into a liquid, ultrasound achieves chemical and physical effects, while the mechanical effects lead to increasing the extraction of nutraceutical components and disrupt or damage the cellular membrane (Jiranek et al., 2008, Clodoveo et al., 2016). Ultrasound assisted extraction is a simple and efficient alternative to conventional extraction techniques. The enhancement in extraction obtained by using ultrasound is mainly attributed to the effect of acoustic cavitations produced in the solvent by the passage of an ultrasound wave (Wang et al., 2008). The forces generated by acoustic cavitations determine the mechanism responsible for the ultrasonic deactivation effect. The application of ultrasound through intracellular cavitations determines a bactericidal effect in liquid foods (Dolatowski et al., 2007).

In food processing, the application of ultrasound includes freezing/crystallization, drying, degassing, extractions, induction of oxidation/reduction reactions, enzyme inactivation, filtration and defoaming (Jiranek et al., 2008, Chemat et al., 2011).

ULTRASOUND IN WINEMAKING PROCESS

In the literature, the latest research showed the potential uses of ultrasound in winemaking including: reducing the fermentation time and increasing the extraction of phenolic compounds (Sacchi et al., 2005, Vilku et al., 2008, Tiwari et al., 2010, Tudose-Sandu-Ville et al., 2012, Coletta et al., 2013, Bautista-Ortin et al., 2017), controlling a variety of wine spoilage organisms (Jiranek et al., 2008, Luo et al., 2012), or wine maturation and ageing (Ferraretto and Celotti, 2016, Zhang et al., 2016). However, when applying ultrasound in winemaking process, changes in aroma profile and sensory properties of beverages might occur, like a slight "burnt" flavor, or a slight metallic character (Nolan, 2016).

During the winemaking, the ultrasound variables are power, frequency, exposure time, amplitude and temperature (Clodoveo et al., 2016, Zhang et al., 2016). The properties of the medium, treatment parameters and ultrasound parameters are critical processing factors (Dolatowski et al., 2007, Jiranek et al., 2008).

The main stages during traditional winemaking are fermentation and maturation or ageing (Di Lorenzo et al., 2016, Zhang et al., 2016). However, the process starts with the maceration on the skins that is enhancing the extraction of the color and active compounds (Di Lorenzo et al., 2016). Thus, improving extraction using ultrasound during maceration is of great interest to increase their quantity (Coletta et al., 2013).

During the harvest time, the wineries are trying to reduce the maceration time and consequently the quality of the wine would be influenced. Alternatives to accelerate the reactions within the wine and to enhance the extraction of phenolic compounds includes ultrasound, pulsed electric field and high voltage electrical discharges, while the variables includes fermentation temperature, thermovinification, must freezing, enzyme treatments and extended maceration (Sacchi et al., 2005, Martin and Sun, 2013, Bautista-Ortin et al., 2017).

Recently, Bautista-Ortin et al. (2017) studied the application of high power ultrasound (HPU) on reducing the maceration time and increasing the extraction of phenolic compounds from red grapes. They observed that the treated must (2500 W, 28 kHz) had the highest values for phenols, anthocyanins and color intensity and a double concentration of the tannin. They also reported that the ultrasound treatment facilitated the extraction of phenolic compounds from the crushed grapes. In their opinion, the application of ultrasound might be an optimizing technique that allows wineries to reduce the maceration time while keeping the same quality characteristics of the wines.

On the other hand, Tudose-Sandu-Ville et al. (2012) studied the phenolic compounds in Merlot wines obtained through different technologies, including ultrasound. They found that the phenolic extraction was not increased after ultrasound maceration (2000W, 35 kHz, 15 min.) and the quantities of anthocyanins and tannins in the wine were low. Moreover, the influence of ampelotechnical practices and winemaking technologies on phenolic composition and sensory characteristics of red wines have been assayed by (Coletta et al., 2013). They observed that ultrasound treatment on destemmed grapes (37 kHz, 150 W, 15 min at 30 °C) improved the extraction of all phenolic compounds, especially anthocyanins and lead to improved sensory characteristics of wine.

Color, as the easily recognized aspect of a quality red wine together with the astringency, hardness and flavor are influenced by phenolic compounds (Sacchi et al., 2005, Tudose-Sandu-Ville et al., 2012). Phenolic compounds that are located in skins, pulp and seeds of grapes are partially extracted during winemaking (Coletta et al., 2013) and include non-flavonoids and flavonoids among which anthocyanins are responsible for sensory properties

and for chemical stability and tannins that entail astringency (Martin and Sun, 2013, Di Lorenzo et al., 2016).

Other studies (Tiwari et al., 2010) explored effects of amplitude level and treatment time on anthocyanins and color parameters. Variating the amplitude level (24.4–61.0 μm) and treatment time (0–10 min) at a constant frequency of 20 kHz and pulse durations of 5 s on and 5 s off, the authors concluded that a high degree of anthocyanin retention was found in grape juice, considering thus this technique as a preservation one for processing of fruit juice products where is desired a high retention of anthocyanins.

Also, Vilkhu et al. (2008) showed that ultrasonic technology improves the extraction of polyphenols, anthocyanins, aromatic compounds, polysaccharides and functional compounds. High values of amplitude and longer times of treatment lead to a significant increase of catechins (Ferraretto and Celotti, 2016). Ghafoor and Choi (2009) found that compared with other extraction methods ultrasound shortened the extraction time. The optimum extraction conditions for total phenols were 53.14% ethanol concentration, 46.03 °C extraction temperature and 24.03 min extraction time and 53.06% ethanol, 50.65 °C temperature and 25.58 min time for maximum antioxidant activity.

Other authors, Cacciola et al. (2013) observed that the ultrasound treatments on wine lees (5 min and 60 % amplitude and 3 and 5 min at 90 % amplitude) lead to greater effects in terms of colloids and proteins due to an increased release of colloids from the yeast. They concluded that the effects of using ultrasound for few minutes can be comparable to traditional aging on lees by obtaining a rapid extraction of macromolecules from the yeast lees.

Also, Jiranek et al. (2008) mentioned ultrasound technology as potential application during different stages of winemaking process for reduction of spoilage organisms and enhancing extraction of color and flavor compounds. Appling 20 min exposure to HPU influenced the viability of almost all organisms – yeast and bacteria commonly associated with wine spoilage, with different response of genus. Cell numbers decreased for all yeast and bacteria studied, except *Pediococcus* sp. (Luo et al., 2012). However, it was suggested that yeast cells are more susceptible to the effects of cavitations while bacteria are more resistant (Chandrapala et al., 2012).

Cho et al. (2006) reported that compared to conventional solvent extraction, the application of ultrasound-assisted extraction in grape skin can enhance the recovery of functional compounds up to 30% and also prevents the possible chemical degradation of targeted compounds (Wang and Weller, 2006).

Wine ageing consist in maturation that is an oxidative ageing that takes place in tanks or barrels and bottling that is a reductive ageing (Martin and Sun, 2013). Traditional ageing in barrels has some disadvantages including time needed, high cost, thus innovative winemaking and ageing technologies are currently assayed. Ferraretto and Celotti (2016) evaluate the effect of HPU in wine aging. The authors reported that after the treatment (20 kHz frequency, 1, 3 and 5 min. time and 51, 102 and 153 μm amplitude), the anthocyanins suffered no negative consequences, confirming thus their stability. Also, an increase in tannic compounds was observed. The results obtained by the authors suggested that the HPU uses might accelerate the aging process of wines with best results in young, well-colored wines.

Other assays evaluate the influence of ultrasound on physicochemical parameters of red wine (Zhang et al., 2016). By variation of power (120, 150, 180, 210, 240, 270 and 300 W), frequencies (45, 80 and 100 kHz), exposure time (20, 40, 60, 80 and 100 min.) and temperature (20 °C, 30 °C, 40 °C, 50 °C and 60 °C), they concluded that ultrasound can change some physicochemical properties of red wine. The significantly changes were observed on the

concentration of total phenolic compounds and electrical conductivity and the suggested parameters were 240 W, 80 kHz, 20 °C and 80 min. However, the exposure time seem to be the main factor that leads to changes in red wine.

CONCLUSIONS

Ultrasound technique has been used by the 1960′ in the industry and it still is a subject of interest for enhancing or complements the conventional or traditional practices. Ultrasound has been assayed on different stages of the winemaking with different aims including accelerating chemical reactions and increase extraction of the compounds from grape to must. However, in the winemaking, most of the researchers have been carried out experiments at laboratory scale, where the conditions assayed were still limited and thus the results were not standardized related to operating conditions and makes the comparison within studies difficult.

Despite the results that demonstrated the potential application of this emerging technique in winemaking, the complete effect of ultrasound is still to be assayed due to some undesirable reactions or effects that might occur, like off odors in wine. Still, the application of ultrasound in winemaking represents a possibility to optimize maceration, fermentation and maturation stages.

REFERENCES

Bautista-Ortin, A.B., Jimenez-Martinez, M.D., Jurado, R., Iniesta, J.A., Terrades, S, Andres, A., Gomez-Plaza, E. (2017). Application of high-power ultrasounds during red wine vinification. International Journal of Food Science and Technology 2017.

Cacciola, V., Batllò, I. F., Ferraretto, P., Vincenzi, S., Celotti, E. (2013). Study of the ultrasound effects on yeast lees lysis in winemaking. European Food Research and Technology, 236(2): 311–317.

Carrera, C., Ruiz-Rodriguez, A., Palma, M., Garcia-Barroso, C. (2012). Ultrasound assisted extraction of phenolic compounds from grapes. Analytica Chimica Acta, 732: 100–104.

Chandrapala, J., Oliver, C., Kentish, S., Ashokkumar, M. (2012). Ultrasound in food processing – Food quality assurance and food safety. Trends in Food Science and Technology, 26: 88-98.

Chang, A. C., Chen, F. C. (2002). The application of 20 kHz ultrasonic waves to accelerate the aging of different wines. Food Chemistry, 79(4): 501–506.

Chemat, F., Zill-e-Huma, Khan, M.K. (2011). Applications of ultrasound in food technology: Processing, preservation and extraction. Ultrasonics Sonochemistry 18: 813–835.

Clodoveo, M.L., Dipalmo, T., Rizzelo, C.G., Corbo, F., Crupi, P. (2016). Emerging technology to develop novel red winemaking practices. Innovative Food Science and Emerging Technologies 38: 41-56.

Coletta, A., Trani, A., Faccia, M., Punzi, R., Dipalmo, T., Crupi, P., Gambacorta, G. (2013). Influence of viticultural practices and winemaking technologies on phenolic composition and sensory characteristics of Negroamaro red wines. International Journal of Food Science and Technology, 48(11), 2215–2227.

Di Lorenzo, A., Bloise, N., Meneghini, S., Sureda, A., Tenore, G.C., Visai, L., Arciola, C.R., Daglia, M. (2016). Effect of Winemaking on the Composition of Red Wine as a Source of Polyphenols for Anti-Infective Biomaterials. Materials, 9*(5), (316*). DOI:10.3390/ma9050316.

Dolatowski, Z.J., Stadnik, J., Stasiak, D. (2007). Applications of ultrasound in food technology, Acta Sci. Pol. Technol. Aliment. 6(3): 89–99.

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Article

Ultrasound Treatment Influence on Antioxidant Properties of Blueberry Vinegar

Cristina Padureanu [1], Carmen Liliana Badarau [1], Alina Maier [1], Vasile Padureanu [1,*], Mirabela Ioana Lupu [1], Cristina Maria Canja [1], Geronimo Raducu Branescu [1], Oana-Crina Bujor [2], Florentina Matei [1], Mariana-Atena Poiana [3,*], Ersilia Alexa [3] and Anisor Nedelcu [4]

[1] Faculty of Food and Tourism, Transilvania University of Brasov, Castelului 148, 500014 Brasov, Romania; cristina.padureanu@unitbv.ro (C.P.); carmen.badarau@unitbv.ro (C.L.B.); alina.maier@unitbv.ro (A.M.); lupu.mirabela@unitbv.ro (M.I.L.); canja.c@unitbv.ro (C.M.C.); geronimo.branescu@unitbv.ro (G.R.B.); florentina.matei@unitbv.ro (F.M.)

[2] Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Marasti Blvd. 59, 011464 Bucharest, Romania; oana.bujor@qlab.usamv.ro

[3] Faculty of Food Engineering, University of Life Sciences "King Michael I" from Timisoara, Calea Aradului 119, 300645 Timisoara, Romania; ersiliaalexa@usab-tm.ro

[4] Department of Manufacturing Engineering, Transilvania University of Brasov, 29 Eroilor Boulevard, 500036 Brasov, Romania; a.nedelcu@unitbv.ro

* Correspondence: padu@unitbv.ro (V.P.); marianapoiana@usab-tm.ro (M.-A.P.); Tel.: +40-724-031-966 (V.P.); +40-726-239-838 (M.-A.P.)

Abstract: As one of the most widely used acidic condiments in the world, vinegars have demonstrated physiological functions. Due to their polyphenol content, blueberries (*Vaccinium myrtillus* L.) are a valuable source of natural flavours and antioxidants for vinegar production. Ultrasound treatment is recognized as an effective technique for improving the extraction yield of bioactive compounds from various plant materials. The aim of this research was to investigate the antioxidant properties of experimental vinegar variants obtained by an innovative manufacturing recipe using an alcoholic substrate containing blueberry juice for a rapid initiation of acetic fermentation. The substrate was subjected to ultrasound treatment at a frequency of 20 kHz and an amplitude (A) of 40%, 60%, and 80% for 3, 4, and 5 min. Under these conditions, total polyphenol content (TPC), total anthocyanins content (TAC), antioxidant activity based on ABTS and DPPH assays, as well as the sensory attributes in blueberry-vinegar formulations, were evaluated. The level of TPC and TAC and the antioxidant activity of the developed vinegar variants were optimized using response surface methodology (RSM). The obtained results revealed that ultrasound treatment resulted in increased TPC and TAC and improved antioxidant properties and sensory characteristics of blueberry vinegar. Our data revealed that the optimum values of the ultrasound treatment parameters were amplitude A: 78.50% and time t: 3.96 min. The following predicted values were determined for the main parameters: TPC: 628.01 mg GAE/L, TAC: 22.79 mg C3G/L, ABTS: 391.7 µmol/100 mL, and DPPH: 229.17 µmol/100 mL. The results of this study recommend the integration of both the use of an alcoholic substrate containing blueberry juice and the application of ultrasound treatment in vinegar production as innovative technological interventions with practical applicability for a rapid initiation of acetic fermentation and for improving the antioxidant properties of blueberry vinegar. In addition, RSM can be considered a valuable tool to optimize the ultrasound treatment's effect on the antioxidant properties of the vinegar formulations.

Keywords: blueberry vinegars; ultrasound treatment of alcoholic substrate; antioxidant activity; phenolic compounds; anthocyanins; response surface methodology

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1. Introduction

Vinegar is used as a fermented flavouring ingredient and as a special culinary constituent preservative, and also to give flavour to many food products such as salad dressings, mayonnaise, mustard, ketchup, and other food [1]. Due to its vitamins, phenolic compounds, and organic acids in its constitution, vinegar has multiple positive effects on health, especially for appetite stimulation, blood-sugar control, blood-pressure regulation, digestive, antimicrobial, body-weight management, antidiabetic, and lipid metabolism regulation [2–5].

Fruits such as grapes, apples, bananas, lemons, strawberries, rice, orange, pineapple, mulberry, blueberry, blackberry, or vegetables can be used as raw material for vinegar production all around the world [6]. Vinegars are named according to their raw material origin and uptake the properties of the corresponding raw material [7]. Berry vinegar contains numerous bioactive compounds, including phenolic components. The synergic effect of the acetic acid and these bioactive compounds could give to this product its ability to reduce the availability of carbohydrates, which is good for people with diabetes [8]. Vinegars obtained from fruit juices conserve just a fraction of these health-connected compounds [9].

According to the 'Global Vinegar Market Report and Forecast 2021–2026', the global vinegar market reached USD 1.32 billion in 2020. Meanwhile, the global vinegar market it is expected to grow at a Compound Annual Grow Rate (CAGR) of 1.6% between 2021 and 2026. It is expected to grow in the forecast period of 2021–2026 at a CAGR of 1.6% to attain USD 1.43 billion in 2026 [10]. The global vinegar market is led by the rising consumption of food globally. Europe is the largest vinegar market, representing nearly half of the global market. Within Europe, Italy is the leading market for vinegar as well as its major exporter [10,11].

The production of fruit vinegars as a way of making use of fruit byproducts is widely employed by the food industry since it allows them to exploit surplus and low-quality fruits without compromising the quality of the final product. The acetic nature of fruit vinegars and the high sensory impact that this acid produces on the sensorial properties of the product allow almost any type of fruit to be used for its elaboration. Every year, large amounts of fruits are produced and wasted since the excess cannot be consumed or because the fruits are considered of a second- or third-quality category [11].

In line with the Food and Agriculture Organization of the United Nations (FAO) [11], 21.6% of the fruit produced in the world is wasted, starting from the postharvest stage until its distribution. Very often, fruit is rejected simply because of its "imperfect" appearance or inadequate size, even if the fruit is edible. These actions lead to both ecological and economic problems [12]. Considering the functional properties and the perishability of these fruits, the use of such raw material in the production of high-value-added products such as vinegars could be a valuable strategy [13]. Usually, this vinegar is produced from raw materials containing sugar via sequential ethanol and acetic acid fermentations [14,15].

In the last few years, in food production, ultrasonic processing was considered a beneficial method for improving the nutritional properties of products regarding the enhancement of their biologically active compounds level, mostly the phenolic compounds [16]. It was outlined that the use of ultrasonic extraction for vegetable extracts could raise sucrose content, acidity content, phenolic compounds level, and pigmentation of liquid foods [17]. Data reported by other researchers [16,18] demonstrated the increase of the antioxidant potential of different liquid food products, such as tomato juice or cider, after the use of the ultrasound treatment. Currently, the reports of ultrasound treatment effects on the antioxidant potential of vinegars obtained from berry juices are limited, despite its potential.

Response surface methodology (RSM) can reduce workloads and the time consumption of the research work [3,19]. Therefore, RSM was used in this study, being successful in "predicting the model of various process factors in a convenient manner such as food science and engineering, chemical engineering and technology, and environment fields, etc." [19–21].

In this study, an alcoholic substrate containing blueberry juice treated with ultrasound was used to obtain blueberry vinegar. Under these conditions, the intention was to rapidly initiate acetic fermentation. The degree of innovation of this study refers to a new recipe used for obtaining blueberry vinegar and identifying a treatment formula for this product in order to improve the polyphenols and anthocyanins content and the antioxidant properties. Consequently, the goal of this study was to optimize (using RSM) the extraction of polyphenols and anthocyanins, and the antioxidant activity of some vinegar variants obtained from an alcoholic substrate containing blueberry juices treated by ultrasound. Optimization is used in food processes, especially to minimize losses of bioactive ingredients, with RSM being preferred for this purpose. Thus, we aimed to apply the ultrasound treatment for the first time in blueberry-vinegar production by a new method and to optimize the bioactive components (TPC, TAC, DPPH, and ABTS) using RSM.

2. Results and Discussion

The effects of high-power ultrasonic treatment were explored in terms of the content of bioactive compounds such as TPC and TAC, as well as antioxidant activity measured by ABTS and DDPH assays. All the above parameters were studied on blueberry fruits, blueberry juice, juice diluted with water, and blueberry vinegar (B), as well as on the samples subjected to ultrasound treatment at 40, 60, and 80% amplitude for 3, 4, and 5 min, as follows: B403 (A = 40%; t = 3 min), B404 (A = 40%; t = 4 min), B405 (A = 40%; t = 5 min), B603 (A = 60%; t = 3 min), B604 (A = 60%; t = 4 min), B605 (A = 60%; t = 5 min), B803 (A = 80%; t = 3 min), B804 (A = 80%; t = 4 min), and B805 (A = 80%; t = 5 min).

2.1. Analysis of the Raw Material (Blueberry) and Blueberry Juices Obtained

The titratable acidity (TA), pH, and total soluble solids (TSS), expressed in °Brix, of fruit raw material, blueberry juice, and blueberry juice diluted with water are presented in Table 1.

Table 1. Titratable acidity, pH and total soluble solids of blueberry fruits, blueberry juice, and blueberry juice diluted with water.

Sample	Titratable Acidity (%)	TSS (°Brix)	pH	TSS/TA Ratio
Blueberry	1.29 ± 0.02	12.28 ± 0.47	3.02 ± 0.14	9.44
Blueberry juice	0.94 ± 0.01	13.02 ± 0.26	3.17 ± 0.29	12.48
Juice diluted with water	0.75 ± 0.01	10.00 ± 0.00	3.3 ± 0.00	11.76

Each value represents average ± standard deviation (n = 3).

According to Saftner et al. [22], for consumption, the blueberries should have TSS values above 10%, total titratable acidity values between 0.3% and 1.3%, pH values from 2.25 to 4.25, and a TSS/TA ratio between 10 and 33. In our case, the titratable acidity varied around the value of 1.29% with a pH of 3.02, which is acceptable for fresh consumption. However, in relation to acidity and TSS/TA ratio, the value is less than 10% and, hence, their destination for the production of vinegars could be of interest for being explored. The results for pH (3.02) and titratable acidity (1.29%) indicated the fruits were quite acidic. Similar values for titratable acidity (1.58%) were reported for blueberries cultivated in Turkey [23]. Likewise, mature blueberries cultivated in Nova Scotia (Canada) were much less acidic than the blueberries used in this work [24]. High values of blueberry acidity were also reported by de Souza et al. [25] and by da Silva Fonseca et al. [13] for blueberries grown in Brazil (2.56% and 2.04%).

The results for total soluble solids content (12.28 °Brix) were similar to that reported by da Silva Fonseca et al. [13] for Brazilian blueberries and by Almenar et al. [26] who found a value of 12.67 °Brix for high bush blueberries (*V. corymbosum* L.).

Total phenolic content (TPC), total anthocyanin content (TAC), and antioxidant potential, expressed as ABTS and DPPH assay of the berry fruits raw material are presented in Table 2.

Table 2. Total phenolic content, total anthocyanin content, and antioxidant potential of berry fruits.

Fruit Raw Material	TPC (mg GAE/100 g)	TAC (mg C3G/100 g)	ABTS (µmol TE/g)	DPPH (µmol TE/g)
Blueberry	642.37 ± 0.24 [1] 5246.94 ± 19.49 [2]	80.28 ± 0.82 [1] 651.46 ± 6.67 [2]	38.36 ± 6.03 [1] 312.38 ± 0.74 [2]	27.58 ± 0.5 [1] 224.59 ± 5.94 [2]

[1] Values specified at fresh weight FW; [2] Values specified at dry weight DW. Each value represents average ± standard deviation (n = 3).

Table 3 contains data on the total phenolic content (TPC), total anthocyanin content (TAC), and antioxidant potential of the blueberry juices used in the vinegar manufacturing process.

Table 3. Total phenolic content, total anthocyanin content, and antioxidant potential of blueberry juices used in the vinegar manufacturing process.

Raw Material	TPC (mg GAE/L) (mg GAE/100 g DW)	TAC (mg C3G/L) (mg GAE/100 g DW)	ABTS (µmol TE/100 mL) (µmol TE/g DW))	DPPH (µmol TE/100 mL) (µmol TE/g DW)
Blueberry juice	1364.52 ± 46.4 [1] 1048.0 ± 3.56 [2]	195.28 ± 11.54 [1] 149.98 ± 8.82 [2]	798.2 ± 18.43 [3] 68.9 ± 7.40 [4]	653.9 ± 25.18 [3] 50.22 ± 14.72 [4]
Juice diluted with water	972.18 ± 23.4 [1] 972.1 ± 23.4 [2]	142.46 ± 2.80 [1] 142.46 ± 2.80 [2]	600.3 ± 38.19 [3] 60.03 ± 3.80 [4]	482.01 ± 28.30 [3] 48.27 ± 16.50 [4]

[1] Values expressed in mg GAE/L (FW). [2] Values expressed in mg GAE/100 g DW (specified at dry weight DW). [3] Values expressed in µmol TE/100 mL FW. [4] Values expressed in µmol TE/g DW.

Similar amounts of the total phenolic compounds of the blueberry used in the present study (642.37 mg GAE/100 g) were reported by Bunea et al. [27] for the Bluecrop variety (652.27 mg GAE/100 g) and by da Silva Fonseca et al. [13] when using ethanol as an extraction solvent (697.49 mg GAE/100 g).

The results regarding the total anthocyanins tested in our study for the blueberry (80.28 mg/100 g) were similar to those found by Jacques et al. [28] ranging from 72 to 128 mg/100 g and with those obtained by da Silva Fonseca et al. [13] (88.29 mg/100 g). In the case of the anthocyanins content, the results were much less than those obtained by Bunea et al. [27] for the Romanian blueberry *V. corymbosum* L. (100.58–163.40 mg/100 g). The content of anthocyanins depends on many factors (growing area, climatic conditions, harvest time, positioning conditions, etc.). These factors may have contributed to the observed differences in anthocyanin content.

Regarding the antioxidant activity of the blueberry tested in this study using an ABTS assay (38.36 µmol TE/g), higher values were found compared to the values reported by Bunea et al. [27] for several blueberry varieties, ranging from 24.33 to 36.46 TE mmol/g.

2.2. Vinegars Variants Obtained (Acidity, TSS)

The average values of vinegar acidity obtained in this study are presented in Figure 1 and varied between 3.597 and 4.307% after 50 days of fermentation. Generally, the acidity values were similar to the data presented by da Silva Fonseca et al. for blueberry vinegars [13]. The maximum value of acetic acid content (4.307%) was obtained for variant B804 (Figure 1).

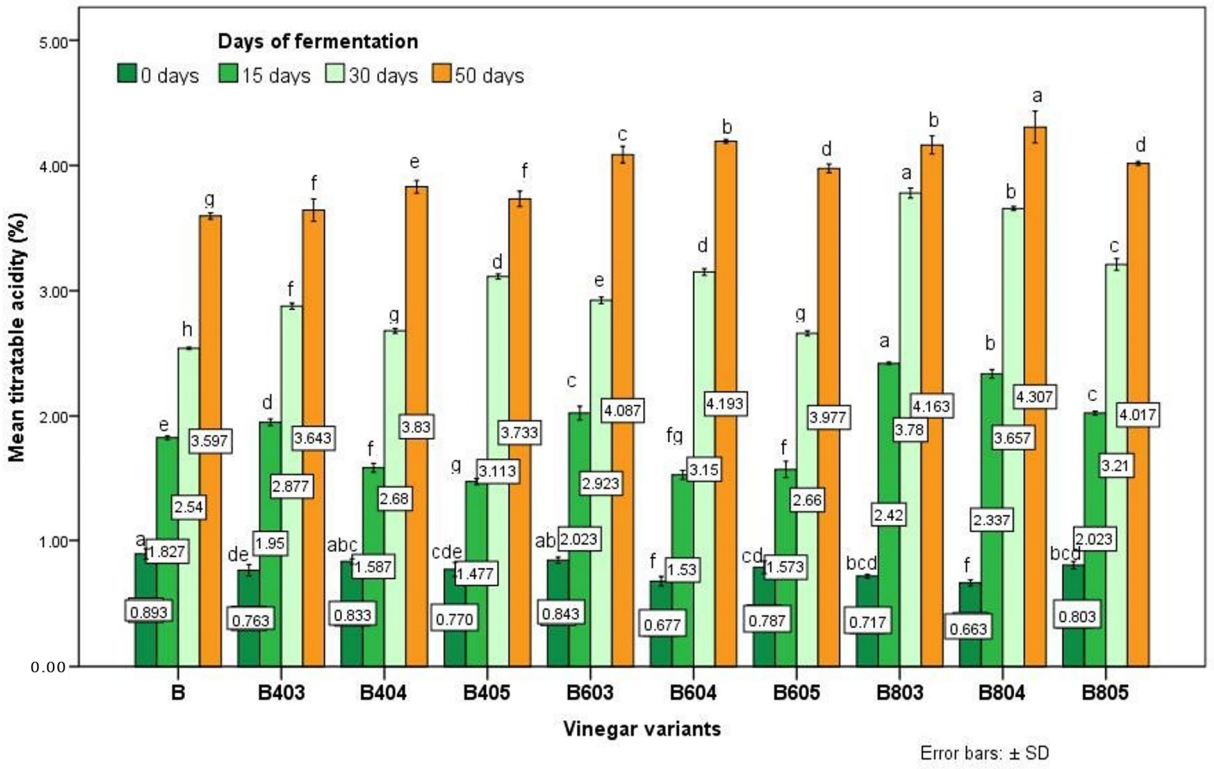


Figure 1. The average value of acidity (acetic acid %) of the blueberry variants obtained using substrates ultrasounds treated (graph of acetic acid formation in different stages of acetic fermentation). Data were expressed as the average values of 3 determinations ± standard deviation. Values with different letters differ significantly by ANOVA and Duncan's test ($p < 0.05$).

According to Tarhon M.C and to Romanian legislation, fruit vinegars must have a minimum acidity of 3.5 g/100 mL acetic acid and can present a maximum residual ethanol content of 1 g/L [29].

The vinegars obtained in this study are in accordance with legislation (Figure 1). The legislation does not establish the maximum value for the acetic acid content of the vinegar, but a vinegar with an acidity exceeding 5.5% can be rejected by consumers [13].

2.3. Total Phenolic Content and Total Antocyanins Content of Blueberry Vvinegars

The average values of total polyphenol content, total anthocyanins content, and antioxidant potential, expressed as ABTS and DPPH assay, of blueberry vinegars obtained using the process described in the chapter material and methods are presented in Table 4.

Table 4. Average values of the total soluble solids, total polyphenol content, total anthocyanins content, and antioxidant potential of blueberry vinegars.

	TSS	TPC		TAC		ABTS		DPPH	
	(°Bx)	(mg GAE/L)	(mg GAE/100 g DW)	(mg C3G/L)	(mg GAE/100 g DW)	(µmol TE/100 mL)	(µmol TE/g DW)	(µmol TE/100 mL)	(µmol TE/g DW)
B	8.6	431.33 ± 24.72 ^{e}	501.55 ± 28.74 ^{e}	13.46 ± 3.22 ^{d}	15.65 ± 3.72 ^{d}	209.18 ± 42.60 ^{e}	24.32 ± 49.53 ^{e}	137.41 ± 11.35 ^{e}	15.98 ± 13.20 ^{e}
B603	8.6	441.41 ± 51.11 ^{e}	513.27 ± 58.75 ^{e}	15.25 ± 1.32 ^{cd}	17.73 ± 1.52 ^{cd}	220.63 ± 37.22 ^{e}	25.65 ± 42.78 ^{e}	146.68 ± 16.42 ^{e}	17.06 ± 18.87 ^{e}
B803	8.6	455.45 ± 38.36 ^{e}	529.59 ± 43.44 ^{e}	16.07 ± 1.96 ^{cd}	18.69 ± 2.22 ^{cd}	244.29 ± 25.87 ^{e}	28.41 ± 29.30 ^{e}	150.66 ± 11.85 ^{e}	17.52 ± 13.42 ^{e}
B403	8.5	486.77 ± 25.30 ^{de}	572.67 ± 29.42 ^{de}	16.45 ± 2.18 ^{cd}	19.35 ± 2.53 ^{cd}	260.34 ± 18.00 ^{de}	30.63 ± 20.9 ^{de}	139.99 ± 19.07 ^{e}	16.47 ± 22.17 ^{e}
B604	8.7	475.88 ± 38.39 ^{e}	546.99 ± 44.13 ^{e}	16.05 ± 2.60 ^{cd}	18.45 ± 2.99 ^{cd}	300.62 ± 34.13 ^{cd}	34.55 ± 39.23 ^{cd}	155.02 ± 13.00 ^{e}	17.82 ± 14.94 ^{e}
B805	8.7	601.84 ± 30.26 ^{ab}	691.77 ± 33.62 ^{ab}	21.08 ± 2.44 ^{ab}	24.23 ± 2.71 ^{ab}	363.69 ± 14.50 ^{b}	41.80 ± 16.11 ^{b}	217.10 ± 12.02 ^{ab}	24.95 ± 13.36 ^{ab}
B605	8.5	547.63 ± 19.22 ^{bc}	644.27 ± 22.61 ^{bc}	18.61 ± 1.50 ^{bc}	21.89 ± 1.76 ^{bc}	350.51 ± 25.26 ^{bc}	41.24 ± 29.72 ^{bc}	187.56 ± 11.07 ^{cd}	22.07 ± 13.02 ^{cd}
B804	8.83	536.53 ± 24.00 ^{cd}	607.62 ± 26.67 ^{cd}	20.74 ± 1.86 ^{ab}	23.49 ± 2.07 ^{ab}	317.31 ± 25.04 ^{bc}	35.94 ± 27.82 ^{bc}	179.96 ± 12.04 ^{d}	20.38 ± 13.38 ^{d}
B404	9.0	643.05 ± 28.05 ^{a}	714.50 ± 32.62 ^{a}	23.29 ± 1.34 ^{a}	25.88 ± 1.56 ^{a}	417.19 ± 37.53 ^{a}	46.35 ± 43.64 ^{a}	238.04 ± 14.18 ^{a}	26.45 ± 16.49 ^{a}
B405	9.0	588.15 ± 27.39 ^{abc}	653.50 ± 32.22 ^{abc}	21.15 ± 2.38 ^{ab}	23.50 ± 2.80 ^{ab}	361.03 ± 17.93 ^{b}	40.11 ± 21.09 ^{b}	204.56 ± 11.33 ^{bc}	22.73 ± 13.33 ^{bc}

Data were expressed as the average of 3 determinations ± standard deviation. Values with different letters differ significantly by ANOVA and Duncan's test ($p < 0.05$).

To understand the influence of ultrasound treatments on the biologically active compounds of blueberry vinegars and to improve the levels of these valuable substances, the response surface methodology (RSM) was used. All polynomial mathematical equations concerning the indicators of the vinegars obtained in this study are given as the results of RSM. Therefore, with the aim to optimize the process for increasing the level of TPC and TAC, the following polynomial model was used:

$$\text{TPC (mg GAE/L)} = 601.81 + 57.73 \times X_1 + 30.90 \times X_2 - 28.46 \times {X_1}^2 - 55.83 \times {X_2}^2 + 1.57 \times X_1 \times X_2 \quad (1)$$

$$\text{TAC (mg C3G/L)} = 21.08 + 2.55 \times X_1 - 0.6538 \times X_2 - 0.7289 \times {X_1}^2 - 1.90 \times {X_2}^2 - 0.1972 \times X_1 \times X_2 \quad (2)$$

where X_1 indicates the ultrasound amplitude and X_2 refers to the time of the treatment.

These models indicate the effect of amplitude and time on the TPC and TAC values in berry vinegar as a result of RSM, using the experimental design presented in Table 5. The TPC values were the highest (643.2 mg GAE/L) for the ultrasound-treated samples using an amplitude of 80% for 4 min. The lowest TPC value was found in the samples treated with an amplitude of 40% for 3 min (441.4 mg GAE/L). Regarding the TAC of the samples, the lowest value was reported at 3 min and with 40% amplitude in sample application 3 (B403) (15.31 mg C3GE/L). The highest TAC value was identified in the variant treated with 80% amplitude for 4 min (23.29 mg C3GE/L) (Table 5).

Table 5. The measured responses in the experimental design (RSM).

Sample Application (ID)	Independent Variables		Dependent Variables			
	Amplitude (X_1)	Time (X_2)	Response 1	Response 2	Response 2	Response 3
			TPC (mg GAE/L)	TAC (mg C3G/L)	ABTS (µmol TE/100 mL)	DPPH (µmol TE/100 mL)
B	-	-	431.33	13.46	209.18	137.41
1 (B603)	60	3	475.88	16.05	300.62	155.02
2 (B803)	80	3	536.53	20.74	317.31	179.96
3 (B403)	40	3	441.41	15.31	220.63	146.68
4 (B604)	60	4	600.19	21.01	363.43	216.94
5 (B604)	60	4	605.32	21.11	364.44	217.88
6 (B805)	80	5	588.15	21.15	361.03	204.56
7 (B605)	60	5	547.63	18.61	350.51	187.56
8 (B604)	60	4	600.21	21.05	363.32	216.44
9 (B804)	80	4	643.05	23.29	417.19	238.04
10 (B604)	60	4	603.28	21.13	363.42	217.88
11 (B404)	40	4	455.45	16.07	244.29	150.66
12 (B604)	60	4	600.19	21.12	363.84	216.37
13 (B405)	40	5	486.77	16.45	260.34	139.99

The results regarding the analysis of variance (ANOVA) for the responses in the case of TPC (mg GAE/L) and TAC (mg C3G/L) of the berry vinegar samples at different levels of amplitude and time are presented in Table 6.

For complying with the level, there was found a quadratic model ($R^2 = 0.9614$ for TPC, respectively, $R^2 = 0.9889$ for TAC). The effect of the amplitude on the TPC and TAC of the samples was statistically significant at $p < 0.001$.

The change of the TPC and TAC values, depending on the US time and amplitude is presented in Figure 2.

Table 6. Analysis of variance (ANOVA) for the responses regarding vinegars TPC and TAC.

Source	SS	DF	MS	*F*-Value	*p*-Value	Significance
Model for TPC (mg GAE/L)	52,678.71	5	10,535.74	60.73	<0.0001	**
X_1 = A-Amplitude	26,658.15	1	26,658.15	153.67	<0.0001	**
X_2 = B-Time	4922.79	1	4922.79	28.38	0.0001	**
AB	9.80	1	9.80	0.0565	0.8190	-
A^2	6520.57	1	6520.57	37.59	0.0005	*
B^2	16,951.25	1	16,951.25	97.72	<0.0001	**
Lack of Fit	1192.03	3	397.34	71.32	0.0006	-
Total	53,893.02	12				-
R^2 = 0.9775; Adjusted R^2 = 0.9614; Std. Dev. = 13.17; Average = 552.62; C.V. % = 2.38						
Model for TAC (mg C3G/L)	82.45	5	16.49	214.12	<0.0001	**
X_1 = A-Amplitude	52.02	1	52.02	675.51	<0.0001	**
X_2 = B-Time	3.42	1	3.42	44.40	0.0003	*
AB	0.1560	1	0.1560	2.03	0.1976	-
A^2	3.70	1	3.70	47.99	0.0002	*
B^2	25.22	1	25.22	327.42	<0.0001	**
Lack of Fit	0.5284	3	0.1761	65.72	0.0007	-
R^2 = 0.9935; Adjusted R^2 = 0.9889; Std. Dev.= 0.2775; Average = 19.46; C.V. % = 1.43						

SS: Sum of squares; DF: degree of freedom; MS: average squares; TPC: total polyphenols content; TAC: total anthocyanin content; * significant at $p < 0.05$ and ** significant at $p < 0.01$

In the optimization model, TPC was determined to be 628.02 mg GAE/L and TAC was found to be 22.79 mg C3G/L, after 3.96 min and 78.50% amplitude treatment (Figure 2), an increase of 45.6% was found for TPC, compared with the untreated control, and, respectively, an increase of 69.3% was identified for the TAC amount value.

Similar results regarding the positive effects of ultrasonic treatments on vinegars TPC were reported by Yikmiș et al. for apple [3] and tomato vinegars [9], by Lieu et al. [17] for grape juice, by Brezan et al. [16] for apple cider, and by Bhat et al. [30] for lime juice.

2.4. Antioxidant Activity of Berry Vinegars

With the aim of optimizing the process to increase the level of ABTS and DPPH, the following polynomial model was used:

$$\text{ABTS (μmol TE/100 mL)} = 363.69 + 55.23 \times X_1 + 19.25 \times X_2 - 26.06 \times {X_1}^2 - 28.64 \times {X_2}^2 + 1.01 \times X_1 \times X_2 \quad (3)$$

$$\text{DPPH (μmol TE/100 mL)} = 217.1 + 27.68 \times X_1 + 9.64 \times X_2 - 14.55 \times {X_1}^2 - 28.41 \times {X_2}^2 + 7.82 \times X_1 \times X_2 \quad (4)$$

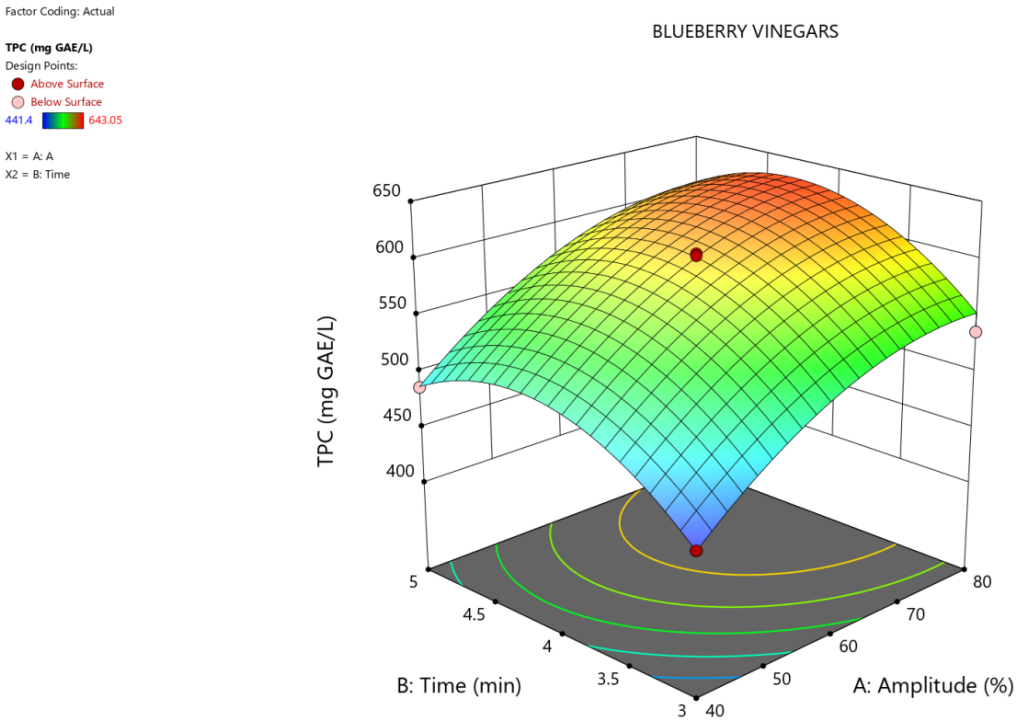
where X_1 indicates the ultrasound amplitude and X_2 refers to the time of the treatment.

Table 7 presents the results of the ANOVA analysis for ABTS (μmol TE/100 mL) and DPPH μmol TE/100 mL) values of the berry-vinegar samples. In this study, a quadratic model was used (R^2 = 0.8607 for ABTS and R^2 = 0.9189 for DPPH) and the values obtained are within the accepted limits.

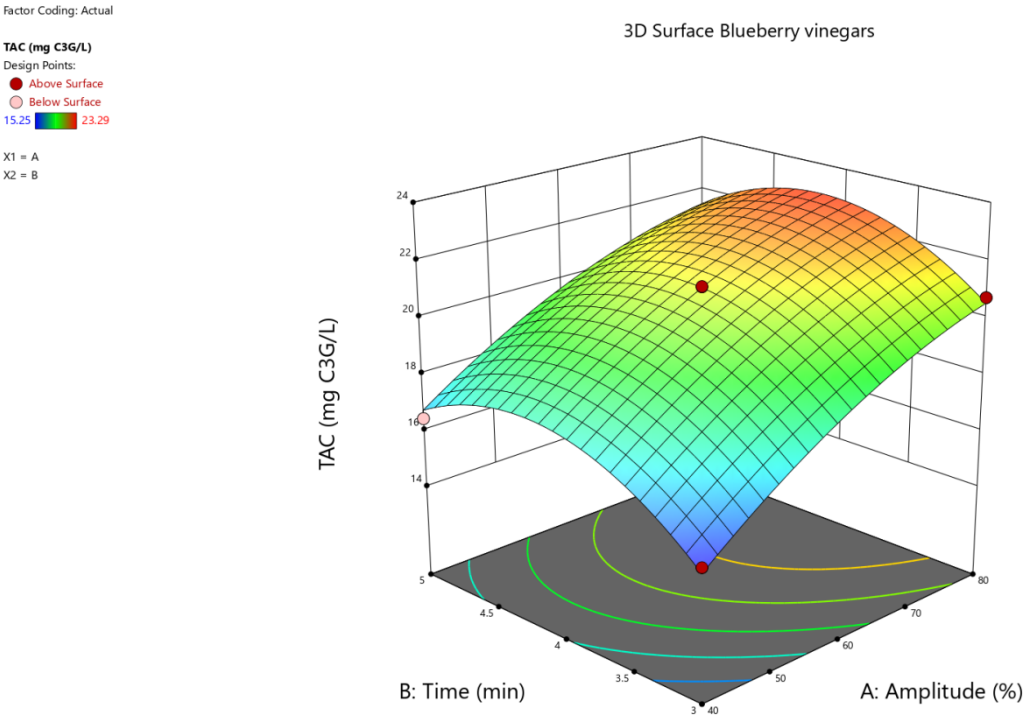
The change of ABTS and DPPH values, depending on the US variables (amplitude and time) is presented in Figure 3.

At the end of optimization, ABTS was found to be 363.69 (μmol TE/100 mL) and DPPH respectively 217.102 (μmol TE/100 mL) for 3.96 min treatment at 78.50% amplitude (Figure 4).

The lowest DPPH (μmol TE/100 mL) value was determined in the sample treated for 5 min with 40% amplitude, application 13 (139.1 μmol TE/100 mL), while the highest DPPH value was detected in the sample treated with 80% amplitude for 4 min (238.9 μmol TE/100 mL).



(**a**)



(**b**)

Figure 2. Response surface showing the effect of time (min) and amplitude (A%) of the ultrasound treatments on bioactive compounds of the blueberry vinegars: (**a**) TPC (mg GAE/L); (**b**) TAC (mg C3G/L).

Table 7. Analysis of variance (ANOVA) of responses for antioxidant activity (evaluated by DPPH and ABTS assays) of the vinegar samples.

Source	SS	DF	MS	*F*-Value	*p*-Value	Significance
Model for ABTS * (µmol TE/100 mL)	36,608.95	5	7321.79	15.83	0.0011	*
X_1 = A-Amplitude	24,406.97	1	24,406.97	52.77	0.0002	**
X_2 = B-Time	2964.31	1	2964.31	6.41	0.0391	*
AB	4.04	1	4.04	0.0087	0.9282	-
A^2	4723.21	1	4723.21	10.21	0.0152	*
B^2	5707.83	1	5707.83	12.34	0.0098	*
Lack of Fit	3236.73	3	1078.91	5004.21	<0.0001	-
Total	39,846.54	12				-
R^2 = 0.9187; Adjusted R^2 = 0.8607; Std. Dev. = 21.15; Average = 330.03; C.V.% = 6.52						
Model for DPPH * (µmol TE/100 mL)	12,791.90	5	2558.38	28.21	0.0002	*
X_1 = A-Amplitude	6128.57	1	6128.57	67.57	<0.0001	**
X_2 = B-Time	511.31	1	511.31	5.64	0.0493	*
AB	244.77	1	244.77	2.70	0.1444	-
A^2	1592.54	1	1592.54	17.56	0.0041	*
B^2	4946.38	1	4946.38	54.53	0.0002	*
Lack of Fit	632.73	3	210.91	381.58	<0.0001	-
R^2 = 0.9527; Adjusted R^2 = 0.9189; Std. Dev. = 9.41; Average = 190.66; C.V. % = 4.94						

SS: Sum of squares; DF: freedom degree; MS: average squares; * significant at $p < 0.05$ and ** significant at $p < 0.01$.

The antioxidant activity mentioned in this study is higher than those reported by Arvaniti et al. [31] for commercial and homemade Greek vinegars (from apple cider) and similar to those specified by da Silva Fonseca et al. [13] for the blueberry and honey vinegars obtained using another fermentation process.

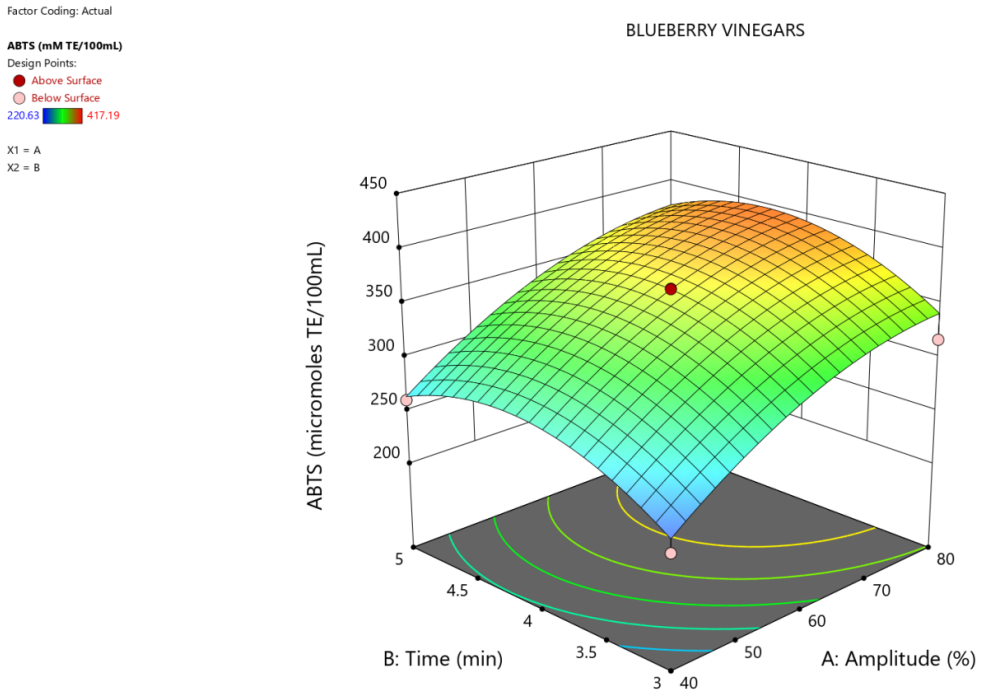
As stated by Wang et al. [32], ABTS and DPPH radicals have a different stereo-chemical composition and consequently confer, after interaction with antioxidants, a qualitatively different reaction for radical deactivation. In this context, more than a single assay is needed to provide the amount of information needed to estimate the antioxidant potential.

Figure 4 showed that a satisfactory antioxidant range is likely to be obtained by applying an ultrasound treatment with an amplitude of 78.50% and a period time of 3.96 min and this fact is most likely owing to the high responsiveness of phenolics to these conditions.

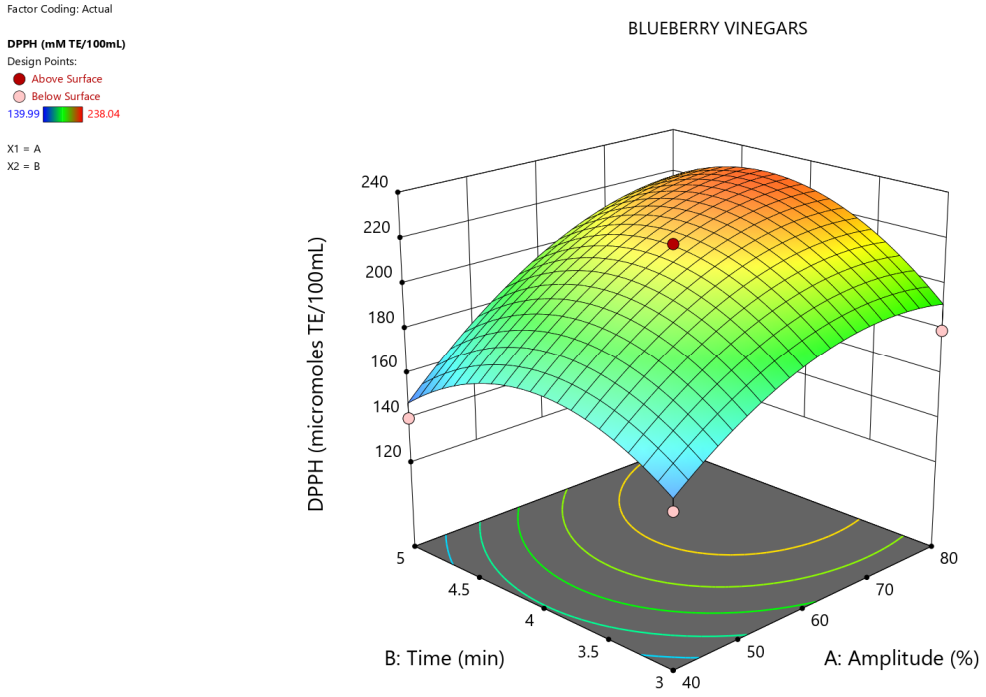
2.5. HPLC Analysis of Phenolic Compounds

In this study, four phenolic compounds (ellagic acid, gallic acid, ferulic acid, and chlorogenic acid) were identified in several samples chosen according to the results obtained using the model optimization. The choice of the study of the four hydroxycinnamic acids was made taking into account data from the literature that reported hydroxycinnamic acids (gallic acid and caffeic acid) as the main phenolic compounds in most types of fruit vinegars [33]. Chou et al. [34] highlighted that the number of flavonoids in vinegars is less. The authors reported that only one flavonoid compound (catechin) was detected when 21 flavonoid standards were analyzed. In persimmon vinegar, hydroxycinnamic acids were reported as major compounds [35], while in pomegranate vinegar, the protocatechuic acid (28.88 ± 0.02 mg/L), followed by gallic acid, were the most abundant phenolic compounds detected [36].

The level of these antioxidants in the vinegars obtained using the US treated substrates A 80% and 60% for 4 and 5 min and the untreated substrate are presented in Table 8.



(**a**)



(**b**)

Figure 3. Response surface showing the effect of time (min) and amplitude (A%) of the ultrasound treatments on the antioxidant potential of the blueberry vinegars: (**a**) ABTS assay; (**b**) DPPH assay.

It can be observed that the level of all phenolics compounds identified by HPLC analysis was the highest in the vinegar sample B804 (ID Table 5). This vinegar sample was obtained using an amplitude of 80% for 4 min and the values of the treatment parameters were very close to those recorded after applying the optimization model (A 78.5%, 3.96 min).

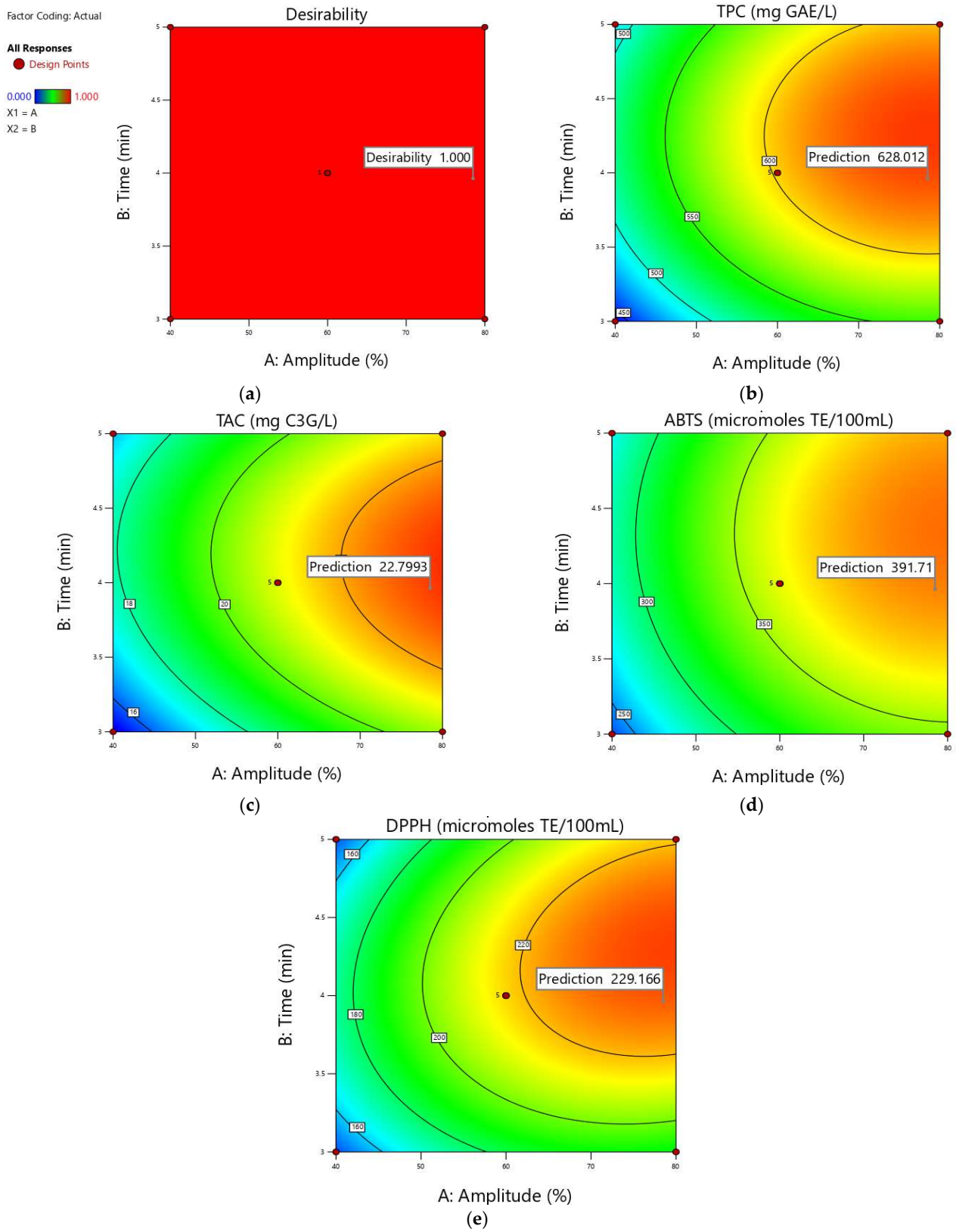


Figure 4. Contour plots showing the optimum predicted values for all the responses (parameters predicted values for amplitude 78.50% and 3.96 min time period of the US treatment): (**a**) desirability; (**b**) predicted value for TPC (612.715; 643.31); (**c**) predicted value for TAC (22.4769; 23.1216); (**d**) predicted value for ABTS (366.732; 416.689); (**e**) predicted value for DPPH (218.104; 240.227).

Table 8. Phenolic compounds in the vinegar samples identified by HPLC.

Sample	Ellagic Acid (μg/100 mL)	Gallic Acid (μg/100 mL)	Ferulic Acid (μg/100 mL)	Chlorogenic Acid (μg/100 mL)
B	1.781 ± 0.085 ^{d}	21.466 ± 2.72 ^{d}	1.506 ± 0.038 ^{d}	17.422 ± 1.083 ^{b}
B804	3.347 ± 0.026 ^{a}	35.22 ± 1.03 ^{a}	2.538 ± 0.116 ^{a}	18.801 ± 0.995 ^{a}
B805	3.271 ± 0.018 ^{b}	34.065 ± 0.85 ^{b}	1.967 ± 0.047 ^{b}	8.588 ± 0.084 ^{d}
B604	3.062 ± 0.104 ^{c}	28.215 ± 1.34 ^{c}	1.59 ± 0.062 ^{c}	14.238 ± 1.027 ^{c}
B605	2.353 ± 0.163 ^{c}	21.914 ± 2.78 ^{d}	1.536 ± 0.009 ^{cd}	8.447 ± 0.082 ^{d}

Data were expressed as the average of 3 determinations ± standard deviation. Values with different letters differ significantly by ANOVA and Duncan's test ($p < 0.05$).

2.6. Sensory Analysis

The results of sensory analysis of blueberry-vinegar samples, based on odour and taste profiles (general impression, pungent sensation, aromatic intensity, taste, and ethyl acetate odour) are presented in Figure 5. Among the judges, there were six experts (three female and three male panellists). The score of the attribute "ethyl acetate odor" is only the score of intensity.

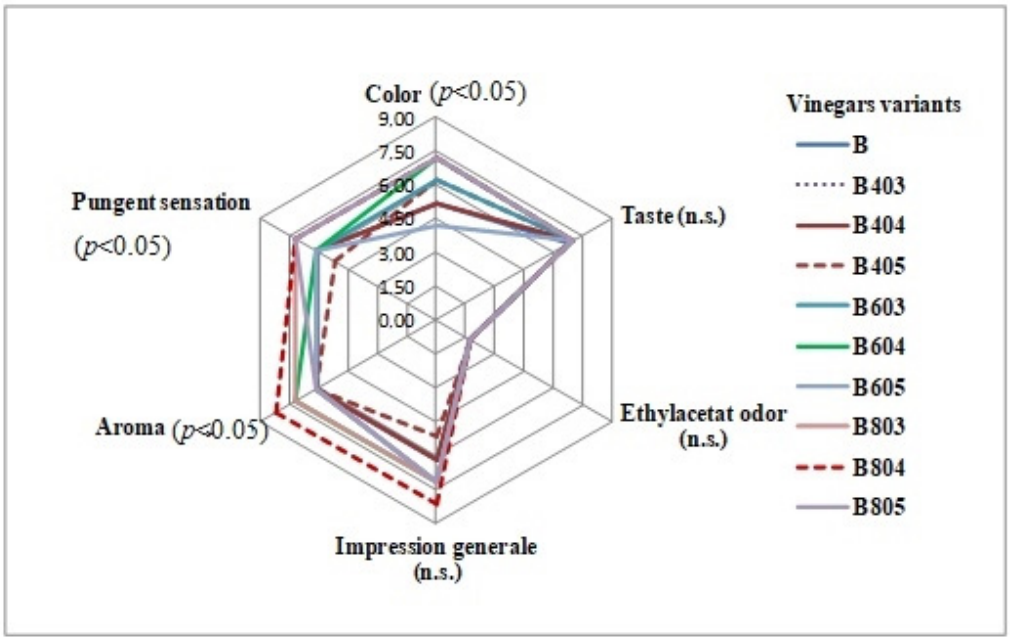


Figure 5. Results of sensory analysis for blueberry vinegars. ID of the samples were presented in Table 5. There were significant differences between samples at $p < 0.05$ and n.s., no statistical difference.

Sensory analysis of the vinegar variants evaluated revealed no abnormal odours. It was observed that there was no statistically significant difference between all samples in the case of the vinegars taste. The intensely sour taste of vinegar led to similar results, with the scores given by the tasters being close (there were no significant differences in taste).

The B804 (ID Table 5) sample was the most preferred sample regarding the evaluation of colour (8.27), aromatic intensity (8.18), pungent sensation (7.07), and general evaluation of impression (8.18) ($p < 0.05$).

The evaluation of the panellists highlights that the samples obtained using ultrasound-treated substrates were generally more liked compared with the sample control (B). Similar data were reported by Yikmuș et al. [4] for US-treated juice vinegars, products generally admired by the panellists. Other researchers reported also an improvement in the sensory

evaluations because of the ultrasound treatments used in the processing of various fruit juices [37].

3. Materials and Methods

3.1. Materials, Reagents

Folin–Ciocalteu reagent, was acquired from Sigma-Aldrich (St. Louis, MI, USA). DPPH (cat. no. D9132, purity 97%) and ABTS (cat. no. A1888, purity 98.6%) were acquired from Sigma-Aldrich (St. Louis, MO, USA). Cyanidin 3 glycoside and gallic acid were acquired from Karl Roth GmbH (Karlsruhe, Germany). Hydrochloric acid, sodium acetate, sodium carbonate, and sodium hydroxide were acquired from POCH BBASIC (Glivice, Poland). Trolox were acquired from Sigma-Aldrich (St. Louis, MO, USA).

Wild blueberry fruits (*Vaccinium myrtillus* L., Wild), sold at a local fruit market, were used as raw materials. The berries were harvested in August from a hilly area situated in Northeast Romania 45°43′18″ N 26°20′15″ E (Comandau, Covasna County, Romania).

3.2. Production of Berry-Vinegar Variants

Fresh blueberries were sorted, mechanically crushed (with a beater), and the berry juice was obtained under laboratory conditions using a Bosch MES3500 (700 W) centrifugal juice extractor (Bosch GmbH, Stuttgart, Germany) following a procedure typically applied at home scale.

The clear blueberry juice obtained was used for the production of vinegar according to the technological flow chart shown in Figure 6 but the method used was not the traditional one. We have tried a new method, our intention being the faster initiation of acetic fermentation, the use of treatments leading to the accentuation of the functional valences of the finished product, and the identification of the optimal parameters of these treatments in order to obtain vinegar with improved antioxidant properties.

First, the berry juices were diluted with water in order to obtain substrates having total soluble substances of 10 °Brix. After that, these substrates were mixed with ethanol (7%, *v*/*v*), glucose (4%, *w*/*v*), and apple vinegar (10%, *v*/*v*) for acidification. A commercial mineral-salts blend (0.03%, *w*/*v*; Acetozym® (Heinrich Frings GmbH & Co. KG, Rheinbach, Germany) was used to supplement the substrates. After the ultrasound treatments, this substrate having enough alcohol for the acetic fermentation was inoculated (in ratio 10%, *v*/*v*) with a culture of acetic bacteria prepared according to da Cunha et al. [1] and da Silva Fonseca [13]. A volume of 200 mL ultrasound-treated substrate was transferred to a 0.5 L capacity glass jar and inoculated with 20 mL acetic bacteria culture inoculum. The acetic bacteria culture and the inoculum were prepared according to da Cunha et al. [1]. The acetic acid bacteria were isolated from red grape (*Vitis vinifera* L.) vinegar produced in the faculty laboratory, by cultivating nonpasteurized vinegar in a GY medium (100 g/L glucose, 10 g/L yeast extract, and 100 mg/L natamycin). For obtaining the inoculum, in 500 mL Erlenmeyer flasks there were mixed the acetic bacteria culture (25 mL) with blueberry wine (150 mL), the dilution of the bacterial culture being 1:7. The flasks were incubated at 30 °C for 24 h in an orbital shaker rotating at 120 rpm for growth and cell adaptation and then added to the alcoholic substrates.

The fermentation temperature was set at 30 °C. During the process were determined the acidity (% acetic acid) and the pH values. The fermentation process was stopped when the acetic acid content was stable for a period of 7 days and until the alcohol content was between 0.5% and 1%. The acetic fermentation experiments were completed in 50 days after the inoculation. As a control (B sample), it used the untreated berry vinegar. Vinegar samples were stored at 4 °C.

3.3. Titratable Acidity, pH, and Total Soluble Solids

The pH, titratable acidity (% acetic acid), and total soluble solids values were determined for the substrates (before fermentation) and for the vinegar samples. The pH was measured using a pH meter (Consort C5020T, Consort, Turnhout, Belgium), the acidity

(% acetic acid) was determined by titration with a solution of 1N NaOH in the presence of phenolphthalein as an indicator and the total soluble solids content (TSS, °Brix) was analysed using a refractometer (OE Germany/OE Swiss/MASS) at room temperature.

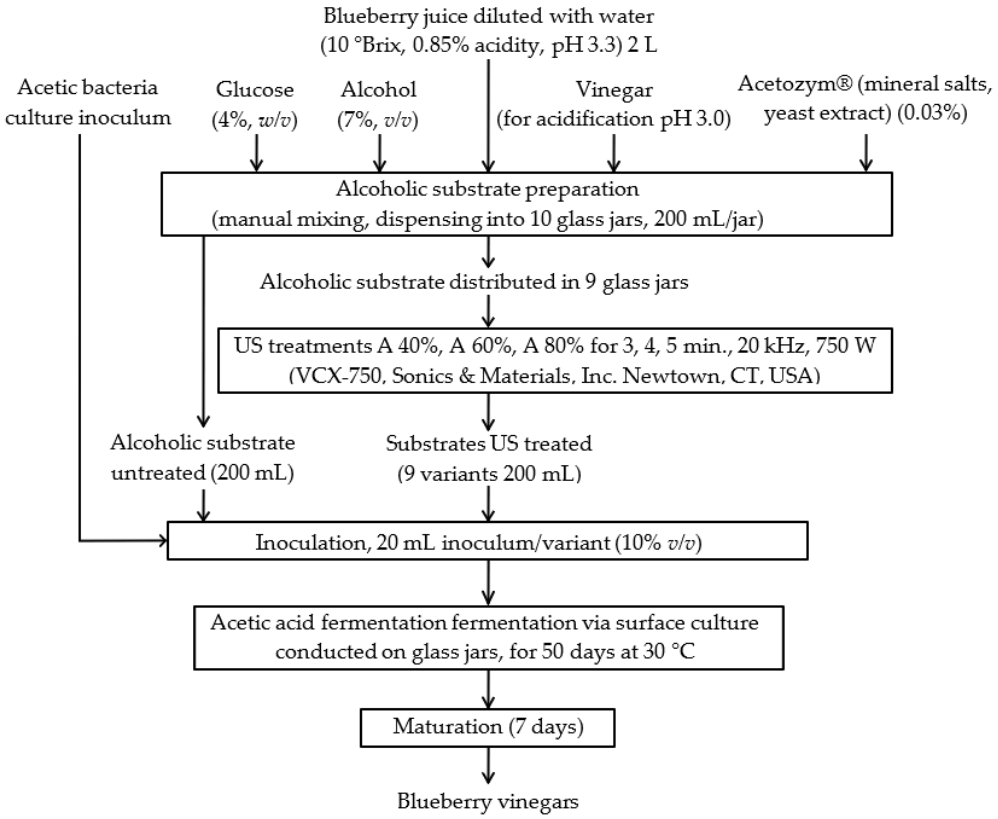


Figure 6. The method used for obtaining blueberry vinegar (US = ultrasound).

3.4. Ultrasound (US) Treatments

The substrates were ultrasonically treated (VCX-750, Sonics & Materials, Inc., Newtown, CT, USA) using a continuous frequency of 20 kHz, an amplitude A of 40%, 60%, and 80%, and a power of 750 W for periods of 3, 4, and 5 min for samples of 19 mm and 500 mL as a mixture [16].

3.5. Factorial Experimental Design

The berry vinegars' parameters were analyzed using Minitab Statistical Analysis Software (Minitab18.1.1) and response surface methodology (RSM) was employed in order to optimize the ultrasound treatment's effect on the antioxidant properties of the products. Table 5 presents the test applications (ID samples) used for optimization.

The response surface method (RSM) was used to understand the effect of ultrasound treatment of blueberry vinegar on bioactive components. Central composite design (CCD) was chosen for RSM. Two factors and 3 levels were determined in the design. There were 13 trial points for RSM optimization. Model adequacy, R^2 and corrected R^2 coefficients, lack-of-fit tests, and ANOVA results were evaluated. The independent variables were duration (X_1) and amplitude (X_2). Dependent variables were total phenolic content (TPC), total anthocyanins content (TAC), and total antioxidants (DPPH and ABTS). MINITAB statistical software (Minitab 18.1, Minitab, Inc., State College, PA, USA, 2017) was used for RSM and its graphs were developed with SigmaPlot 12.0 Statistical Analysis Software (Systat Software, Inc., San Jose, CA, USA). A polynomial was used to create model equations.

3.6. Total Phenolic Content (TPC) and Total Monomeric Anthocyanins Content (TAC)

Total phenolic content (TPC) of the samples was analyzed by the Folin–Ciocalteu method [38] using a plate reader spectrophotometer (Tecan, SunRise™, software Magellan™, Männedorf, Switzerland). The TPC was calculated as milligrams of gallic acid equivalents (GAE) per litre (L) and per 100 g dry weight (DW).

Total anthocyanins monomers were determined using the differential pH method and the absorbance was measured by the use of a spectrophotometer DR2800 type (Hach Lange, Loveland, CO, USA) [39]. The results were reported as mg cyanidin 3 glycoside (C3G) per litre of vinegar, respectively, per 100 g DW.

3.7. Antioxidant Activity

The antioxidant activity was determined by two different methods, respectively, ABTS and DPPH assays.

3.7.1. ABTS Assay

The antioxidant potential of vinegar variants in reaction with ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical (ABTS +) was determined by referring to a method related by Re et al. [40]. Trolox standards were applied to generate the calibration curve. Results were reported in µmol Trolox Equivalents per 100 mL of vinegar (µmol TE/100 mL) and per g dry weight (µmol TE/g DW).

3.7.2. DPPH Assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was formulated by a method described by Kumaran et al. [41]. Absorbance was analyzed at 517 nm (spectrophotometer DR2800, Hach Lange, Loveland, USA) considering the methanol as a witness sample. The antioxidant activity of the samples against DPPH radicals was expressed as micromoles of Trolox equivalents per 100 mL of vinegar (µmol TE/100 mL) and per g dry weight (µmol TE/g DW) using a calibration curve previously created.

3.8. Polyphenols Profiles by HPLC Analysis

The polyphenolic compounds profile was determined by high-performance liquid chromatography (HPLC) according to the method described by Abdel-Hameed et al. [42]. Separations were performed using an Agilent Technologies 1200 chromatograph equipped with a UV-DAD detector, using a 250 mm × 4 mm Licrocart (Licrospher PR-18 5 µm) column (Merck, Darmstadt, Germany) operated at 30 °C. All recorded data were processed using Agilent Chem Station B.04.03 software (Agilent, Santa Clara, USA). The mobile phase consisted of water/acetic acid (97:3, *v*/*v*) (eluent A) and acetonitrile (eluent B) at the flow rate of 1 mL/min. The linear gradient profile was as follows: 97% A (0 min), 97–91% A (5 min), 91–84% A (5–15 min), 84–65% A (15–20.8 min), 65–64.5% A (20.8–36 min), 64.5–50% A (36–37 min), 50% A (37–38 min), 50–97% A (38–39 min), and 97% A (42 min). The injection volume was 20 µL.

3.9. Sensory Analysis

The overall acceptability of the vinegar samples was evaluated by considering odor and taste profiles (general impression, pungent sensation, aromatic intensity, taste, and ethyl acetate odor). Twelve female and ten male panellists participated in the vinegar samples evaluation. Each sample was dosed in glass containers 20 ± 1 g and placed at 4–6 °C until serving. For performing the analysis, a 9–point hedonic scale was used. Scale scores were the following: excellent, 9; very good, 8; good, 7; acceptable, 6; and poor, <6.

3.10. Statistical Analysis

In order to optimize the antioxidant potential of blueberry vinegar, the RSM (Minitab 18.1, Minitab, Inc.) was used. There were obtained three-dimensional graphs (Sigma

Plot 12.0 Statistical Analysis Software, Systat Software, Inc.). Samples were prepared in triplicate.

4. Conclusions

This study provides strong evidence for the integration of ultrasound treatment of alcoholic substrate containing blueberry juices as an effective step in vinegar production to increase the extraction of bioactive compounds and, consequently, to enhance the antioxidant properties of the resulting products. The added value of this study consists both in the use of a new recipe to obtain blueberry vinegar, using an alcoholic substrate containing fruit juice and in the optimization, based on the response surface methodology, of the extraction process of antioxidant compounds from blueberry. The obtained data proved that the blueberry vinegars ultrasound-treated and optimized were of superior quality in relation to the polyphenols and anthocyanins content having, at the same time, better antioxidant properties. For the optimum values of all these parameters, the better values found for US amplitude and duration were 78.50% and 3.95 min, respectively. The sensory analysis of blueberry-vinegar variants revealed that the samples obtained using ultrasound-treated substrates were generally more liked compared with the control sample. The vinegar sample obtained following ultrasound treatment at 80% amplitude for 4 min, which showed the highest TPC and TAC levels and the strongest antioxidant properties assessed by the DPPH and ABTS tests, was the most preferred sample in terms of colour, aromatic intensity, pungent sensation, and overall impression. Blueberry vinegars obtained by ultrasound treatment of the raw material are a good source of polyphenolic compounds that contribute to the daily intake of antioxidants. We plan to extend the research for a more indepth analysis of polyphenolic compounds in blueberry-vinegar variants obtained using ultrasound treatments. Our results highlight that the use of an ultrasonically treated alcoholic substrate represents innovative items with practical applicability for a rapid start of acetic fermentation and for improving the antioxidant properties of blueberry vinegar.

Limitations of this study could be due to the experimental design with only three levels of variable design (multivariate). Therefore, future approaches are considered to improve the design in order to meet the optimal condition for obtaining the best blueberry-vinegar variant with a high level of valuable bioactive compounds. In addition, any study on the antioxidant and TPC potential of blueberry vinegars should take into account the structure of the antioxidants, their contribution, raw material, technology, and the ageing process. In light of this approach, we intend to continue research on blueberry vinegars as products with a promising antioxidant potential and multiple benefits for human health.

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References

1. Da Cunha, M.A.A.; De Lima, K.P.; Santos, V.A.Q.; Heinz, O.L.; Schmidt, C.A.P. Blackberry Vinegar Produced by Successive Acetification Cycles: Production, Characterization And Bioactivity Parameters. *Braz. Arch. Biol. Technol.* **2016**, *59*, 1–10. [CrossRef]
2. Chen, H.; Chen, T.; Giudici, P.; Chen, F. Vinegar Functions on Health: Constituents, Sources, and Formation Mechanisms. *Compr. Rev. Food Sci. Food Saf.* **2016**, *15*, 1124–1138. [CrossRef]
3. Yıkmış, S. Optimization of Uruset Apple Vinegar Production Using Response Surface Methodology for the Enhanced Extraction of Bioactive Substances. *Foods* **2019**, *8*, 107. [CrossRef] [PubMed]
4. Yıkmış, S.; Bozgeyik, E.; Şimşek, M.A. Ultrasound processing of verjuice (unripe grape juice) vinegar: Effect on bioactive compounds, sensory properties, microbiological quality and anticarcinogenic activity. *J. Food Sci. Technol.* **2020**, *57*, 3445–3456. [CrossRef]
5. Liu, Q.; Tang, G.-Y.; Zhao, C.-N.; Gan, R.-Y.; Li, H.-B. Antioxidant activities, phenolic profiles, and organic acid contents of fruit vinegars. *Antioxidants* **2019**, *8*, 78. [CrossRef]
6. Solieri, L.; Giudici, P. Vinegars of the World. In *Vinegars of the World*, 1st ed.; Solieri, P., Giudici, L., Eds.; Springer: Milan, Italy, 2009; pp. 1–16.
7. Catsberg, C.M.E.; Dommelen, G.J.M.K.-V. Vinegar. In *Food Handbook*; Springer: Dordrecht, The Netherlands, 1990; pp. 360–362.
8. Driss, O. The Nutritional and Antioxidant Potential of Artisanal and Industrial Apple Vinegars and Their Ability to Inhibit Key Enzymes Related to Type 2 Diabetes In Vitro. *Molecules* **2022**, *27*, 567.
9. Yıkmış, S.; Aksu, F.; Altunatmaz, S.S.; Çöl, B.G. Ultrasound processing of vinegar: Modelling the impact on bioactives and other quality factors. *Foods* **2021**, *10*, 1703. [CrossRef] [PubMed]
10. Expert Market Research. Available online: https://www.expertmarketresearch.com/reports/vinegar-market (accessed on 9 January 2023).
11. The State of Food and Agriculture 2019. Moving Fordward on Food Loss and Waste Reduction. Available online: https://scholar.google.com/scholar_lookup?title=The+State+of+Food+and+Agriculture+2019:+Moving+Fordward+on+Food+Loss+and+Waste+Reduction&author=Food+and+Agriculture+Organization&publication_year=2019 (accessed on 15 January 2023).
12. Luzón-Quintana, L.M.; Castro, R.; Durán-Guerrero, E. Biotechnological processes in fruit vinegar production. *Foods* **2021**, *10*, 945. [CrossRef]
13. Da Silva Fonseca, M.; Santos, V.A.Q.; Calegari, G.C.; Dekker, R.F.H.; De Melo Barbosa-Dekker, A.; Da Cunha, M.A.A. Blueberry and honey vinegar: Successive batch production, antioxidant potential and antimicrobial ability. *Braz. J. Food Technol.* **2018**, *21*. [CrossRef]
14. Ho, C.W.; Lazim, A.M.; Fazry, S.; Zaki, U.K.H.H.; Lim, S.J. Varieties, production, composition and health benefits of vinegars. A review. *Food Chem.* **2017**, *221*, 1621–1630. [CrossRef]
15. Trček, J.; Mahnič, A.; Rupnik, M. Diversity of the microbiota involved in wine and organic apple cider submerged vinegar production as revealed by DHPLC analysis and next-generation sequencing. *Int. J. Food Microbiol.* **2016**, *223*, 57–62. [CrossRef] [PubMed]
16. Brezan, B.; Badarau, C.; Woinaroschy, A. Effects of Blueberry and Black Carrot Extracts Addition on Antioxidant Properties and Protein-Precipitating Capacity of Ultrasound-Treated Cider. *Processes* **2020**, *8*, 812. [CrossRef]
17. Lieu, L.N.; Le, V.V.M. Application of ultrasound in grape mash treatment in juice processing. *Ultrason. Sonochem.* **2010**, *17*, 273–279. [CrossRef] [PubMed]
18. Wen, C.; Zhang, J.; Zhang, H.; Dzah, C.S.; Zandile, M.; Duan, Y.; Ma, H.; Luo, X. Advances in ultrasound assisted extraction of bioactive compounds from cash crops. A review. *Ultrason. Sonochem.* **2018**, *48*, 538–549. [CrossRef]
19. Xu, J.; Yang, H.; Zhang, C.; Liu, C. Optimised preparation and characterisation of lotus root starch oxidised with sodium hypochlorite (NaOCl) using response surface methodology. *Czech J. Food Sci.* **2022**, *40*, 61–68. [CrossRef]
20. Xu, F.; Wang, B.; Hong, C.; Telebielaigen, S.; Nsor-Atindana, J.; Duan, Y.; Zhong, F. Optimization of spiral continuous flow-through pulse light sterilization for Escherichia coli in red grape juice by response surface methodology. *Food Control* **2019**, *105*, 8–12. [CrossRef]
21. Zhang, X.; Zuo, Z.; Yu, P.; Li, T.; Guang, M.; Chen, Z.; Wang, L. Rice peptide nanoparticle as a bifunctional food-grade Pickering stabilizer prepared by ultrasonication: Structural characteristics, antioxidant activity, and emulsifying properties. *Food Chem.* **2021**, *343*, 128545. [CrossRef]
22. Saftner, R.; Polashock, J.; Ehlenfeldt, M.; Vinyard, B. Instrumental and sensory quality characteristics of blueberry fruit from twelve cultivars. *Postharvest Biol. Technol.* **2008**, *49*, 19–26. [CrossRef]
23. Colak, N.; Torun, H.; Gruz, J.; Strnad, M.; Subrtova, M.; Inceer, H.; Ayaz, F.A. Comparison of Phenolics and Phenolic Acid Profiles in Conjunction with Oxygen Radical Absorbing Capacity (ORAC) in Berries of *Vaccinium arctostaphylos* L. and *V. myrtillus* L. *Polish J. Food Nutr.* **2016**, *66*, 85–91. [CrossRef]

24. Gibson, L.; Rupasinghe, H.P.V.; Forney, C.F.; Eaton, L. Characterization of Changes in Polyphenols, Antioxidant Capacity and Physico-Chemical Parameters during Lowbush Blueberry Fruit Ripening. *Antioxidants* **2013**, *2*, 216–229. [CrossRef]
25. de Souza, V.R.; Pereira, P.A.; da Silva, T.L.; de Oliveira Lima, L.C.; Pio, R.; Queiroz, F. Determination of the bioactive compounds, antioxidant activity and chemical composition of Brazilian blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits. *Food Chem.* **2014**, *156*, 362–368. [CrossRef] [PubMed]
26. Almenar, E.; Samsudin, H.; Auras, R.; Harte, B.; Rubino, M. Postharvest shelf life extension of blueberries using a biodegradable package. *Food Chem.* **2008**, *110*, 120–127. [CrossRef] [PubMed]
27. Bunea, A.; Rugina, O.D.; Pintea, A.M.; Sconţa, Z.; Bunea, C.I.; Socaciu, C. Comparative Polyphenolic Content and Antioxidant Activities of Some Wild and Cultivated Blueberries from Romania. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2011**, *39*, 70–76. [CrossRef]
28. Jacques, A.C.; Pertuzatti, P.B.; Barcia, M.T.; Zambiazi, R. Nota científica: Compostos bioativos em pequenas frutas cultivadas na região sul do Estado do Rio Grande do Sul. *Braz. J. Food Technol.* **2009**, *12*, 123–127. [CrossRef]
29. Tarhon, M.C. *Tehnologia Fabricării Oțetului*; Fundației Universitare "Dunărea de Jos": Galați, Romania, 2006.
30. Bhat, R.; Shuaidda, N.; Min-Tze, L.; Karim, A.A. Sonication improves kasturi lime (*Citrus microcarpa*) juice quality. *Ultrason. Sonochem.* **2011**, *18*, 1295–1300. [CrossRef] [PubMed]
31. Arvaniti, O.S.; Mitsonis, P.; Siorokos, I.; Dermishaj, E.; Samaras, Y. The physicochemical properties and antioxidant capacities of commercial and Homemade Greek Vinegars. *Acta Sci. Pol. Technol. Aliment.* **2019**, *18*, 225–234.
32. Wang, C.C.; Chu, C.Y.; Chu, K.O.; Choy, K.W.; Khaw, K.S.; Rogers, M.S.; Pang, C.P. Trolox-equivalent antioxidant capacity assay versus oxygen radical absorbance capacity assay in plasma. *Clin. Chem.* **2004**, *50*, 952–954. [CrossRef]
33. Xia, T.; Zhang, B.; Duan, W.; Zhang, J.; Wang, M. Nutrients and bioactive components from vinegar: A fermented and functional food. *J. Funct. Foods* **2020**, *64*, 103681. [CrossRef]
34. Chou, C.H.; Liu, C.W.; Yang, D.J.; Wu, Y.H.; Chen, Y.C. Amino acid, mineral, and polyphenolic profiles of black vinegar, and its lipid lowering and antioxidant effects in vivo. *Food Chem.* **2015**, *168*, 63–69. [CrossRef]
35. Zou, B.; Xiao, G.; Xu, Y.; Wu, J.; Yu, Y.; Fu, M. Persimmon vinegar polyphenols protect against hydrogen peroxide-induced cellular oxidative stress via Nrf2 signalling pathway. *Food Chem.* **2018**, *255*, 23–30. [CrossRef]
36. Kharchoufi, S.; Gomez, J.; Lasanta, C.; Castro, R.; Sainz, F.; Hamdi, M. Benchmarking laboratory-scale pomegranate vinegar against commercial wine vinegars: Antioxidant activity and chemical composition. *J. Sci. Food Agric.* **2018**, *98*, 4749–4758. [CrossRef] [PubMed]
37. Jambrack, A.R.; Šimunek, M.; Petrovic, M.; Bedic, H.; Herceg, Z.; Juretic, H. Aromatic profile and sensory characterisation of ultrasound treated cranberry juice and nectar. *Ultrason. Sonochem.* **2017**, *38*, 783–793. [CrossRef] [PubMed]
38. Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Meth. Enzym.* **1999**, *299*, 152–178.
39. Wang, H.; Guo, X.; Hu, X.; Li, T.; Fu, X.; Liu, R.H. Comparison of phytochemical profiles, antioxidant and cellular antioxidant activities of different varieties of blueberry (*Vaccinium* spp.). *Food Chem.* **2017**, *217*, 773–781. [CrossRef] [PubMed]
40. Re, R.; Nicoletta, P.; Anna, P.; Ananth, P.; Min, Y.; Catherine, R.-E. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef] [PubMed]
41. Kumaran, A.; Karunakaran, R.J. Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. *Food Chem.* **2006**, *97*, 109–114. [CrossRef]
42. Abdel-Hameed, E.-S.S.; Bazaid, S.A.; Salman, M.S. Characterization of the Phytochemical Constituents of Taif Rose and Its Antioxidant and Anticancer Activities. *BioMed Res. Int.* **2013**, *2013*, 345465. [CrossRef] [PubMed]

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Insights on the Potential of Carob Powder (*Ceratonia siliqua* L.) to Improve the Physico-Chemical, Biochemical and Nutritional Properties of Wheat Durum Pasta

Mirabela Ioana Lupu [1], Cristina Maria Canja [1,*], Vasile Padureanu [1], Adriana Boieriu [1], Alina Maier [1], Carmen Badarau [1], Cristina Padureanu [1], Catalin Croitoru [2], Ersilia Alexa [3] and Mariana-Atena Poiana [3,*]

[1] Faculty of Food and Tourism, Transilvania University of Brasov, Castelului 148, 500014 Brasov, Romania
[2] Faculty of Materials Science and Engineering, Transilvania University of Brasov, Eroilor 29 Blvd., 500039 Brasov, Romania
[3] Faculty of Food Engineering, University of Life Sciences "King Mihai I" from Timisoara, Calea Aradului 119, 300645 Timisoara, Romania
* Correspondence: canja.c@unitbv.ro (C.M.C.); marianapoiana@usab-tm.ro (M.-A.P.); Tel.: +40-723-246-331 (C.M.C.); +40-726-239-838 (M.-A.P.)

Abstract: The aim of this research was to improve the physical-chemical properties and processability of wheat durum pasta while adding supplementary nutritional benefits. This was accomplished by incorporating carob powder into the conventional wheat pasta recipe. The study investigated the properties of pasta made with different proportions of carob powder (2%, 4%, 6% *w*/*w*) and evaluated its nutritional profile, texture, dough rheological properties and the content of bioactive compounds such as phenolic compounds. The physical and chemical properties (total treatable acidity, moisture content, and protein content), compression resistance, rheological properties of the dough and sensory analysis were also analyzed. Results showed that incorporating up to 4% carob powder improved the sensory and functional properties of the pasta. Additionally, the study found that the pasta contained phenolic compounds such as Gallic, rosmarinic, rutin and protocatechuic acids, ferulic, coumaric, caffeic acid, resveratrol and quercetin, and increasing the percentage of carob powder improved the polyphenolic content. The study concluded that it is possible to create innovative value-added pasta formulas using carob powder. Thus, the information revealed by this study has the potential to expand the portfolio of functional pasta formulations on the food market.

Keywords: carob powder; pasta; bioactive compounds; pasta properties; compression resistance; sensory analysis

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1. Introduction

Pasta represents one of the most consumed food products throughout the world, being distinguished by easiness and quickness of preparation [1]. It is an important source of carbohydrates (mainly starch), it contains proteins, a variable number of fibers and a very low concentration of fat [2].

The last report of the International Pasta Organization (IPO) indicated that 14.5 million tons of pasta were produced worldwide in 2018 [3] and there is an expected growth rate of 2.7% annually until 2025 [4]. Pasta is a popular food product in Europe, with high rates of consumption being reported in the Western region, in Italy, Spain and France; the Balkan region, in Greece; and in the Southern region; as well as in Chile, Argentina, Peru and Venezuela, according to a study conducted by Statista Global Consumer Survey [5] There is an increasing demand for food products with functional properties. These dietary items, also referred to as functional foods, can be enhanced, enriched or fortified.

To increase the nutritional value of the pasta, different studies have been carried out by adding different cereals (e.g., barley and pigmented cereals) [6,7], maize bran, grape marc

and brewers spent grain flour [8], legume and pseudocereal flours [9–11] and ingredients from various origins, such as oregano and carrot leaf meal [12].

Plant phytochemicals and phenolic antioxidants, such as those found in fruits, vegetables, herbs and spices, have been identified as active ingredients for use in functional foods [4]. This aspect launched the opportunity to create new types of pasta, involving novel ingredients [13].

Therefore, in our study, we wanted to highlight the influence and benefits of carob powder on the quality of pasta. Carob, scientifically denominated *Ceratonia siliqua* L., is an ancient crop, cultivated in countries located in the Mediterranean area. The plant belongs to the Leguminosae (Fabaceae) family, being used by humans for nourishment since early times, according to archeologists. Carob powder can be used as an additive for enhancing the aromatic profile of products, as a coloring thickener agent, as a sweetener and as an anticoeliac agent [14] in the pharmaceutical industry. The nutritional value of carob was considered relevant due to its high content of dietary fiber and phenolic compounds [15]. Several studies have investigated the effect of the addition of carob flour in bread and bakery products, revealing the functional profile of the products [16–19].

Due to its sweetness and flavor similar to chocolate, as well as its low price, the carob milled into flour is widely used in the Mediterranean region as a cocoa substitute for sweets, biscuits and processed drinks production [20–23]. Additionally, the advantage of using carob powder as a cocoa substitute is that it does not contain caffeine and theobromine [24]. In the last years, carob pods have gained considerable attention because of their high carbohydrate and mineral content: many high-value-added products, such as lactic acid, mannitol, citric acid and pullulans, were produced from carob fermentation [25]. In parallel, carob pods have been used as a resource for bioethanol production. In Lebanon, carob pulp is mainly used for the preparation of carob syrup or carob molasses denoted "dibs", which is consumed by the Lebanese population as a sweetener [26].

The idea behind this study focused on the fact that, to date, little information is available on the usefulness of carob as an unconventional ingredient for improving the physicochemical, biochemical and nutritional properties of durum wheat pasta. Only a few attempts to use carob flour as an alternative source of polyphenolic compounds to design new pasta formulations have been reported. In this respect, the studies by Biernacka et al. [27] and Seczyk et al. [28] are a valuable starting point for our research. In terms of dough processability and sensory properties of the pasta, a major influence is given by the level of ingredients incorporated and requires in-depth studies in order to optimize formulations. It has also been noted that the fortification of pasta with functional ingredients rich in polyphenolic compounds allows for increasing the content of bioactive compounds, but this effect may be limited to a different extent by several factors, including the binding of polyphenolic compounds with food matrix constituents as a result of interactions of these compounds with proteins and starch. [29].

Following the above information, the goal of this research was to highlight the beneficial properties of carob powder by using it in the production of composite flours in the proportions of 2%, 4% and 6% (*w*/*w*) to develop functional wheat durum pasta. For this purpose, the content of bioactive compounds such as individual phenolic compounds were studied, as well the physicochemical properties (total titratable acidity, moisture content, protein content), dough rheological properties and compression resistance, and the sensory analysis was conducted.

2. Materials and Methods

2.1. Raw Materials

The raw material used in the technological process of carob pasta is durum wheat semolina flour produced by Valse Mollen Denmark. Premium semolina is a grist of coarse, relatively uniformly sized particles (200–425 µm) of the endosperm with minimal fines (flour) and bran content. In the process of milling durum wheat to obtain flour of particle size 300–500 µm, wheat bran and germ are removed from the flour [30].

The commercial profile of semolina, according to the common European semolina specifications, included: moisture 15%, protein 12%, ash 0.88%, granulation: over 500 µm

(0%), over 355 µm (20%), over 250 µm (45%), over 180 µm (20%), under 180 µm (15%) [30]. Supplementary nutritional parameters were provided by the producer: 1.9% fat, 68% carbohydrates, 3.5% fibers and 0.01% salt.

Carob (*Ceratonia siliqua* L.) flour used for sample preparation produced by Sanovita Valcea was purchased from a local specialized shop and stored in a dry place until use. The nutritional profile offered by the producer was: proteins (5.1%), lipids (0.3% of which was saturated), carbohydrates (80.7%) and fibers (10.8%).

Iodized salt used in pasta production was purchased from a local market and tap water used was collected from the national water distribution. Eggs were achieved from a local farm, produced in an organic method.

2.2. Pasta Preparation

The recipes of pasta with the addition of carob included mixtures in which the share of the mass of carob powder was 2%, 4% and 6% (m/m) of the mass of the mixture (100 g). A control sample with no addition of carob powder was also prepared. Thus, four pasta formulations were prepared. The recipe of the control sample consists of 100 g wheat flour, 37.5 mL of water, 1 g salt and 21 g egg. In pasta formulas with added carob, the wheat flour was replaced by a mixture of 98 g wheat flour and 2 g carob powder, 96 g wheat flour and 4 g carob powder and 94 g wheat flour and 6 g carob powder. Pasta sample coding is released in Table 1.

Table 1. Sample coding.

Type	Coding	Percentage (%) of Carob Flour Used in the Pasta Formulations
Control sample	PM	0
Sample with 2% carob flour	P2CP	2
Sample with 4% carob flour	P4CP	4
Sample with 6% carob flour	P6CP	6

The pasta manufacturing process is shown in Figure 1.

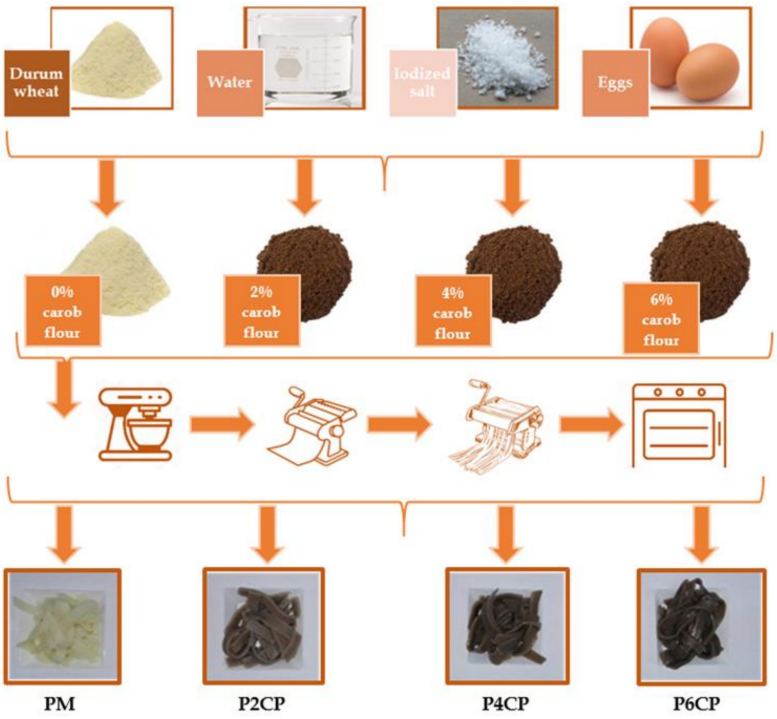

Figure 1. Pasta production method. PM: control sample; P2CP: sample with 2% (*w*/*w*) carob powder; P4CP: sample with 4% (*w*/*w*) carob powder; P6CP: sample with 6% (*w*/*w*) carob powder.

The raw materials and ingredients were dosed, weighted and scaled before being mixed at 250 rpm for 15 min in a laboratory mixer (Tefal Wizzo–QB307, Tefal, Rumilly, France) until a homogenous and firm dough was obtained. The dough was then divided into two 50 g pieces and laminated with a laminating machine (Laica PM2000, LAICA S.p.A., Barbarano Mossano (VI), Italy), pressed to eliminate air bubbles and excessive water deposit until the moisture content of the dough was 12 percent and the thickness of 2.5 mm was achieved. Then, the dough sheet was cut into tagliatelle shapes with a width of 4 mm and then dried by using a drying rack (KitchenAid—Pasta Drying Rack 5KPDR) and a laboratory drying oven at 50 °C (Deca PT-40) until pasta moisture reached the value of 12% moisture/100 g of pasta. The samples were carried out in triplicate for each type of flour.

2.3. Rheological Properties of the Dough

The biaxial stretching rheological properties of the dough at constant hydration were determined with a Chopin alveograph (Chopin Technologies, F Model Alveographe NG, Chopin, France). The method is based on the tensile strength of a dough sheet that is rested for 20 min and then subjected to a constant air pressure current until it forms a bubble and subsequently breaks. The air pressure inside the bubble is recorded until the bubble breaks and is then extrapolated graphically as a curve that reflects the dough's resistance to deformation [31].

The following parameters were recorded for each dough formulation: maximum overpressure P [mmH_2O], swelling index G (mm) and deformation energy W (J). The indicator L (mm) represents the dough extensibility (mm), estimating its handling properties. The P/L ratio indicates the ratio between the dough's toughness and its extensibility and is an important indicator along with W for characterizing flours for different bread and pastry products.

2.4. Extraction of Polyphenolic Compounds

The extraction of polyphenolic compounds was performed according to the method by Gaita et al. [29] by grinding the dry pasta samples and then performing in a hydroalcoholic medium with ethanol 45% (*v*/*v*). The solid ratio: solvent of 1:20 was stirred at a temperature of 25 °C for one hour, using the shaker Heidolph Promax 1020 (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). The extraction was filtered, and the obtained clear fractions were used for further analysis.

2.5. Determination of Total Phenolic Content and Polyphenolic Compounds Profile

Folin–Ciocalteu assay was used to determine the total phenolic content [32]. We used 0.5 mL from the extraction of each type of pasta treated with 1.25 mL of Folin–Ciocalteu reagent (Merck, Darmstadt, Germany) diluted 1:10 with water for this purpose. After incubating the sample for 5 min at room temperature, 1 mL Na_2CO_3 60 g/L was added. A UV-VIS spectrophotometer was used to measure sample absorption at 750 nm after 30 min of incubation at 50 °C (Analytic Jena Specord 205). The calibration curve was created with Gallic acid as the standard at concentrations ranging from 5–250 g/mL.

The results of total phenolic content (TPC) were expressed in micrograms of Gallic acid equivalents (GAE) per g of investigated sample.

The profile of polyphenolic compounds was determined using high-performance liquid chromatography coupled with mass spectrometry (LC-MS) as described by Abdel-Hameed et al. [33]. The main polyphenols in carob flour pasta samples were determined using LC-MS with SPD-10A UV (Shimadzu) and LC-MS 2010 detectors, column EC 150/2 NUCLEODUR C18 Gravity SB 150 × 2 mm × 5 µm. The following were the chromatographic conditions: mobile stages A: water with formic acid at pH-3, B: acetonitrile with formic acid at pH-3, gradient program: 0.01–20 min 5% B, 20.01–50 min 5% B, 5–55 min 5% B, 55–60 min 5% B. Temperature 20 °C, mobile phase 0.2 mL/min The monitoring wavelengths were 280 nm and 320 nm. The curve calibration was drawn between 20 and 50 µg/mL. The results were given in µg/mL.

2.6. Determination of Total Titratable Acidity (TTA)

The samples of pasta were weighed to 15 g and chopped into small pieces for an easement of the homogenization process. The resulting samples were mixed with 100 mL of distilled water in a glass flask until homogenization. Meanwhile, the titration agent was formulated (Sodium Hydroxide 0.1 N Belle Chemical LLC, Billings, MT, USA). Three drops of phenolphthalein 1% in alcoholic solution were added and the titration started drop by drop, continuing until the indicator turned light pink and persisted for 30 s. The results were the average value of the two measurements. Total titratable acidity (acidity degrees/100 g product) was evaluated using the relationship presented in Equation (1):

$$\text{Total Titratable Acidity (acidity degrees/100 g products)} = \frac{V \times 0.1}{m} \times 100 \quad (1)$$

2.7. Determination of Moisture Content

The moisture content of pasta samples was determined by using the gravimetric method reported by Rios et al. [34] and detailed in ISO 712:2009 [35]. Samples were weighed to 5 g using an analytical balance into a weighting vial and dried using a drying stove at 130 °C until reaching constant weight. Each sample was analyzed in triplicate and the results were expressed as average. The results were reported as weight percentage (%).

2.8. Determination of Compression Resistance

The crushing resistance of pasta was performed using a compression kit tester (Zwick-Roell Z005, Zwick, Ulm-Einsingen, Germany) [27]. Each sample was tested in triplicate and the results were expressed as averages. Measurements were carried out using cooked pasta and chilled until reaching the room temperature of 22.5 °C. Each sample was placed on the inferior machine pan (Zwich Z005) and compressed using a compression kit (2.5 mm thick) at a speed of 25 mm/min. The test was performed for determining the maximum force necessary for sample compression until the structure is smashed at 70% strain.

2.9. Determination of Protein and Fiber Content

The protein content of obtained pasta samples was evaluated by using the Kjeldahl mineralization method after nitrogen analysis using spectrophotometric analysis, according to the method reported by Heeger et al. [36] and Rios et al. [34]. The calibration was performed using ammonium chloride (NH_4Cl) and a conversion factor corresponding to the analyzed category was applied (5.7) to determine the protein content of the samples. The analysis was performed in triplicate and the results were expressed as the average of the three trials. The result was expressed as weight percentage (%). The fiber content was calculated taking into account the fiber content of raw materials and the recipe of pasta fabrication according to [37].

2.10. Sensory Evaluation

The overall acceptability of the pasta was evaluated by considering two profiles—visual profile and taste profile [28]. The visual profile included attributes such as color, appearance, attractiveness and overall acceptability, and the taste profile included flavor, taste, texture, consistency and aftertaste.

For performing the analysis, a 9-point hedonic scale was used. Each scale corresponded to a perception in order to facilitate data processing as follows: 1—"Dislike extremely"; 2—"Dislike very much"; 3—"Moderately dislike"; 4—" Slightly dislike"; 5—"Neither like nor dislike"; 6—" Slightly like"; 7—" Moderately like"; 8—"Like very much"; and 9—"Like extremely".

Sensory analysis was performed at the Faculty of Food and Tourism, Brasov, Romania. Panelists who declared suffering from different digestive problems were excluded from the study due to the high risk of aggravation of the diseases. While performing the analysis,

the panelists were presented with a total of four samples of the product, as indicated in Table 1.

A number of 30 panelists aged 18–60 were requested to evaluate the samples. The participants in the study are persons with experience and competencies in sensory analysis, teachers and students who have studied and taught the mentioned subject. Panelists were asked to evaluate each sample for the assessed parameters. Panelists received the samples by turn in order to diminish the similarity and interdependence between samples with a 5-min rest before each sample tasting. Samples were coded accordingly to the codification mentioned above and each sample was distributed in glass containers at 20 ± 1 g and placed at a temperature in the range of 18–20 °C until serving. Panelists were served a glass of water as a palate cleanser. Consent for each panelist was required.

2.11. Statistical Analysis

Data analyses were performed using SPSS software (Statistical Package for Social Sciences Statistics, version 25.0.0, IBM, 2009, New York, NY, USA) and analyzed by ANOVA and Duncan's multiple range test (scored as significant if $p < 0.05$). The analysis was made in triplicate and the results were reported as mean value ± standard deviation.

3. Results and Discussion

3.1. Dough Rheological Properties

The rheological behavior of doughs and the quality attributes of final goods are greatly influenced by the water absorption capacity of flours, which varies among different flour sources [38].

The rheological parameters of pasta dough formulations are given in Table 2.

Table 2. Rheological data for the pasta dough formulations.

Parameter	PM	P2CP	P4CP	P6CP
P (mmH_2O)	170 ± 0.15 ^{a}	103 ± 0.09 ^{b}	101 ± 0.03 ^{b}	93 ± 0.01 ^{c}
L (mm)	22 ± 0.05 ^{a}	33 ± 0.12 ^{b}	33 ± 0.09 ^{b}	27 ± 0.06 ^{c}
G (mm)	10.4 ± 0.11 ^{a}	12.8 ± 0.13 ^{b}	12.8 ± 0.07 ^{b}	11.6 ± 0.05 ^{c}
W (10^{-4} J)	169 ± 0.25 ^{a}	130 ± 0.28 ^{b}	125 ± 0.21 ^{c}	100 ± 0.14 ^{d}
P/L	7.73 ± 0.13 ^{a}	3.12 ± 0.09 ^{b}	3.06 ± 0.18 ^{b}	3.44 ± 0.05 ^{b}

PM: control sample; P2CP: sample with 2% (w/w) carob powder; P4CP: sample with 4% (w/w) carob powder; P6CP: sample with 6% (w/w) carob powder. P: the maximum overpressure; L: extensibility; G: swelling index; W: strain energy; P/L: indicates the ratio between the tenacity of the dough and its extensibility. Values followed by different letters differ significantly by ANOVA test ($p < 0.05$).

In the case of using 4% w/w carob flour, it can be noticed that there was an improvement in the extensibility (L) of the dough and the swelling index (G) by up to 33%. The extensibility of the dough is influenced by the raw materials of the dough preparation. Dube et al. [39] reported that the extensibility of 100% wheat control dough progressively decreased from 156 mm to 77 mm for 40% sorghum wheat dough, while Sibanda et al. [40] also recorded similar results of a decrease in extensibility from 132 mm for 100% wheat control dough to 36 mm for 30%. The pasta supplemented with 10% carob fruit showed better texture parameters (less hard, less sticky and less adhesive) [41].

Figure 2 illustrates the variation of rheological parameters P and W with the amount of carob added to the pasta formulations.

Dough containing carob flour has the ability to retain gas and has a low capacity for extension without breaking. The dough with a more extensible character is particularly essential for improved gas retention during the process of baking which results in a good loaf volume [40].

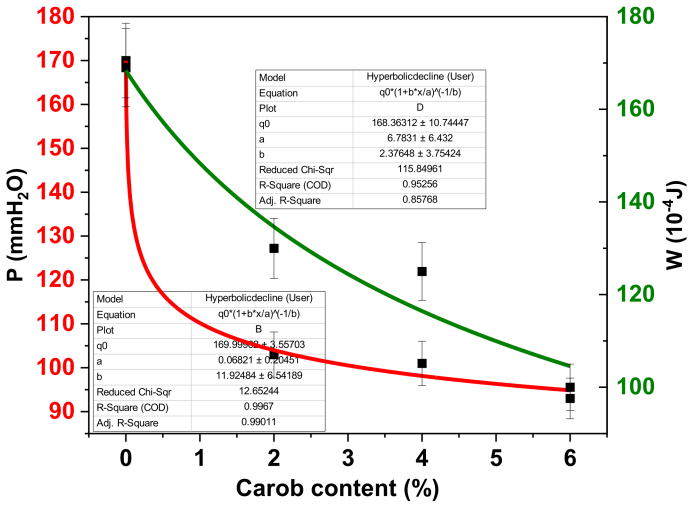

Figure 2. Variation of rheological parameters P and W with the amount of carob.

The tenacity of the dough decreases by up to 40% with increasing carob content (the P and W values, Figure 2 and Table 2), which is probably due to the higher fiber content of the additive compared to neat semolina flour. However, this decrease is normal for a non-gluten additive and does not significantly alter the processability of the dough. The increased extensibility of the carob-containing flour can be attributed to the plasticizing effect of the carbohydrates present in the product. The formulations with 2% and 4% carob flour may offer a good balance between processability and extensibility [42].

The beneficial effects of enrichment with different flours/powders after frozen storage conditions have been reported in the literature [43]. The physical characteristics of frozen dough and semi-baked frozen samples with the addition of commercial soluble fibers or whole oat flour were determined after baking and compared with the fresh samples. The results highlighted that in semi-baked frozen samples the crumb elasticity increased by 18% in comparison to the respective fresh ones. Additionally, samples containing whole oats presented an increased water adsorption capacity. Further studies are needed to assess the influence of carob powder on the quality of frozen dough.

3.2. Physical and Chemical Properties

Titratable acidity, protein content, moisture and TPC of obtained samples are reported in Table 3.

Table 3. Samples assessment after 2 h from the pasta production process (dry pasta).

Parameter	2 Hours			
	PM	**P2CP**	**P4CP**	**P6CP**
Titratable acidity (%)	2.21 ± 0.015 ^{a}	1.80 ± 0.010 ^{b}	1.42 ± 0.015 ^{c}	1.21 ± 0.015 ^{d}
Protein content (%)	14.03 ± 0.058 ^{c}	14.17 ± 0.153 ^{bc}	14.30 ± 0.100 ^{ab}	14.45 ± 0.050 ^{a}
Moisture (%)	30.23 ± 0.257 ^{c}	32.03 ± 0.057 ^{b}	33.07 ± 0.057 ^{b}	35.87 ± 0.152 ^{a}
TPC (μg GAE/g)	239.07 ± 0.010 ^{d}	311.53 ± 0.025 ^{c}	406.80 ± 0.100 ^{b}	461.10 ± 0.100 ^{a}
Fiber (%) *	2.00	2.04	2.08	2.12

PM: control sample; P2CP: sample with 2% (*w*/*w*) carob powder; P4CP: sample with 4% (*w*/*w*) carob powder; P6CP: sample with 6% (*w*/*w*) carob powder. Values followed by different letters differ significantly by ANOVA test ($p < 0.05$), * the fiber content was calculated according to the fiber content of raw materials and the recipe of pasta fabrication.

Samples assessed after 2 h containing different proportions of carob flour showed higher titratable acidity contents compared to the control sample. It can be observed that, between samples, the differences were notable, with the highest value being recorded for the sample without carob powder (PM-2.21%) and the lowest value being recorded for

the pasta with the highest concentration of carob powder P6CP—1.21%. Samples P4CP and P2CP showed lower values of 1.42% and 1.80%. The protein content showed slight differences between samples with the addition of carob flour and the blank sample. The most relevant development was recorded for sample P6CP—14.45% protein content due to the amount of carob flour added. Gopalakrishnan et al. [44] reported that the addition of sweet potato and fish powder in pasta raised the protein content to 12.84%.

The highest moisture content was recorded in the case of the sample with the highest content of carob powder, P6CP, (35.87%) and the lowest moisture content was recorded in the case of pasta without carob powder, PM, with 30.23%. Biernacka et al. [27] have stated that the pasta enriched with carob flour in various increasing proportions (1% to 5%) showed a certain correlation between the total phenolic content and antioxidant activity in pasta samples. The antioxidant profile of carob flour added in pasta samples revealed that the greatest value of total polyphenol content was noted for the sample containing the highest proportion of carob flour—6%—a fact that supports the statement that the addition of carob flour improves the antioxidant profile of samples. Values were considerably superior compared to the control sample. Similar to the present study, research developed by Biernacka et al. [27], Boroski et al. [12] and Zhu et al. [45] showed that the addition of carob powder in pasta in various proportions showed positive correlations concerning the TPC value. Makris et al. [46] reported that carob powder contains a higher ratio of antioxidants than red wines and can be considered a valuable source for pharmaceutical products due to its polyphenolic content. Issaoui et al. [47] reported that carob flour showed lower moisture values (13.40%, 13.50% and 13.700%) for carob pulp powder, (14.0%, 14.20%) respectively, for carob seed powder that was reflected in the final product's moisture content.

It was observed that the higher the concentration of carob is in a food product, the higher the phenolic concentration will be. For example, Aydin et al. [48] reported that the addition of 42% carob flour in spread samples was 615.28 mg GAE/100 g, considerably higher compared to the results obtained in the current study, in which the highest addition content was 6% of carob powder. Additionally, Sebecic et al. [49] mentioned that the total phenol content of biscuit samples obtained with the addition of 25% carob flour was 5.53 g/kg biscuit compared to the sample with wheat flour, with 1.10 g/kg biscuit, and 1.60 g/kg of wheat whole grain flour.

The present study reported a superior value of TPC for the sample containing carob flour in proportion of 6% (461.10 mg GAE/100 g) in contrast with the control sample, with 239.07 mg GAE/100 g. The increase in the total polyphenol content of the samples containing carob powder was closely related to the method for obtaining the carob powder, the amount of carob powder introduced into the products, the methodology used to obtain the products and the biochemical reactions developed in the product matrix [50–52].

The results shown in Table 4 show that fortifying pasta with carob increased the level of individual phenolic compounds.

All analyzed compounds are present in the P6CP sample, which contains the highest level of carob. Similar values were obtained when pasta was fortified with grape pomace [53]. The addition of 3–9% grape marc to the pasta recipe resulted in an increase of individual polyphenolic compounds depending on the percentage added. For example, Gaita et al. [29] reported the following values for individual polyphenols in pasta enriched with grape marc: gallic acid (27.95–88.22 μg/g), caffeic acid (0.66–58.11 μg/g), epicatechin (10.29–14.92 μg/g), coumaric acid (0. 32–0.85 μg/g), ferulic acid (6.33–15.75 μg/g), rutin (0.14–18.56 μg/g), rosmarinic acid (26.1–38.53 μg/g), resveratrol (31.15–34.38 μg/g), quercetin (2.89–7.3 μg/g), kaempferol (19.73–54.24 μg/g).

The addition of carob flour has been found to increase the content of polyphenols; however, the increase is not linear but exponential.

Table 4. Polyphenolic compounds identified in the dry pasta samples.

Compound	Ion Mode	Polyphenolic Compounds (μg/g d.s.)			
		PM	P2CP	P4CP	P6CP
Gallic acid	169	nd	nd	6.97 ± 0.07 [a]	31.44 ± 0.18 [b]
Protocatechuic acid	153	nd	nd	19.93 ± 0.16 [a]	51.14 ± 0.08 [b]
Caffeic acid	179	nd	nd	0.86 ± 0.05 [a]	8.56 ± 0.11 [b]
Epicatechin	289	nd	nd	17.15 ± 0.13 [a]	24.31 ± 0.17 [b]
p-Coumaric acid	163	nd	nd	0.30 ± 0.03 [a]	0.30 ± 0.09 [a]
Ferulic acid	193	nd	nd	nd	0.23 ± 0.13 [a]
Rutin	609	nd	nd	1.07 ± 0.06 [a]	1.41 ± 0.16 [b]
Rosmarinic acid	359	nd	nd	nd	224.24 ± 0.32 [a]
Resveratrol	227	nd	nd	nd	0.85 ± 0.07 [a]
Quercetin	301	nd	0.54 ± 0.02 [a]	11.49 ± 0.11 [b]	243.66 ± 0.25 [c]
Kaempferol	285	nd	nd	63.76 ± 0.17 [a]	231.51 ± 0.32 [b]

nd—not detected; PM: control sample; P2CP: sample with 2% (*w*/*w*) carob powder; P4CP: sample with 4% (*w*/*w*) carob powder; P6CP: sample with 6% (*w*/*w*) carob powder. Values followed by different letters differ significantly by ANOVA test ($p < 0.05$).

This has been determined for quercetin, for which detectable values are available for all compositions. The increase may depend on the degree of mixing of the carob flour with the semolina dough and the distribution of these compounds in the flour.

Our findings are consistent with those of other authors such as Gaita et al. [29], who have stated that the improvement with natural phenolics can be influenced by a lot of factors, among which includes binding with food matrix components. In addition, Gaita et al. [29] and Sęczyk et al. [28] specified that the content of bioactive compounds may be influenced by the combination of proteins and phenolics content, such as hydrophobic interactions and hydrogen and ionic bonding. Frühbauerova et al. [54] conducted a study on the bioaccessibility of phenolics from carob pod powder prepared by cryogenic and vibratory grinding, and they showed that, from the 13 compounds involved in the UHPLC analysis, three were phenolic acids (vanillic, ferulic and cinnamic), three flavons (luteolin, apigenin and chrysoeriol), naringenin (flavanone) and quercitrin (glycoside form of flavonoid quercetin). The highest amount of quercitrin (44.54–64.68 μg/g), cinnamic acid (27.48–31.40 μg/g) and chrysoeriol (8.60–9.82 μg/g) have been found. The amount of the rest of the phenolic constituents ranged from 1.88 to 10.14 μg/g [55–57].

In our study, the highest number of compounds is also found in quercitrin, in P6CP (243.66 μg/g). The results shown in Table 4 show that fortifying pasta with carob increased the level of phenolic compounds.

3.3. Compression Resistance

The textural characteristics of products are crucial for fulfilling the acceptance of the consumers. During mastication, the brain processes the food's physical features and evaluates its texture. The sensation of food texture is important in influencing consumers' liking and preference for a food product [58].

Analyzing the values from the chart in Figure 3, it can be observed that the evolution of the values required for the crushing force of carob pasta is similar.

The differences were observed for the maximum values of crushing force, with the highest value being recorded for the sample with the lowest amount of carob powder added—P2CP with 2% of carob powder. The lowest value of the crushing force was recorded for the sample containing 6% carob powder—P6CP—compared to the control sample, where the crushing force was lower.

Regarding the pasta with 6% added carob powder, the elastoplastic curve remains constant for a longer period of time and the deformation of the carob pasta reaches 2.12 mm. After exceeding the flow area, the distinctive curves develop a new ascending tendency, the gradient being approximately identical.

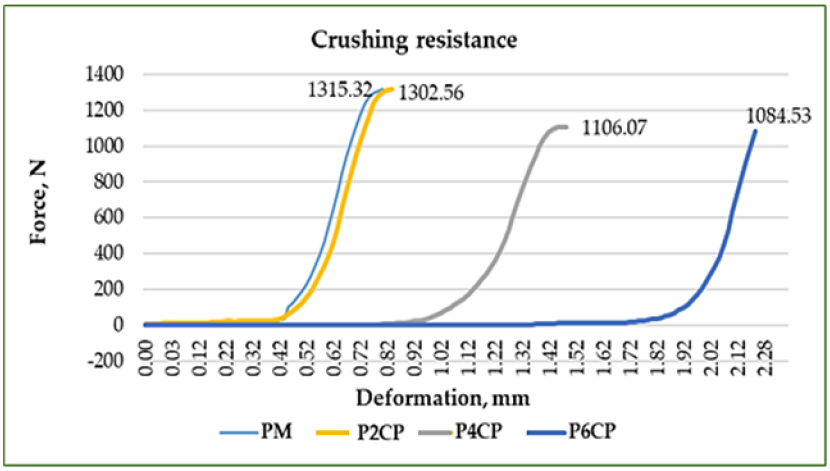

Figure 3. Crushing resistance of pasta samples with the addition of carob powder. PM: control sample; P2CP: sample with 2% (*w*/*w*) carob powder; P4CP: sample with 4% (*w*/*w*) carob powder; P6CP: sample with 6% (*w*/*w*) carob powder.

Some researchers [58] used seedless carob flour for obtaining pastry filling and observed that the major content of carob flour added to the samples' composition resulted in an increase in consistency and firmness, a fact that can be compared to the results of the crushing test from the current study, where the greatest deformation of samples was observed for the highest addition of carob powder—2.12 mm for samples with 6% carob powder addition. Additionally, a linear decrease in pasta cutting force was observed as the proportion of carob fiber increased. The same findings regarding the decreasing of crushing resistance from 1315.32 N in control to 1084.53 N in P6CP were observed in our study and can be attributed to the increase in the fiber content in the sample by 6% carob. The fiber content increased from 2.00 to 2.12% (Table 3) with the addition of carob powder in the pasta manufacturing recipe.

Research conducted by Biernacka et al. [27] showed that the cutting force required for carob flour pasta decreased while the content of carob flour in samples increased. In line with the current research, it can be stated that the high fiber content contributes to a weaker structure of the products due to the addition of fiber in the starch structure.

3.4. Sensory Analysis

Figure 4 illustrates the sensory characteristics in terms of overall acceptability, consistency, attractiveness, aftertaste, appearance, color, flavor, taste and texture of pasta samples with different percentages of carob flour.

It can be observed that the pasta with a medium carob powder, P4CP, with the addition of 4% of carob powder was the most preferred due to its attributes, with a mean value of 8.62 $\pm$ 3.8 out of 9 points. This indicates that the consumer prefers a small amount of carob powder in pasta. The addition of a higher amount of carob powder was rejected; samples with 6% carob flour were undervalued by panelists. Based on texture, taste, color and consistency, sample P4CP was the most appreciated, being ranked with the highest value out of a possible 9 points. This was very close to sample P6CP, which had flavor, color and texture scoring 8 $\pm$ 7.3 points. Samples with 6% carob powder addition received the lowest scores due to the affected attractiveness and appearance. The sensory analysis is an important parameter to evaluate the adaptation of several pulses' capacity in fresh pasta and if the addition of novel constituents in the structure of products influences the properties of final products and consumers' perception.

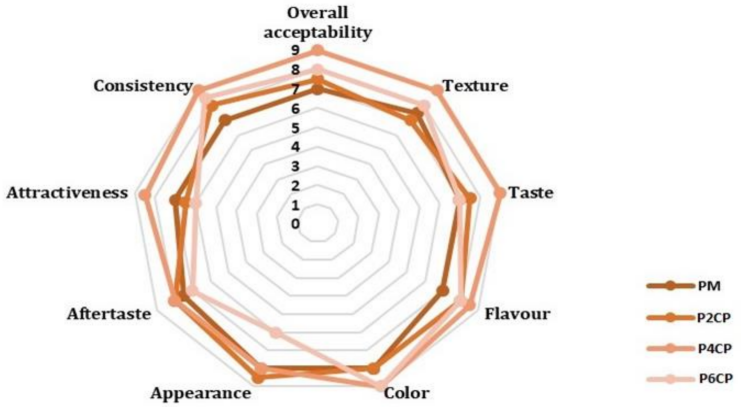

Figure 4. Sensory analysis score of pasta samples with the addition of carob powder. PM: control sample; P2CP: sample with 2% (*w*/*w*) carob powder; P4CP: sample with 4% (*w*/*w*) carob powder; P6CP: sample with 6% (*w*/*w*) carob powder.

It has been demonstrated that the addition of gluten-free flour to a pasta recipe alters the gluten network and reduces the overall structure of the pasta, resulting in a negative effect on sensory properties [53,55]. Furthermore, it may result in increased solid substance losses from pasta in cooking water. Darker color was observed when pasta was supplemented with 10% carob fruit which negatively influenced consumers' perceptions [41]. Consumers rejected products with excessively high concentrations due to their dark color and bitter taste [58]. According to Dulger Altiner, D. and Hallac, S., 2020, the highest values in terms of sensory analysis were determined in the 20% carob flour addition to pasta [41].

4. Conclusions

The addition of 2%, 4% and 6% (*w*/*w*) carob flour to pasta recipes resulted in an increase in total polyphenol content. A more significant increase in the individual polyphenolic content is observed from the concentration of 4% carob flour. At a concentration of 2%, compared to the control sample, the increase was very small. It was also found that all the individual polyphenolic compounds analyzed are present in the P6CP sample, which contains the highest concentration of carob. Up to 6% carob flour in durum wheat flour strongly influenced the color of pasta. However, due to the difficulties that could arise during pasta processing, it is not recommended to use a concentration of carob flour greater than 6% in the pasta recipe because the consistency of the pasta dough will no longer be achieved based on the recipe used. In order to use a larger amount of carob flour, the basic recipe must be modified and more flour added, as the pasta dough becomes softer as the concentration of carob flour increases. Carob flour affects the kneading, modeling and drying processes due to a reduction in the elasticity of the dough caused by a reduction in the gluten content. The sample with a medium carob flour addition (4%) was the most appreciated for its properties, with an average score of 8.62 out of 9 points. At a lower concentration (2%), the color was much more intense compared to the sample without carob flour, but the taste was not very permeating. At a concentration of 6% carob flour, the color of the pasta was very intense and the taste was very carob. The results of the physico-chemical analyses carried out on all the pasta types were within the limits set by the standards.

Regarding the recommended amount of carob flour to be used in the pasta technological process, correlating the results of the physico-chemical properties, dough rheological properties, compression resistance and sensory analysis, a concentration of 4% is the most recommended. It can be concluded that carob flour has enhanced health-promoting properties and could be a useful additive for the production of pasta from common wheat.

In order to improve the functionality of carob pasta, future studies will be considered to supplement the fiber intake, but also to improve the sensory properties by adding natural

aromatic compounds. The synergism created by the active principles of the ingredients will also be another direction for future research.

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References

1. Kaur, G.; Sharma, S.; Nagi, H.P.S.; Ranote, P.S. Enrichment of pasta with different plant proteins. *J. Food Sci. Technol.* **2013**, *50*, 1000–1005. [CrossRef]
2. Fuad, T.; Prabhasankar, P. Role of Ingredients in Pasta Product Quality: A Review on Recent Developments. *Crit. Rev. Food Sci. Nutr.* **2010**, *50*, 787–798. [CrossRef]
3. IPO. 2019. Available online: https://internationalpasta.org/annual-report/ (accessed on 5 March 2023).
4. Falciano, A.; Sorrentino, A.; Masi, P.; Di Pierro, P. Development of Functional Pizza Base Enriched with Jujube (Ziziphus jujuba) Powder. *Foods* **2022**, *11*, 1458. [CrossRef] [PubMed]
5. Statista Global Consumer Survey. Available online: https://www.statista.com/chart/19776/gcs-pasta-consumption/ (accessed on 6 October 2020).
6. Montalbano, A.; Tesoriere, L.; Diana, P.; Barraja, P.; Carbone, A.; Spano, V.; Parrino, B.; Attanzio, A.; Livrea, M.A.; Cascioferro, S.; et al. Quality characteristics and in vitro digestibility study of barley flour enriched ditalini pasta. *LWT* **2016**, *72*, 223–228. [CrossRef]
7. Abdel-Aal, E.-S.M.; Young, J.C.; Rabalski, I. Anthocyanin Composition in Black, Blue, Pink, Purple, and Red Cereal Grains. *J. Agric. Food Chem.* **2006**, *54*, 4696–4704. [CrossRef]
8. Spinelli, S.; Padalino, L.; Costa, C.; Del Nobile, M.A.; Conte, A. Food by-products to fortified pasta: A new approach for optimization. *J. Clean. Prod.* **2019**, *215*, 985–991. [CrossRef]
9. Fares, C.; Menga, V. Effects of toasting on the carbohydrate profile and antioxidant properties of chickpea (*Cicer arietinum* L.) flour added to durum wheat pasta. *Food Chem.* **2012**, *131*, 1140–1148. [CrossRef]
10. Lorusso, A.; Verni, M.; Montemurro, M.; Coda, R.; Gobbetti, M.; Rizzello, C.G. Use of fermented quinoa flour for pasta making and evaluation of the technological and nutritional features. *LWT* **2017**, *78*, 215–221. [CrossRef]
11. Rizzello, C.G.; Verni, M.; Koivula, H.; Montemurro, M.; Seppa, L.; Kemell, M.; Katina, K.; Coda, R.; Gobbetti, M. Influence of fermented faba bean flour on the nutritional, technological and sensory quality of fortified pasta. *Food Funct.* **2017**, *8*, 860–871. [CrossRef]
12. Boroski, M.; de Aguiar, A.C.; Boeing, J.S.; Rotta, E.M.; Wibby, C.L.; Bonafé, E.G.; de Souza, N.E.; Visentainer, J.V. Enhancement of pasta antioxidant activity with oregano and carrot leaf. *Food Chem.* **2011**, *125*, 696–700. [CrossRef]
13. Teterycz, D.; Sobota, A.; Zarzycki, P.; Latoch, A. Legume flour as a natural colouring component in pasta production. *J. Food Sci. Technol.* **2020**, *57*, 301–309. [CrossRef] [PubMed]
14. Testa, M.; Malandrino, O.; Santini, C.; Supino, S. Chapter 6—Nutraceutical and functional value of carob-based products The LBG Sicilia Srl Case Study. In *Case Studies on the Business of Nutraceuticals, Functional and Super Foods*; Woodhead Publishing Series in Consumer Science & Strategy Market; Woodhead Publishing: Sawston, UK, 2023; pp. 107–120, ISBN 9780128214084.
15. Ortega, N.R.; Marcia, A.; Romero, M.; Reguant, J.; Motilva, M.J. Matrix composition effect on the digestibility of carob flour phenols by an in-vitro digestion model. *Food Chem.* **2011**, *124*, 65–71. [CrossRef]
16. Azab, A. Carob (*Ceratonia siliqua*): Health, medicine and chemistry. *Eur. Chem. Bull.* **2017**, *610*, 456–469. [CrossRef]
17. Boublenza, I.; Lazouni, H.A.; Ghaffari, L.; Ruiz, K.; Fabiano-Tixier, A.S.; Chemat, F. Influence of Roasting on Sensory, Antioxidant, Aromas, and Physicochemical Properties of Carob Pod Powder (*Ceratonia siliqua* L.). *J. Food Qual.* **2017**, *2017*, 1–10. [CrossRef]
18. Papaefstathiou, E.; Agapiou, A.; Giannopoulos, S.; Kokkinofta, R. Nutritional characterization of carobs and traditional carob products. *Food Sci. Nutr.* **2018**, *6*, 2151–2161. [CrossRef]
19. Tsatsaragkou, K.; Yiannopoulos, S.; Kontogiorgi, A.; Poulli, E.; Krokida, M.; Mandala, L. Effect of Carob Flour Addition on the Rheological Properties of Gluten-Free Breads. *Food Bioproc. Tech.* **2014**, *7*, 868–876. [CrossRef]

20. Ayaz, F.A.; Torun, H.; Glew, R.H.; Bak, Z.D.; Chuang, L.T.; Presley, J.M.; Andrews, R. Nutrient content of carob pod (*Ceratonia siliqua* L.) flour prepared commercially and domestically. *Plant Foods Hum. Nutr.* **2009**, *64*, 286–292. [CrossRef]
21. Bengoechea, C.; Romero, A.; Villanueva, A.; Moreno, G.; Alaiz, M.; Millán, F.; Puppo, M.C. Composition and structure of carob (*Ceratonia siliqua* L.) germ proteins. *Food Chem.* **2008**, *107*, 675–683. [CrossRef]
22. Biner, B.; Gubbuk, H.; Karhan, M.; Aksu, M.; Pekmezci, M. Sugar profiles of the pods of cultivated and wild types of carob bean (*Ceratonia siliqua* L.) in Turkey. *Food Chem.* **2007**, *100*, 1453–1455. [CrossRef]
23. Durazzo, A.; Turfani, V.; Narducci, V.; Azzini, E.; Maiani, G.; Carcea, M. Nutritional characterisation and bioactive components of commercial carobs flours. *Food Chem.* **2014**, *153*, 109–113. [CrossRef]
24. Kumazawa, S.H.K.; Taniguchi, M.A.S.A.T.; Suzuki, Y.A.S.; Shimura, M.A.S.; Kwon, M.I.U.N.K.; Nakayama, T.S.N. Antioxidant activity of polyphenols in carob pods. *J. Agricult. Food Chem.* **2002**, *50*, 373–377. [CrossRef] [PubMed]
25. Germec, M.; Turhan, I.; Karhan, M.; Demirci, A. Ethanol production via repeated-batch fermentation from carob pod extract by using Saccharomyces cevisiae in biofilm reactor. *Fuel* **2015**, *161*, 304–311. [CrossRef]
26. Haddarah, A.; Ismail, A.; Bassal, A.; Hamieh, T.; Ioannou, I.; Ghoul, M. Morphological and chemical variability of Lebanese carob varieties. *Eur. Sci. J.* **2013**, *9*, 353–369.
27. Biernacka, B.; Dziki, D.; Gawlik-Dziki, U.; Roozy, R.; Siastala, M. Physical, sensorial, and antioxidant properties of common wheat pasta enriched with carob fiber. *LWT* **2017**, *77*, 186–192. [CrossRef]
28. Seczyk, L.; Swieca, M.; Gawlik-Dziki, U. Effect of carob (*Ceratonia siliqua* L.) flour on the antioxidant potential, nutritional quality, and sensory characteristics of fortified durum wheat pasta. *Food Chem.* **2016**, *194*, 637–642. [CrossRef] [PubMed]
29. Gaita, C.; Alexa, E.; Moigradean, D.; Conforti, F.; Poiana, M.A. Designing of high value-added pasta formulas by incorporation of grape pomace skins. *Rom. Biotechnol. Lett.* **2020**, *25*, 1607–1614. [CrossRef]
30. Gruber, W.; Sarkar, A. Chapter 8—Durum Wheat Milling. In *American Associate of Cereal Chemists International, Durum Wheat*, 2nd ed.; Sissons, M., Abecassis, J., Marchylo, B., Carcea, M., Eds.; AACC International Press: Washington, DC, USA, 2012; pp. 139–159.
31. Hellin, P.; Escarnot, E.; Mingeot, D.; Gofflot, S.; Sinnaeve, G.; Lateur, M.; Godin, B. Multiyear evaluation of the agronomical and technological properties of a panel of spelt varieties under different cropping environments. *J. Cereal Sci.* **2023**, *109*, 103615. [CrossRef]
32. Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Meth. Enzymol.* **1999**, *299*, 152–178.
33. Abdel-Hameed, E.S.; Bazaid, S.A.; Salman, M.S. Characterization of the phytochemical constituents of Taif rose and its antioxidant and anticancer activities. *Biomed Res. Int.* **2013**, *2013*, 1–13. [CrossRef]
34. Rios, M.B.; Iriondo-DeHond, A.; Iriondo-DeHond, M.; Herrera, T.; Velasco, D.; Gomez-Alonso, S.; Callejo, M.J.; Dolores del Castillo, M. Effect of Coffee Cascara Dietary Fiber on the Physicochemical, Nutritional and Sensory Properties of a Gluten-Free Bread Formulation. *Molecules* **2020**, *25*, 1358. [CrossRef]
35. ISO 712:2009 Cereals and Cereal Products–Determination of Moisture Content–Reference Method. Available online: https://www.sis.se/api/document/preview/911751/ (accessed on 15 December 2021).
36. Heeger, A.; Kosinska-Cagnazzo, A.; Cantergiani, E.; Andlauer, W. Bioactives of coffee cherry pulp and its utilisation for production of Cascara beverage. *Food Chem.* **2017**, *221*, 969–975. [CrossRef] [PubMed]
37. Calculator de Calorii—Numararea Caloriilor—Klorii. Available online: https://klorii.ro/ (accessed on 8 March 2023).
38. Yazar, G.; Duvarci, O.; Tavman, S.; Kokini, J.L. Non-linear rheological behavior of gluten-free flour doughs and correlations of LAOS parameters with gluten-free bread properties. *J. Cereal Sci.* **2017**, *74*, 28–36. [CrossRef]
39. Dube, N.M.; Xu, F.; Zhao, R. The efficacy of sorghum flour addition on dough rheological properties and bread quality: A short review. *Grain Oil Sci. Technol.* **2022**, *4*, 164–171.
40. Sibanda, T.; Ncube, T.; Ngoromani, N. Rheological properties and bread making quality of white grain sorghum-wheat flour composites. *Int. Food Sci. Nutr. Eng.* **2015**, *5*, 176–182.
41. Arribas, C.; Cabellos, B.; Cuadrado, C.; Guillamón, E.; Pedrosa, M.M. Cooking Effect on the Bioactive Compounds, Texture, and Color Properties of Cold-Extruded Rice/Bean-Based Pasta Supplemented with Whole Carob Fruit. *Foods* **2020**, *9*, 415. [CrossRef]
42. Bchir, B.; Karoui, R.; Danthine, S.; Blecker, C.; Besbes, S.; Attia, H. Date, Apple, and Pear By-Products as Functional Ingredients in Pasta: Cooking Quality Attributes and Physicochemical, Rheological, and Sensorial Properties. *Foods* **2022**, *11*, 1393. [CrossRef] [PubMed]
43. Mandala, I.; Polaki, A.; Yanniotis, S. Influence of frozen storage on bread enriched with different ingredients. *J. Food Eng.* **2009**, *92*, 137–145. [CrossRef]
44. Gopalakrishnan, J.; Menon, R.; Padmaja, G.; Sajeev, M.S.; Moorthy, S.N. Nutritional and Functional Characteristics of Protein-Fortified Pasta from Sweet Potato. *Food Sci. Nutr.* **2011**, *2*, 944–955. [CrossRef]
45. Zhu, F.; Li, J. Physicochemical and sensory properties of fresh noodles fortified with ground linseed (Linum usitassimum). *LWT* **2019**, *101*, 847–853. [CrossRef]
46. Makris, D.P.; Kefalas, P. Carob pods (*Ceratonia siliqua* L.) As a source of polyphenolic antioxidants. *Food Technol. Biotechnol.* **2004**, *42*, 105–108.
47. Issaoui, M.; Flamini, G.; Delgado, A. Sustainability Opportunities for Mediterranean Food Products through New Formulations Based on Carob Flour (*Ceratonia siliqua* L.). *Sustainability* **2021**, *13*, 8026. [CrossRef]

48. Aydin, S.; Ozdemir, Y. Development and Characterization of Carob Flour Based Functional Spread for Increasing Use as Nutritious Snack for Children. *J. Food Qual.* **2017**, *2017*, 1–7. [CrossRef]
49. Sebecic, B.; Vedrina-Dragojevic, I.; Vitali, D.; Hecimovic, M.; Dragicevic, I. Raw Materials in Fibre Enriched Biscuits Production as Source of Total Phenols. *Agric. Conspec. Sci.* **2007**, *72*, 265–270.
50. Bastida, S.; Sánchez-Muniz, F.J.; Olivero, R.; Pérez-Olleros, L.; Ruiz-Roso, B.; Jiménez-Colmenero, F. Antioxidant activity of Carob fruit extracts in cooked pork meat systems during chilled and frozen storage. *Food Chem.* **2009**, *116*, 748–754. [CrossRef]
51. Bissar, S.; Ozcan, M.M. Determination of quality parameters and gluten free macaron production from carob fruit and sorghum. *Int. J. Gastron. Food Sci.* **2022**, *27*, 100460. [CrossRef]
52. Brassesco, M.E.; Brandao, T.R.S.; Silva, C.L.M.; Pintado, M. Carob bean (*Ceratonia siliqua* L.): A new perspective for functional food. *Trends Food Sci. Technol.* **2021**, *114*, 310–322. [CrossRef]
53. Tolve, R.; Pasini, G.; Vignale, F.; Favati, F.; Simonato, B. Effect of Grape Pomace Addition on the Technological, Sensory, and Nutritional Properties of Durum Wheat Pasta. *Foods* **2020**, *9*, 354. [CrossRef]
54. Frühbauerova, M.; Cervenka, L.; Hajek, T.; Pouzar, M.; Palarcík, J. Bioaccessibility of phenolics from carob (*Ceratonia siliqua* L.) pod powder prepared by cryogenic and vibratory grinding. *Food Chemistry* **2022**, *377*, 131968. [CrossRef]
55. Benković, M.; Bosiljkov, T.; Semić, A.; Ježek, D.; Srečec, S. Influence of Carob Flour and Carob Bean Gum on Rheological Properties of Cocoa and Carob Pastry Fillings. *Foods* **2019**, *8*, 66. [CrossRef]
56. Rayas-Duarte, P.; Mock, C.M.; Satterlee, L.D. Quality of spaghetti containing buckwheat, amaranth, and lupin flours. *Cereal Chem.* **1996**, *73*, 381–387.
57. Marinelli, V.; Padalino, L.; Nardiello, D.; Del Nobile, M.A.; Conte, A. New approach to enrich pasta with polyphenols from grape marc. *J. Chem.* **2015**, *2015*, 1–8. [CrossRef]
58. Chen, J.; Rosenthal, A. Modifying food texture. In *Volume 1: Novel Ingredients and Processing Techniques*; Woodhead Publishing: Cambridge, UK, 2015; pp. 3–22.

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THE IMPACT OF THE INFUSION METHOD OF CHOKEBERRY POWDER IN WHITE TEA

Mirabela Ioana Lupu[1], Cristina Maria Canja[1]✉, Alina Maier[1], Vasile Padureanu[1], Geronimo Raducu Branescu[1] and Ana-Maria Manolica [1,2]

[1]Faculty of Food and Tourism, Transilvania University of Brasov, Romania
[2] Faculty of Biotechnology, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
✉*canja.c@unitbv.ro*

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ABSTRACT

The objective of this study was to evaluate the impact of chokeberry powder on the properties of white tea, with the aim of developing a new product with high bioactive compounds. Initially, the influence of the infusion technique on the characteristics of white tea was investigated. A comparative analysis was conducted between hot and cold infusion methods for both plain white tea and white tea with chokeberry powder at varying concentrations (0.6%, 0.8%, 1%). In the course of the experimental research, the total polyphenol content, antioxidant activity, viscosity, and pH of the tea were evaluated. A sensory analysis was also conducted on all varieties of tea presented in this paper. The experimental research demonstrated that the incorporation of chokeberry powder has a beneficial impact on the properties of white tea, resulting in a notable increase in polyphenol content (1% chokeberry powder in a cold infusion resulted in a total phenol concentration of 12.7 ± 0.6 mg GAE/100 mL). This enhancement in polyphenol content was accompanied by an increase in the beneficial effects of tea on the human body. Additionally, the sensory analysis indicated that the 1% chokeberry powder cold infusion (TC3) was the most preferred tea among consumers. This suggests that the TC3 sample exhibited the optimal balance between bioactive properties and consumer acceptance.

1.Introduction

Tea is a beverage with a global popularity that transcends geographical boundaries. It is consumed in a variety of forms, including hot and cold. The most commonly consumed teas (green tea, black tea, oolong tea, white tea, and yellow tea) are derived from the leaves and buds of the *Camellia sinensis (L.)* plant, belonging to the *Theaceae* family. These teas are distinguished by variations in harvesting, processing, and the degree of oxidation of the polyphenols present in fresh tea leaves (Sharangi, 2009; Unachukwu et al., 2010). As stated by some authors (Damiani et al., 2014), the two most prevalent grades, Silver Needle and White Peony, are commercially accessible. However, there are numerous other varieties with diverse trade names.

The Silver Needle (Bai Hao Yinzhen in its traditional name) is manufactured exclusively from the unopened buds of the plant, without any leaves. As its name indicates, it is characterized by a silver-white hue and comprises long, thin needles. The buds are initially sun-dried on sieves or drying mats for a period of approximately 24 hours, representing the initial phase of the processing method. This is then followed by baking over a low fire until the buds are fully desiccated. The final product exhibits a subtle flavor profile and a pale-yellow

hue. The White Peony (traditional name Bai Mudan) is manufactured from the bud and one or two leaves derived from the plant's vegetative apex. The processing of the leaves involves two simple phases: withering (sun drying/airing/low temperature) and basket drying. The resulting tea exhibits a light golden-brown color and a pleasant roasted aroma. The flowers and leaves of the *Camellia sinensis* plant contain a variety of bioactive substances, including nutrients (carbohydrates, proteins, and minerals), alkaloids (methylxanthines), and phenolic compounds (phenolic acids, flavonoids, and tannins) (Sharma et al., 2021). White tea (WT) is described as "tea for one year, medicine for three years, and treasure for seven years" (Cheng et al., 2021), which indicates the increasing nutritional and functional values of aging WT. White tea (WT) has been demonstrated to exert beneficial effects on human health, including the prevention and treatment of diabetes, cancer, bacterial infections, and obesity (Olcha et al., 2022).

The chokeberry, also known as Aronia, is a *Rosaceae* shrub that is native to North America and was introduced to Europe approximately a century ago (Chrubasik et al., 2010; Sidor et al., 2019). Black chokeberries are a rich source of polyphenols, which contribute to their high biological activity. The polyphenols present in these berries include anthocyanins, flavonols, flavanols, proanthocyanidins, and phenolic acids (Tolić et al., 2017). A previous study conducted by Kokotkiewicz identified the phenolic chemicals present in chokeberry fruit, including procyanidins (0.7-5.2%) and anthocyanins (0.6-2.0%), as the primary classes with therapeutic characteristics (Kokotkiewicz et al., 2010). The high concentrations of anthocyanin and polyphenols may exert a protective effect against the development of cancer, diabetes, gastrointestinal disease, and cardiovascular disease (Burdejova et al., 2020). Given the popularity of chokeberry and its associated health benefits, a variety of preparations have been developed on an industrial scale, including concentrated extracts, juices, and Aronia food products. One particularly valuable product is fruit powder, which is obtained by drying and grinding the fruit. The primary advantage of fruit drying is the extension of the product's shelf life.

Given the numerous advantages offered by both white tea and chokeberry powder, our objective is to enhance the biological activity of white tea by developing a new product. This will be achieved by incorporating chokeberry powder into white tea, thereby investigating the impact of this addition on the tea's properties, including pH, acidity, and viscosity. Additionally, a sensory analysis of the teas was conducted.

2. Materials and methods

2.1. Materials

2.1.1. Raw materials

The white tea utilized in the experimental research is a dry white peony tea procured from the supermarket and produced by the Basilur company. The chokeberry powder is an organic powder from the Aronia Charlottenburg company, procured from a commercial establishment. The nutritional information, as indicated on the product label, is as follows: The energy value is 1013kJ/242kcal, with fat comprising 2.5g-6.0g, of which saturated fatty acids account for 0.4g. Carbohydrates constitute 40-8.0g, with sugars amounting to 1.8-2.8g. Fiber is present in quantities of 70.0-80.0g, while proteins are found in amounts of 5.0-10.0g. Salt is present in quantities of less than 0.01g.

2.1.2. Sample preparation

In the experimental research, two infusion methods were employed in accordance with the methodology illustrated in Figure 1: hot infusion and cold infusion. Hot tea infusions were prepared by adding 100 mL of water at 70° C to 2.5 g of white tea and allowing the infusion to proceed for seven minutes (Damiani et al., 2014). The cold infusion was prepared by adding 100 mL of water at room temperature to 2.5 g of white tea and allowing the infusion to stand at room temperature (20–25 °C) for two hours, agitating continuously using a magnetic stirrer (IKA, RET basic, Germany) (Damiani et

al., 2014). The same recipes were used for both types of infusion, with the same chokeberry powder concentrations. The concentrations of the chokeberry powder were 0%, 0.6%, 0.8%, and 1%. Prior to analysis, all samples were filtered through a 0.45 µm membrane filter.

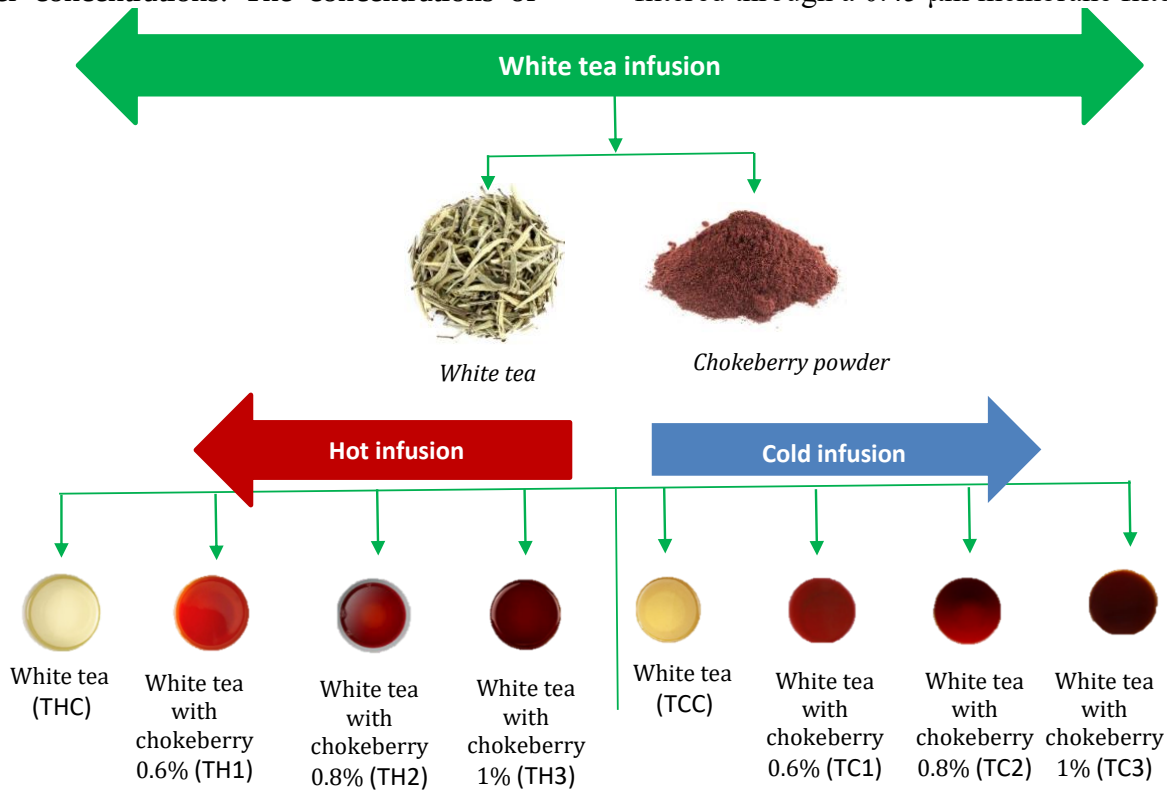

Figure 1. Diagram of the infusion process of white tea with chokeberry powder. THC -White tea hot infusion; TH1- White tea with chokeberry powder 0.6% hot infusion; TH2- White tea with chokeberry powder 0.8% hot infusion; TH3- White tea with chokeberry powder 1% hot infusion; TCC -White tea cold infusion; TC1 - White tea with chokeberry powder 0.6% cold infusion; TC2 - White tea with chokeberry powder 0.8% cold infusion; TC3 - White tea with chokeberry powder 1 % cold infusion.

2.2. Methods

2.2.1. Total polyphenolic content

Using gallic acid as a reference, the total polyphenolic content (TPC) was calculated using the Folin-Ciocalteu spectrophotometric method and represented as milligrams of gallic acid equivalents per milliliter (µg GAE/mL) (Singleton et al., 1999). Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), 1.25 mL, was applied to a 0.5 mL sample after being diluted 1:10 (v/v) with distilled water. 1 mL of Na_2CO_3 60 g/L was added after the mixture had been incubated for 5 minutes at room temperature. The sample absorbance at 750 nm was measured using a UV-VIS spectrophotometer (Specord 205, Analytik Jena Inc., Jena, Germany) after 30 min of incubation at 50 °C. Gallic acid was used as the standard, with concentrations ranging from 5 to 25 mg GAE/mL, to create the calibration curve.

2.2.2. Antioxidant activity (ABTS assay)

The ABTS assay measurement of the different teas was performed according to the method described by (Damiani et al., 2014; Re et al., 1999). A 7.0 mmol/L aqueous ABTS [2,20-azinobis-(3-ethylbenzothiazoline-6)-diammonium salt] solution was mixed in a 0.9:0.1 ratio with a 2.45 mmol/L aqueous solution of potassium persulfate as an oxidizing agent to quantify the radical cation (ABTS•+).

Prior to use, the combination was stored at room temperature in the dark for 12–16 hours. To get an absorbance at 734 nm ranging from 0.6 to 0.8, the ABTS•+ solution stock was 80-fold diluted with water before to use. 0.025 mL of previously diluted tea, adequately diluted Trolox (6-hydroxy-2,5,7,8-tetramethylchro- man-2-carboxylic acid) standard ethanolic solution, or water as a control were added to 2.475 mL of this ABTS•+ solution, and the mixture was then added. The samples were kept at room temperature in the dark for two hours, and the samples' absorbance was measured against water at 734 nm. The following equation was used to compute inhibition percentage values (Equation 1):

$$Inhibition\ of A_{734}(\%) = \left(1 - \frac{A_C}{A_0}\right) \cdot 100 \quad (1)$$

where Ac is an absorbance of the samples, A0 is an absorbance of the control. Antioxidant activity was expressed as mmol/L Trolox Equivalents (TE) using the linear regression value obtained from the Trolox calibration curve.

2.2.3. pH measurement

The pH was measured using the electrochemical method (Webster, 2003) with a potentiometer (Consort C1010, Consort, Turnhout, Belgium).

2.2.4. Viscosity

The viscosity (η [cP]) was determined using the Brookfield rheometer (Brookfield Engineering Laboratories, Inc., Middleborough, MA, USA). The torque required to drive a disk, immersed at a predetermined depth, in the liquid whose viscosity is to be determined, into a rotational movement with a certain speed, will be measured. It is established that a thin layer of liquid adheres to the surface of a body in contact with a liquid due to adhesion forces. This layer, known as the boundary layer, moves as a single entity with the surface to which it has adhered, thus exhibiting the same velocity. The molecular cohesion forces exerted by the molecules in the layer under consideration will cause the molecules in the neighboring layer to move at a slower speed due to the sliding between the layers of molecules.

The accuracy of the Brookfield viscometer was evaluated using a standard-viscosity liquid provided by the Cannon Instrument Company (State College, PA). The results demonstrated that the viscosity measurement data shear rate of 300 s^{-1} from the Brookfield viscometer was found to be within 5% of the standard viscosity value. The torque output became unstable at the shear rate of 300 s^{-1}, which equaled 40 rpm. This instability is likely due to secondary flow caused by centrifugal force in the gap between the cone and plate geometries (Lee et al., 2012).

2.2.5. Sensory analysis

A total of one hundred evaluators, comprising both male and female participants between the ages of 21 and 60, were invited to score the tea samples on a 5-point hedonic scale, ranging from least favored (1, "dislike very much") to most liked (5, "like very much"). This was done in order to ascertain consumer preference for the tested tea samples. Infusions were evaluated considering the criterion of color, brightness, clarity, astringency, aroma and bitterness. The panelists received eight distinct tea samples in 150-ml tea cups, each bearing a randomly assigned number. Tea samples were interspersed with opportunities for participants to rinse their palates with water. The sensory analysis was conducted at room temperature in a facility equipped with LED lighting. The evaluators were selected from the personnel and student body at Transilvania University of Brașov.

2.2.6. Statistical analysis

Each tea sample was tested in triplicate, and the results of the three separate tests were averaged to yield a single value for each sample. All data are presented as the mean of the three replicates, followed by the standard deviation (SD). The significance of mean differences was assessed by one-way ANOVA. Tukey's test ($p \leq 0.05$) was used to compare mean differences. The correlation analysis was employed to estimate the degree of correlation between the data sets, while the regression analysis was utilized to model the relationship between the

predictor variables and the investigated parameters (JASP Team, 2023).

3.Results and discussions

As illustrated in Figure 1, eight distinct tea varieties were obtained through both cold and hot infusion methods. These teas were then subjected to comprehensive analysis, evaluating their polyphenol content, antioxidant activity, viscosity, and pH. The findings from this experimental research were collated and presented in Table 1.

Table 1. The properties of white tea

Analysis	Hot infusion				Cold infusion			
	THC	**TH1**	**TH2**	**TH3**	**TCC**	**TC1**	**TC2**	**TC3**
TPC [mg/mL]	4.05± 0.02^{a}	8.28± 0.78^{b}	9.11± 0.56^{b}	11.13± 0.27^{bc}	8.25± 0.21^{b}	19.4± 0.09^{c}	21.9± 0.20^{c}	33.4± 0.71^{d}
Antioxidant activity [mmol/L]	14.98± 1.76^{a}	31.27± 1.23^{c}	33.03± 1.02^{c}	36.31± 1.76^{c}	26.3± 1.01^{b}	71.78± 1.98^{d}	74.32± 1.65^{d}	82.45± 1.89^{d}
pH	7.82± 0.76^{a}	7.78± 0.98^{ab}	7.75± 0.02^{b}	7.73± 0.98^{b}	8.08± 0.09^{c}	7.97± 0.97^{d}	7.95± 0.1^{d}	7.92± 0.12^{d}
η [cP]	1.11± 0.97^{a}	1.14± 0.21^{ab}	1.15± 0.05^{ab}	1.21± 0.87^{b}	1.23± 0.11^{b}	1.27± 0.78^{c}	1.31± 0.23^{d}	1.34± 0.55^{d}

THC-White tea hot infusion; TH1-White tea with chokeberry powder 0.6% hot infusion; TH2- White tea with chokeberry powder 0.8% hot infusion; TH3-White tea with chokeberry powder 1% hot infusion; TCC-White tea cold infusion; TC1-White tea with chokeberry powder 0.6% cold infusion; TC2-White tea with chokeberry powder 0.8% cold infusion; TC3-White tea with chokeberry powder 1 % cold infusion; TPC-Total polyphenolic content; η-Viscosity. The results are expressed as the mean value of the three replicates ± the standard deviation (SD). Data with different superscripts reported in the same row are significantly different (one-way ANOVA, $p < 0.05$). Data within a row with the same superscripts are not significantly different (one-way ANOVA, $p > 0.05$).

3.1. Total polyphenolic content

Table 1 presents a summary of the total phenol contents (TPC) of the tea infusions, as determined by Folin-Ciocalteu's reagent. A comparison of the total phenol contents (TPC) of hot and cold teas reveals that the latter consistently exhibits a significantly higher TPC, a phenomenon particularly pronounced in the case of the higher concentration of chokeberry powder TC3, with a TPC of 33.4 ± 0.71 mg GAE/mL. A comparison of the control samples (THC and TCC) revealed that the TPC of white tea obtained by cold extraction (8.25±0.21 mg GAE/mL) was significantly different ($p<0.05$) than that of white tea obtained by hot infusion (4.05±0.02 mg GAE/mL). This suggests that thermal treatment has a significant impact on total phenol content. These values are consistent with those reported in the literature (Damiani et al., 2014; Dasdemir et al., 2023; Perera et al., 2015; Ramalho et al., 2013). The infusion method and fruit concentration have a significant impact on the total phenol content (TPC). Furthermore, an examination of the samples with varying chokeberry powder content revealed a significant increase ($p<0.05$) in total polyphenol (TPC) content in those obtained through both hot and cold infusion methods.

Research on white and green tea infusions reveals that brewing conditions significantly impact the extraction of bioactive compounds and antioxidant capacity. Cold infusion (20-25°C) was found to be more efficient in extracting bioactive compounds compared to

hot infusion (80°C) (de Carvalho Rodrigues et al., 2015). However, brewing at 98°C for 7 minutes yielded optimal antioxidant polyphenols in white tea (Pérez-Burillo et al., 2018). White teas exhibited the highest concentrations of chlorophylls, carotenoids, and total phenolic compounds (Popoviciu & Mălureanu, 2022). Total catechin content varied widely among white and green teas, with some white teas containing comparable levels to green teas (Unachukwu et al., 2010). Particle size also influenced extraction, with milled leaves producing greater antioxidant activity than whole leaves (Castiglioni et al., 2015). Cold brewing for 120 minutes or hot brewing at 90°C resulted in maximum extraction efficiency, particularly for whole, large leaves (Castiglioni et al., 2015).

White teas exhibited the highest concentrations of chlorophylls, carotenoids, and total phenolic compounds (Popoviciu & Mălureanu, 2022).

3.2. Antioxidant activity (ABTS assay)

The antioxidant activity of the tea infusions was evaluated using the ABTS method. As evidenced in Table 1, the ABTS assay results demonstrate that all hot tea infusions exhibit significantly diminished antioxidant activity (14.98-36.31 mmol/L TE) in comparison to cold tea infusions (26.3-82.45 mmol/L TE). The highest antioxidant activity was observed in the case of the cold tea infusion with a 1% chokeberry powder concentration, which exhibited an antioxidant capacity of 82.45 ± 1.89 mmol/L TE. This evolution of antioxidant capacity in cold tea in comparison with hot tea was also observed by other authors (Damiani et al., 2014). Moreover, the antioxidant activity of the samples obtained through hot and cold infusion with an identical chokeberry powder content was found to be significantly different ($p<0.05$).

Research on white tea's antioxidant activity in cold and hot infusions reveals varying results across studies. White tea generally demonstrates high antioxidant capacity, with some studies finding prolonged hot steeping or cold extraction to be most effective (Castiglioni et al., 2015; Hajiaghaalipour et al., 2016). However, one study reported optimal antioxidant activity at 70°C for white tea, decreasing at higher temperatures (Chernousova et al., 2018). Cold extraction (20-25°C) was found to be more efficient in extracting bioactive compounds compared to hot extraction (80°C) in some cases (de Carvalho Rodrigues et al., 2015). Factors influencing antioxidant activity include steeping time, temperature, and particle size, with milled leaves generally yielding higher antioxidant activity than whole leaves (Castiglioni et al., 2015). White tea often exhibits greater antioxidant capacity than black tea and comparable or higher levels than green tea (Chernousova et al., 2018; Hajiaghaalipour et al., 2016). Additionally, some white tea extracts have shown bacteriostatic activity against S. aureus and E. coli (de Carvalho Rodrigues et al., 2015).

3.3. pH measurement

The pH values for both methods fell within the range of 7.73 to 8.08, exhibiting minimal discrepancy. A slight decrease in pH was observed in both types of infusion. The sample TCC exhibited the highest pH value. A reduction in pH was similarly documented by other authors (Lunkes & Hashizume, 2014; S. Zhang et al., 2023). The pH of the white tea hot infusion sample differs significantly from that of the white tea cold infusion sample ($p<0.05$). The hot and cold infusion samples with chokeberry powder do not exhibit a significant difference in pH ($p>0.05$), whereas a significant difference is observed between the hot and cold infusion samples with the same addition of chokeberry powder ($p<0.05$).

pH values of tea infusions were generally mildly acidic, ranging from 3.85 to 6.45 (Kaczmarek, 2004), with white teas showing similar pH levels to other teas, except for highly acidic hibiscus tea (Popoviciu & Mălureanu, 2022).

3.4. Viscosity

In regard to viscosity, it was determined that as the concentration of chokeberry powder increases, the viscosity of the tea for both infusion methods also increase. The lowest viscosity was observed in the case of the hot infusion, with a value of THC, 1.11 ± 0.97 cP. A comparison of the control samples from the hot and cold infusions reveals that the viscosity of the cold tea (TCC: 1.23 ± 0.11) is higher than that of the hot tea (THC: 1.11 ± 0.97 cP). Viscosity decreases as temperature increases. This is due to the fact that the powder particles increase the viscosity of the tea, thus demonstrating that the viscosity of the tea is directly proportional to the concentration of the powder (Pérez et al., 2022). Another explanation is that, as tea heats up, the molecules within the liquid move more quickly, reducing the friction between them, and thus decreasing viscosity. Cold tea, by contrast, will have a slightly higher viscosity than hot tea.

3.5. Correlation of infusion method and chokeberry powder concentration

The statistical analysis of the data indicates that the investigated parameters were affected by two factors: infusion method and chokeberry powder concentration, either independently or in combination. The analysis of variance conducted on the analytical parameters for different infusion methods and chokeberry powder concentrations showed significant differences in total polyphenolic content, antioxidant activity, pH, and viscosity.

It is noteworthy that the correlation coefficient (R) for TPC is 0.935, for pH it is 0.992, and for viscosity it is 0.995. Furthermore, the antioxidant activity also appears to exhibit variability, with a correlation coefficient of 0.958. The adjusted R^2 for the two predictors, infusion method and chokeberry powder concentration, indicates that they can predict 70.8% of the variation in TPC results, 81% of the variation in antioxidant activity results, 96.3% of the variation in pH results, and 97.8% of the variation in viscosity results.

The results of the ANOVA indicate that the model is statistically significant. The predictors introduced into the model, both individually and in combination, exerted a notable influence on the analyzed parameters, as detailed below: The total polyphenolic content was found to be significantly influenced ($p < 0.05$) only by the infusion method, while antioxidant activity, pH, and viscosity were significantly influenced ($p < 0.05$) by both the infusion method and the concentration of chokeberry powder.

3.6. Correlation of the analyzed parameters

Figure 2 illustrates a correlation heat map for Pearson r. Pearson's product-moment correlation coefficient is a measure of the linear relationship between two variables. The correlation analysis enables the estimation of the parameters of the correlation. As can be observed, the heatmap
is symmetric along the diagonal. Furthermore, the color blue represents positive correlation coefficients, while the color red represents negative correlation coefficients. The saturation of colors is indicative of the absolute value of the correlation coefficient. The significant correlations are marked with: *$p < 0.05$ if the correlation is significant at alpha=0.05 level; **$p < 0.01$ if the correlation is significant at alpha=0.01 level and ***$p < 0.001$ if the correlation is significant at alpha=0.001 level.

3.7. Sensory evaluation of tea samples

The success of a novel product or formula is primarily contingent upon consumer demand and acceptability based on sensory perception. To ascertain consumer approval and identify any shortcomings in sensory attributes, a sensory evaluation was conducted in conjunction with an assessment of the product's intrinsic qualities.

The outcomes of the hot and cold infusion processes for all eight samples are presented in Figure 3. The graph demonstrated a positive correlation between color, brightness, clarity, astringency, aroma, and bitterness. The tea with the highest level of appreciation was the one with a 1% concentration of chokeberry powder cold infusion (TC3), which received the

maximum score (5 points) for the majority of the analyzed properties, with the exception of clarity, which received 4 points.

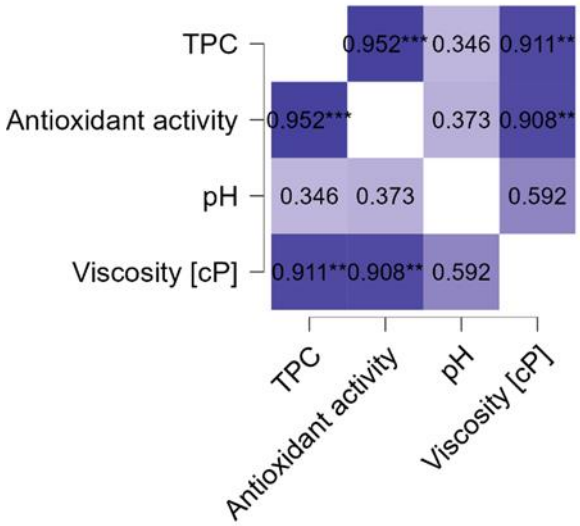

Figure 2. Heatmap for Pearson's r. TPC-Total polyphenolic content

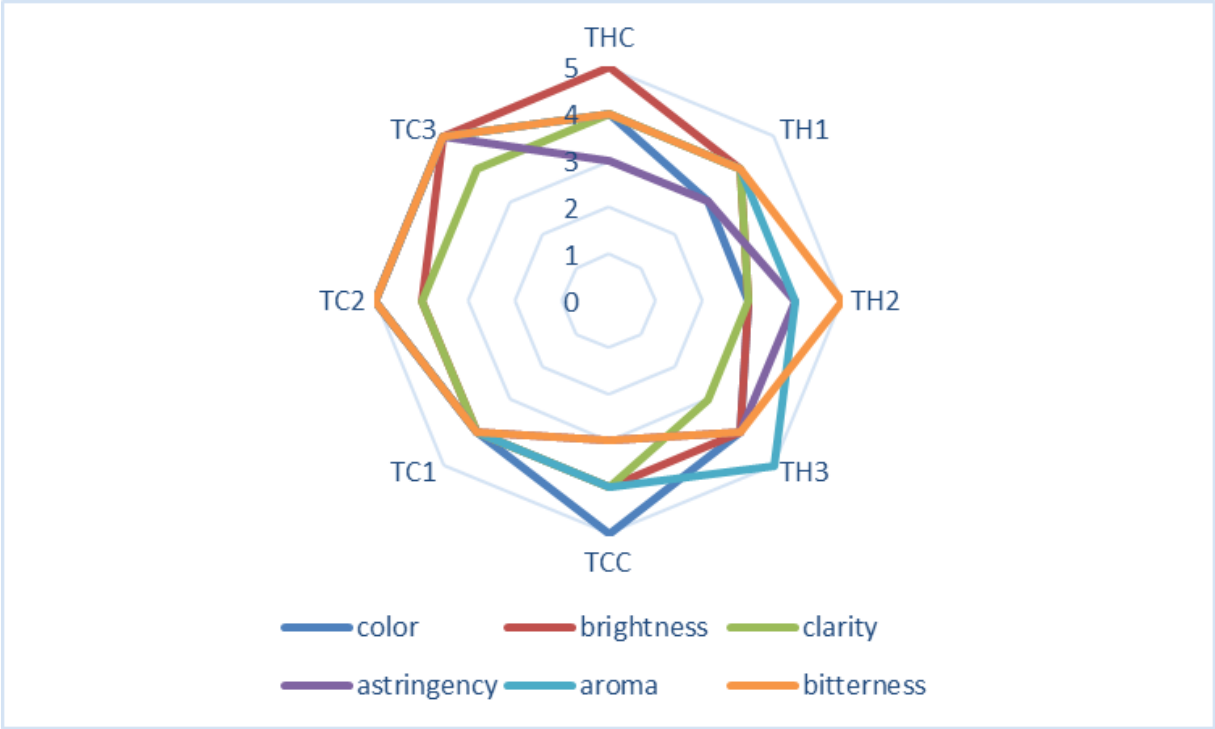

Figure 3. Sensory evaluation of tea samples. THC -White tea hot infusion; TH1- White tea with chokeberry powder 0.6% hot infusion; TH2- White tea with chokeberry powder 0.8% hot infusion; TH3- White tea with chokeberry powder 1% hot infusion; TCC -White tea cold infusion; TC1 - White tea with chokeberry powder 0.6% cold infusion; TC2 - White tea with chokeberry powder 0.8% cold infusion; TC3 - White tea with chokeberry powder 1 % cold infusion

White tea infusions have been studied for their sensory properties and antioxidant content under various brewing conditions. Cold brewing for 120 minutes or hot brewing at 90°C for 7 minutes yielded the highest antioxidant activity, with milled leaves providing greater extraction (Castiglioni et al., 2015). However, whole leaf infusions were preferred in sensory evaluations, particularly for cold-brewed white teas (Castiglioni et al., 2015). Optimal conditions for both antioxidant content and sensory properties were found to be 98°C for 7 minutes (Pérez-Burillo et al., 2018). For Fuding white tea, a 3-minute infusion at 100°C with a 1:50 tea-to-water ratio produced the highest sensory scores (H. Zhang et al., 2017). In cold infusions of Taiwanese teas, consumers could distinguish between unfermented/lightly fermented and heavily/fully fermented teas, with lightly fermented teas preferred for their balanced bitterness, astringency, fresh flavor, and late sweetness (Liu et al., 2021).

4. Conclusion

The findings of the experimental research indicated that cold tea infusion is an effective method for enhancing the active biological properties of tea. A comparison of the total phenol contents (TPC) of hot and cold teas indicates that cold teas consistently have a significantly elevated TPC, most notable in the greater concentration of chokeberry powder TC3, which has a TPC of 33.4 ± 0.71 mg GAE/mL.

It was shown that the application of heat treatment leads to a reduction of these compounds,obtaining a TPC value of 11.13±0.27 mg GAE/mL (TH3) for the highest concentration of aronia powder.

The ABTS assay results indicate that all hot tea infusions display markedly reduced antioxidant activity (14.98-36.31 mmol/L TE) relative to cold tea infusions (26.3-82.45 mmol/L TE). The pH values for both procedures ranged from 7.73 to 8.08, demonstrating negligible variation, with a small reduction in pH noted in both infusion modalities. A comparison of the control samples from the hot and cold infusions indicates that the viscosity of the cold tea (TCC: 1.23 ± 0.11 cP) surpasses that of the hot tea (THC: 1.11 ± 0.97 cP), demonstrating that viscosity diminishes with rising temperature. In the context of sensory analysis, the tea infused with a 1% concentration of chokeberry powder (TC3) achieved the highest score, attaining the maximum rating of 5 points for most of the evaluated attributes.

In conclusion, chokeberry fruits represent a valuable resource for the tea industry, offering multiple health benefits and a distinctive taste and aroma. The exploitation of these fruits can bring advantages from both an economic and a health and sustainability perspective, through the development of a prosperous chokeberry tea sector and the promotion of a healthy and sustainable lifestyle.

5.References

Burdejova, L., Tobolkova, B., & Polovka, M. (2020). Effects of Different Factors on Concentration of Functional Components of Aronia and Saskatoon Berries. *Plant Foods for Human Nutrition*, *75*(1), 83–88. https://doi.org/10.1007/s11130-019-00780-4

Castiglioni, S., Damiani, E., Astolfi, P., & Carloni, P. (2015). Influence of steeping conditions (time, temperature, and particle size) on antioxidant properties and sensory attributes of some white and green teas. *International Journal of Food Sciences and Nutrition*, *66*(5), 491–497. https://doi.org/10.3109/09637486.2015.1042842

Cheng, L., Wang, Y., Zhang, J., Zhu, J., Liu, P., Xu, L., Wei, K., Zhou, H., Peng, L., Zhang, J., Wei, X., & Liu, Z. (2021). Dynamic changes of metabolic profile and taste quality during the long-term aging of Qingzhuan Tea: The impact of storage age. *Food Chemistry*, *359*, 129953. https://doi.org/10.1016/j.foodchem.2021.129953

Chernousova, O. V., Krivtsova, A. I., & Kuchmenko, T. A. (2018). The study of antioxidant activity of white tea.

Proceedings of the Voronezh State University of Engineering Technologies, *80*(1), 133–139. https://doi.org/10.20914/2310-1202-2018-1-133-139

Chrubasik, C., Li, G., & Chrubasik, S. (2010). The clinical effectiveness of chokeberry: a systematic review. *Phytotherapy Research*, *24*(8), 1107–1114. https://doi.org/10.1002/ptr.3226

Damiani, E., Bacchetti, T., Padella, L., Tiano, L., & Carloni, P. (2014). Antioxidant activity of different white teas: Comparison of hot and cold tea infusions. *Journal of Food Composition and Analysis*, *33*(1), 59–66. https://doi.org/10.1016/j.jfca.2013.09.010

Dasdemir, Y., Findik, B. T., Yildiz, H., & Birisci, E. (2023). Blueberry-added black tea: Effects of infusion temperature, drying method, fruit concentration on the iron-polyphenol complex formation, polyphenols profile, antioxidant activity, and sensory properties. *Food Chemistry*, *410*, 135463. https://doi.org/10.1016/j.foodchem.2023.135463

de Carvalho Rodrigues, V., da Silva, M. V., dos Santos, A. R., Zielinski, A. A. F., & Haminiuk, C. W. I. (2015). Evaluation of hot and cold extraction of bioactive compounds in teas. *International Journal of Food Science & Technology*, *50*(9), 2038–2045. https://doi.org/10.1111/ijfs.12858

Hajiaghaalipour, F., Sanusi, J., & Kanthimathi, M. S. (2016). Temperature and Time of Steeping Affect the Antioxidant Properties of White, Green, and Black Tea Infusions. *Journal of Food Science*, *81*(1). https://doi.org/10.1111/1750-3841.13149

Kaczmarek, U. (2004). [PH values and fluoride levels in some tea brands]. *Annales Academiae Medicae Stetinensis*, *50 Suppl 1*, 58–61.

Kokotkiewicz, A., Jaremicz, Z., & Luczkiewicz, M. (2010). A Review of Traditional Use, Biological Activities, and Perspectives for Modern Medicine. *Journal of Medicinal Food*, *13*(2), 255–269. https://doi.org/10.1089/jmf.2009.0062

Lee, D. H., Jung, J. M., Kim, S. Y., Kim, K. T., & Cho, Y. I. (2012). Comparison tests for plasma viscosity measurements. *International Communications in Heat and Mass Transfer*, *39*(10), 1474–1477. https://doi.org/10.1016/j.icheatmasstransfer.2012.10.018

Liu, S.-L., Jaw, Y.-M., Wang, L.-F., Chuang, G. C.-C., Zhuang, Z.-Y., Chen, Y.-S., & Liou, B.-K. (2021). Evaluation of Sensory Quality for Taiwanese Specialty Teas with Cold Infusion Using CATA and Temporal CATA by Taiwanese Consumers. *Foods*, *10*(10), 2344. https://doi.org/10.3390/foods10102344

Lunkes, L. B. F., & Hashizume, L. N. (2014). Evaluation of the pH and titratable acidity of teas commercially available in Brazilian market. *RGO, Rev. Gaúch. Odontol. (Online)*, *62*(no.1 Porto Alegre).

Olcha, P., Winiarska-Mieczan, A., Kwiecień, M., Nowakowski, Ł., Miturski, A., Semczuk, A., Kiczorowska, B., & Gałczyński, K. (2022). Antioxidative, Anti-Inflammatory, Anti-Obesogenic, and Antidiabetic Properties of Tea Polyphenols—The Positive Impact of Regular Tea Consumption as an Element of Prophylaxis and Pharmacotherapy Support in Endometrial Cancer. *International Journal of Molecular Sciences*, *23*(12), 6703. https://doi.org/10.3390/ijms23126703

Perera, G. A. A. R., Amarakoon, A. M. T., Illeperuma, D. C. K., & Muthukumarana, P. K. P. (2015). Effects of raw material on the chemical composition, organoleptic properties, antioxidant activity, physical properties and the yield of instant black tea. *LWT - Food Science and Technology*, *63*(1), 745–750. https://doi.org/10.1016/j.lwt.2015.03.060

Pérez, E., Alviso, D., Manrique, E., & Artana, G. (2022). Estimation of the rheological curve of HPAM solutions from measurements using the Brookfield viscometer. *Journal of Petroleum Science*

and Engineering, *216*, 110793. https://doi.org/10.1016/j.petrol.2022.110793

Pérez-Burillo, S., Giménez, R., Rufián-Henares, J. A., & Pastoriza, S. (2018). Effect of brewing time and temperature on antioxidant capacity and phenols of white tea: Relationship with sensory properties. *Food Chemistry*, *248*, 111–118. https://doi.org/10.1016/j.foodchem.2017.12.056

Popoviciu, D. R., & Mălureanu, R. A. (2022). Comparative quantitative analysis of some classes of bioactive compounds in some tea sortiments and tea like infusions available on the Romanian market. *Annals of The University of Craiova, Biology, Horticulture, Food Products Processing Technology, Environmental Engineering*, *27*(63). https://doi.org/10.52846/bihpt.v27i63.52

Ramalho, S. A., Nigam, N., Oliveira, G. B., de Oliveira, P. A., Silva, T. O. M., dos Santos, A. G. P., & Narain, N. (2013). Effect of infusion time on phenolic compounds and caffeine content in black tea. *Food Research International*, *51*(1), 155–161. https://doi.org/10.1016/j.foodres.2012.11.031

Re, R., Nicoletta, P., Anna, P., Ananth, P., Min, Y., & Catherine, R.-E. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*((9-10)), 1231–1237.

Sharangi, A. B. (2009). Medicinal and therapeutic potentialities of tea (Camellia sinensis L.) – A review. *Food Research International*, *42*(5–6), 529–535. https://doi.org/10.1016/j.foodres.2009.01.007

Sharma, R., Verma, S., & Kumar, D. (2021). Polyphenolics and therapeutic insights in different tissues extract and fractions of Camellia sinensis (L.) Kuntze (Kangra Tea). *Food Bioscience*, *42*, 101164. https://doi.org/10.1016/j.fbio.2021.101164

Sidor, A., Drożdżyńska, A., & Gramza-Michałowska, A. (2019). Black chokeberry (Aronia melanocarpa) and its products as potential health-promoting factors - An overview. *Trends in Food Science & Technology*, *89*, 45–60. https://doi.org/10.1016/j.tifs.2019.05.006

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Meth Enzymol*, *299*, 152–178.

Tolić, M.-T., Krbavčić, I., Vujević, P., Milinović, B., Jurčević, I., & Vahčić, N. (2017). Effects of Weather Conditions on Phenolic Content and Antioxidant Capacity in Juice of Chokeberries (Aronia melanocarpa L.). *Polish Journal of Food and Nutrition Sciences*, *67*(1), 67–74. https://doi.org/10.1515/pjfns-2016-0009

Unachukwu, U. J., Ahmed, S., Kavalier, A., Lyles, J. T., & Kennelly, E. J. (2010). White and Green Teas (Camellia sinensis var. sinensis): Variation in Phenolic, Methylxanthine, and Antioxidant Profiles. *Journal of Food Science*, *75*(6). https://doi.org/10.1111/j.1750-3841.2010.01705.x

Webster, D. (2003). pH – Principles and measurement. In *Encyclopedia of Food Sciences and Nutrition* (pp. 4501–4507). Elsevier. https://doi.org/10.1016/B0-12-227055-X/00913-5

Zhang, H., Li, Y., Lv, Y., Jiang, Y., Pan, J., Duan, Y., Zhu, Y., & Zhang, S. (2017). Influence of brewing conditions on taste components in Fuding white tea infusions. *Journal of the Science of Food and Agriculture*, *97*(9), 2826–2833. https://doi.org/10.1002/jsfa.8111

Zhang, S., Li, Q., Wen, S., Sun, L., Chen, R., Zhang, Z., Cao, J., Lai, Z., Li, Z., Lai, X., Wu, P., Sun, S., & Chen, Z. (2023). Metabolomics reveals the effects of different storage times on the acidity quality and metabolites of large-leaf black tea. *Food Chemistry*, *426*, 136601.

https://doi.org/10.1016/j.foodchem.2023.136601

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Conflicts of Interest:

The authors have no conflict of interest regarding the content of this paper.