Food Chemistry: X 27 (2025) 102412

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x

Advancing sustainable food preservation: Ultrasound and thermosonication as novel approaches to enhance nutritional and bioactive properties of broccoli juice

Seydi Yıkmış[a,*], Melikenur Türkol[b], Dilek Dülger Altıner[c], Aylin Duman Altan[d], Kübra Sağlam[e], Gholamreza Abdi[f,*], Nazlı Tokatlı[g], Güler Çelik[h], Rana Muhammad Aadil[i,*]

[a] Department of Food Technology, Tekirdag Namık Kemal University, 59830 Tekirdag, Türkiye
[b] Nutrition and Dietetics, Faculty of Health Sciences, Halic University, 34060, Istanbul, Türkiye
[c] Department of Gastronomy and Culinary Arts, Tourism Faculty, Kocaeli University, 41400, Kartepe, Kocaeli, Türkiye
[d] Department of Industrial Engineering, Tekirdag Namık Kemal University, 59860 Tekirdağ, Türkiye
[e] Department of Food Processing, Food Technology Program, Istanbul Gelisim Vocational School, Istanbul Gelisim University, 34310, Istanbul, Türkiye
[f] Department of Biotechnology, Persian Gulf Research Institute, Persian Gulf University, Bushehr, 75169, Iran
[g] Department of Computer Engineering, Faculty of Engineering and Natural Sciences, Istanbul Health and Technology University, 34421, Istanbul, Türkiye
[h] The Scientific and Technological Research Council of Turkey, Bursa Test and Analysis Laboratory (Tubitak Butal), Bursa 16190, Türkiye
[i] National Institute of Food Science and Technology, University of Agriculture, Faisalabad 38000, Pakistan

A R T I C L E I N F O

Keywords:
Non-thermal technologies
Sustainable food processing
Ultrasound
Thermosonication
Broccoli juice
Bioactive compounds
Circular food systems

A B S T R A C T

To meet the challenges of sustainability and nutritional quality, innovative food processing technologies are essential. This study investigates the application of ultrasound and thermosonication- emerging non-thermal preservation techniques- to improve the functional properties of broccoli juice. Using Response Surface Methodology (RSM) and Adaptive Neuro-Fuzzy Inference System (ANFIS), the processes were optimised to maximize chlorophyll *a*nd ascorbic acid content. Optimal ultrasound parameters (4 min, 91.1 % amplitude) achieved 12.29 mg/100 mL chlorophyll and 79.38 mg/100 g ascorbic acid. Thermosonication (6.9 min, 66 % amplitude, 40 °C) gave comparable results. Both treatments significantly improved phenolic composition and mineral content, demonstrating superior preservation of bioactive compounds and reduced nutrient degradation compared to traditional methods. The results highlight the potential of ultrasound and thermosonication for sustainable food systems by improving nutritional quality and shelf life, thereby contributing to reduced food waste and environmentally friendly processing. This research provides valuable insights into the integration of non-thermal technologies in the production of functional beverages, supporting the development of circular food systems and sustainable innovation.

1. Introduction

Plant and its parts contain different type of bioactive coimpounds, which has beneficial impact of human and animals (Abduallah et al., 2023; Choudhary & Tahir, 2023; Dalal et al., 2023; Turan et al., 2023) Amogn these, Cruciferous vegetables are recognized as being abundant in bioactive compounds. These include fibre, flavonoids, and other antioxidants (Kurilich et al., 1999; Plumb et al., 1997). Rich in nutrients and bioactive compounds such as vitamins, fibre, phenolics and glucosinolates, broccoli is scientifically known as *Brassica Oleracea* L. *var.* Italica (Miao et al., 2020; Thomas et al., 2018). Due to glucosinolates, a specific phytochemical, diets high in consumption of foods such as broccoli are linked to reduced inflammation and cancer risk (Li et al., 2010; Li et al., 2011; Tse & Eslick, 2014). *Brassica* includes broccoli, cauliflower, cabbage and mustard (Guo et al., 2014). Glucosinolates, compounds containing sulphur and nitrogen, are found in these vegetables. Various glucosinolates have differing pendant groups, resulting in different biological effects (Tang et al., 2013).

As a new technique to reduce the number of processing steps, improve the safety and quality of food and beverages, ultrasound (UT) is gaining popularity (Awad et al., 2012). Ultrasound treatment is widely recognized as an eco friendly, innovative, cost effective, rapidly

* Corresponding authors.
E-mail addresses: syikmis@nku.edu.tr (S. Yıkmış), abdi@pgu.ac.ir (G. Abdi), muhammad.aadil@uaf.edu.pk (R.M. Aadil).

https://doi.org/10.1016/j.fochx.2025.102412
Received 23 January 2025; Received in revised form 18 March 2025; Accepted 23 March 2025
Available online 25 March 2025

developing and scalable technology (Tahir et al., 2024; Wen et al., 2018). Thermosonication (TS), also known as ultrasonic or acoustic heating, involves applying acoustic energy and heat to cell structures. While preserving the nutritional and physiological benefits of vegetables and fruit, this technique facilitates the elimination of pathogens, spoilage micro-organisms and endogenous micro-enzymes (Abdulstar et al., 2023; Urango et al., 2022). The beneficial effects of thermosonication have been demonstrated on freshly squeezed verjuice (*Vitis vinifera* L.) (Çöl et al., 2023), orange juice (Oliveira et al., 2022), hog plum (*Spondias mombin L.*) juice (Oladunjoye et al., 2021), black grape juice (*Vitis vinifera L*) (Yıkmış et al., 2023), parsley juice (Dulger Altıner et al., 2024). A number of researchers have been using thermosonication and ultrasound to preserve and improve the quality of fresh fruit juices (Das et al., 2023; Hoque et al., 2024; Rios-Romero et al., 2021).

RSM combines statistical and mathematical techniques to build models, estimate the impact of multiple independent parameters, and determine the optimal values of these parameters (Breig & Luti, 2021). ANFIS is prominent among neuro-fuzzy techniques, seamlessly combining the structural design and learning abilities typical of artificial neural networks. ANFIS leverages a pre-existing dataset throughout the training process and employs a fuzzy inference system to analyze and interpret the learned weight parameters (Karaboga et al., 2019).

In this study, innovative non-thermal treatments such as ultrasound and thermosonication were used to enhance the functional properties of broccoli juice while preserving its nutritional value. In order to evaluate the nutritional value and functional properties of broccoli juice, chlorophyll, ascorbic acid (vitamin C), total phenolic compounds and mineral contents were examined. These nutrients are directly related to the health benefits of broccoli and are known for their strong antioxidant and bioactive properties. The literature search did not identify any studies on the optimization by RSM of ascorbic acid and total chlorophyll content in broccoli juice. The aim of this research is to optimize the content of chlorophyll and ascorbic acid in broccoli juice by combining RSM and ANFIS with exposure to ultrasound and thermosonication. In addition, the aim of the research is to investigate the phenolic compounds, the total bioactive, and the minerals present in C-BJ, P-BJ, UT-BJ, and TS-BJ.

2. Materials and methods

2.1. Preparation of broccoli juice

Broccoli juice samples were obtained from local producers in Tekirdag, Türkiye. They were stored at 4 °C until the experiments. Stems and mature parts were removed. The broccoli was blended using a Waring commercial blender (model HGB2WTS3, USA). Sample was then filtered, vortex-mixed and labeled as control (C-BJ).

2.2. Ultrasound processing

The broccoli juice samples were subjected to different ultrasonic parameters. Ultrasonic processing was performed on 100 mL of juice using a Hielscher Ultrasonics UP200St unit (Berlin, Germany). The unit was operated at 26 kHz and 200 W power. The procedure included different amplitudes (60 %, 70 %, 80 %, 90 % and 100 %) and processing times in continuous mode (4, 6, 8, 10 and 12 min). An ice water bath was used during the process to avoid overheating. After ultrasonic treatment, the samples were immediately cooled in an ice bath. They were stored at −18 ± 1 °C until further analysis. Once optimised, the samples were referred to as ultrasonically treated broccoli juice (UT-BJ).

2.3. Thermal pasteurization

The collected broccoli juice samples were transferred into 100 mL glass containers and pasteurized at 85 ± 1 °C for 2 min using a water bath system (Wisd model WUC-D06H, Daihan, Wonju, Korea). After pasteurization, the samples were cooled to 20 ± 1 °C at room temperature, labeled as pasteurized broccoli juice (P-BJ), and stored at −20 ± 1 °C until subsequent analysis.

2.4. Thermosonication treatment

For thermosonication (TS) treatment, 100 mL of P-BJ was subjected to ultrasound using a Hielscher Ultrasonics device (model UP200St, Berlin, Germany) operating at 26 kHz and 200 W. Process variables included amplitude settings of 60 %, 70 %, 80 %, 90 % and 100 %; treatment times of 4, 6, 8, 10 and 12 min; and temperatures of 40, 45, 50, 55 and 60 °C, all performed in continuous mode. An ice water cooled bath was used throughout the procedure to prevent overheating. Following the TS treatment, the samples (TS-BJ) were quickly quenched in an ice bath and stored at −18 ± 1 °C before further evaluation.

2.5. Ultrasound modeling procedure for RSM and ANFIS

This study aimed to evaluate the effect of ultrasound processing on the chlorophyll and ascorbic acid content in broccoli. For this purpose, RSM and ANFIS were utilized. In the case of ultrasound treatment, the independent variables were processing time (X_1: 4–12 min) and amplitude (X_2: 60–100 %), while the response variables included total chlorophyll (mg/100 mL) and ascorbic acid (mg/100 g). To optimize the ultrasound process for RSM, the Box-Behnken design was employed using Minitab software (version 19, Minitab Inc., State College, PA, USA). 13 experimental runs were generated based on the 2-factor Box-Behnken design. The results are shown in Table 1. Each experiment was performed in triplicate.

2.6. Thermosonication modeling procedure for RSM and ANFIS

This study used RSM and ANFIS to investigate the influence of thermosonication on broccoli chlorophyll and ascorbic acid levels. Independent variables for the thermosonication process included time (X_1: 4–12 min), amplitude (X_2: 60–100 %), and temperature (X_3: 40–60 °C). The dependent variables were the total chlorophyll (mg/100 mL) and ascorbic acid (mg/100 g). To optimize the thermosonication process using response surface methodology (RSM), Minitab software applied a central composite design (version 19, Minitab Inc., State College, PA, USA). The design generated 20 experimental runs based on three factors, with each experiment run in three times. The results are shown in Table 2. The model's statistical relevance was examined through variance analysis (ANOVA) with a significance level of $p < 0.05$. Model fit was evaluated using lack-of-fit tests, R^2, adjusted R^2, and ANOVA results. Within the time (X_1), amplitude (X_2), and temperature (X_3) ranges, the independent variables were optimised. A numerical optimization approach was applied to maximize the values of ascorbic acid (mg/100 mL) and total chlorophyll (mg/100 mL), which were set as target responses.

2.7. Modeling procedure for ANFIS

ANFIS is an important machine learning method that uses a combination of neural networks and fuzzy logic systems to work effectively on uncertain data sets, especially in complex data analysis and system modeling. In this hybrid model, fuzzy logic rules, usually expressed using sugeno-type rules, define the input variables and the output variable in an if-then format (Tokatlı Demirok et al., 2024). This study examined the effects of ultrasound and thermosonication on chlorophyll (mg/100 mL) and ascorbic acid (mg/100 mL) concentrations in broccoli, using these two parameters as the dependent variables. In the context of the preliminary investigation to ascertain the predictive capacity of ultrasound processing on dependent variables, the proposed ANFIS model operated with two variables: amplitude (%), and time (in minutes). In this second phase of the investigation, the ANFIS model was employed to

Table 1
Ultrasound RSM and ANFIS analysis of dependent and independent variables and ascorbic acid and total chlorophyll results.

Run no. [a]	Independent variables		Dependent variables					
	Time (X_1) (min)	Amplitude (X_2) (%)	Total chlorophyll (mg/100 mL)			AA (mg/100 mL)		
			Experimental data	RSM predicted	ANFIS predicted	Experimental data	RSM predicted	ANFIS predicted
1	4	80	11.76	11.71	11.76	76.250	75.84	76.06
2	8	80	10.96	10.87	10.82	71.280	70.71	70.82
3	8	80	10.65	10.87	10.82	70.150	70.71	70.82
4	8	60	9.67	9.63	9.96	62.860	62.37	63.57
5	8	80	11.02	10.87	10.82	71.580	70.71	70.82
6	10	90	8.92	8.82	8.92	58.130	57.34	57.36
7	12	80	8.85	8.91	8.56	57.252	57.43	57.84
8	8	80	10.82	10.87	10.82	70.356	70.71	70.82
9	8	100	8.76	8.81	8.55	56.948	57.20	56.95
10	6	90	11.40	11.41	11.40	74.118	73.99	75.13
11	6	70	10.54	10.63	10.27	68.480	69.13	68.49
12	8	80	10.88	10.87	10.82	70.752	70.71	70.82
13	10	70	10.43	10.42	10.43	67.386	67.37	67.39
UT-BJ (RSM optimization parameters)	4 min	91.1 % amplitude	12.29			79.38		
Experimental values			12.55			75.63		
% Difference			2.07 %			4.72 %		
UT-BJ (ANFIS optimization parameters)	4 min	91.1 % amplitude	12.50			75.70		
Experimental values			12.55			75.63		
% Difference			0.00 %			0.00 %		

*X_1: time; X_2: amplitude; RSM: Response surface methodology; AA: Ascorbic acid; ANFIS: adaptive neuro-fuzzy inference system; UT-BJ: ultrasound-treated broccoli juice.

Table 2
Results of thermosonication using RSM and ANFIS analysis of dependent and independent parameters, total chlorophyll, and ascorbic acid.

Run no	Independent variables			Dependent variables					
	Time (X1)	Amplitude (X_2)	Tempature (X_3)	Total chlorophyll (mg/100 mL)			AA (mg/100 mL)		
				Experimental data	RSM predicted	ANFIS predicted	Experimental data	RSM predicted	ANFIS predicted
1	6	70	45	9.79	9.69	9.790	72.26	71.61	72.260
2	8	80	50	9.34	9.48	9.494	64.78	65.56	65.429
3	6	90	45	7.34	7.40	7.335	63.91	63.44	63.910
4	10	90	45	7.21	7.19	7.205	59.37	58.79	59.370
5	6	70	55	8.62	8.68	8.790	69.44	70.24	69.680
6	10	70	55	6.88	6.86	6.960	55.42	56.11	54.940
7	6	90	55	9.47	9.41	9.270	70.22	69.61	69.170
8	8	80	50	9.55	9.48	9.494	65.68	65.56	65.429
9	8	80	50	9.52	9.48	9.494	65.37	65.56	65.429
10	10	90	55	7.88	8.02	7.880	56.70	57.58	56.700
11	4	80	50	8.44	8.45	8.440	68.02	68.67	68.020
12	12	80	50	6.53	6.42	6.525	50.59	49.89	50.590
13	8	60	50	7.71	7.68	7.710	61.35	60.71	61.350
14	8	100	50	6.62	6.54	6.740	53.40	54.00	52.920
15	8	80	40	9.24	9.21	9.240	74.61	75.22	74.610
16	8	80	60	9.12	9.04	9.170	73.30	72.64	72.380
17	8	80	50	9.37	9.48	9.494	65.66	65.56	65.429
18	8	80	50	9.64	9.48	9.494	66.38	65.56	65.429
19	8	80	50	9.64	9.48	9.494	65.38	65.56	65.429
20	10	70	45	8.95	9.05	8.950	64.03	64.87	64.03
TS-BJ (RSM optimization parameters)	6.9 min	66 amplitudes	40 °C	10.46			79.27		
Experimental values				10.18			75.33		
% Difference				2.67 %			4.97 %		
TS-BJ (ANFIS optimization parameters)	6.9 min	66 amplitudes	40 °C	10.4			75.7		
Experimental values				10.18			75.33		
% Difference				2.12 %			3.17 %		

*RSM: Response surface methodology AA: ascorbic acid ANFIS: adaptive neuro-fuzzy inference system; TS-BJ: thermosonication-treated broccoli juice.

predict the dependent variables based on the thermosonication process. The model utilized amplitude (%), duration (min), and temperature as input variables for the prediction. The data partitioning method was to allocate 80 % of the data set for ANFIS model building and the remainder for model evaluation.

2.8. RSM and ANFIS comparison

A comparison between ANFIS and RSM models was performed to evaluate their predictions, focusing on metrics such as power, coefficient of determination (R^2), root mean square error (RMSE) and mean absolute percentage error (MAPE). Based on the literature (Table 3), models with MAPE values below 10 %, R^2 values approaching 1, and RMSE values near 0 are considered to demonstrate high accuracy and validity.

2.9. Total chlorophyll

The Hiscox and Israelstam estimation method was used to estimate chlorophyll content. (Hiscox & Israelstam, 2011). Broccoli juice (3 mL) was combined with 3 mL of 80 % (v/v) acetone and passed through Whatman filter paper three times for filtration. The absorbance at 645 nm and 663 nm was observed in the resulting filtrate. The total chlorophyll content was determined according to the formula below:

$$Chlorophylla = (11.85 \times A664) - (1.54 \times A647) \quad (4)$$

$$Chlorophyllb = (21.03 \times A664) - (5.43 \times A647) \quad (5)$$

$$Total\ chlorophyll = (chlorophylla) - (chlorophyllb) \quad (6)$$

2.10. Ascorbic acid

The method described was used to determine the ascorbic acid content (Ordóñez-Santos & Vázquez-Riascos, 2010). Thirty ml of broccoli juice was combined with 0.2 g of oxalic acid ($C_2H_2O_4$). Then, 10 ml of this solution was titrated with 2,6-dichloroindophenol (DPIP) reagent until a stable dark purple color appeared. The concentration of ascorbic acid was calculated from Eq. 7.

$$Ascorbic\ acid\ (mg/100ml) = \mathrm{MVC} \times \mathrm{MDPIP} \times \mathrm{VDPIP}/10 \times \mathrm{VS} \quad (7)$$

M_{VC} = molar mass (g/mol) of ascorbic acid, CDPIP = molar concentration (mol/L) of 2,6-dichloroindophenol (DPIP), VS = sample volume (l), VDPIP = volume of DPIP (l).

2.11. Total phenolic compounds (TPC)

The quantification of the TPC was performed according to the Folin-Ciocalteau method. (Singleton & Rossi, 1965). TPC in broccoli juice samples, 2 ml of each was premixed with 8 ml of 80 % methanol and centrifuged at 4000 rpm for 20 min using a fivefold concentration dilution factor (calculated as 10/2). After centrifugation, add 100 μL Folin-Ciocalteu reagent and 1500 μL deionized water to 50 μL supernatant. This mixture was left to stand for 10 min. At the end of the incubation period, 50 μL of a 20 % sodium carbonate solution (Na_2CO_3) was added. The mixture was then left to react in the dark for 2 h. The absorbance of the broccoli juice samples was measured at 765 nm using a blank for calibration. Results are expressed as mg gallic acid equivalents per 100 ml sample.

2.12. Antioxidant activity

Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, which blocks the DPPH by making certain modifications. (Grajeda-Iglesias et al., 2016). 2.9 mL of a 0.1 mM DPPH solution (dissolved in ethanol) was combined with 0.1 mL of broccoli juice. The mixture was vortexed thoroughly and then left to incubate for 30 min at room temperature, shielded from light. The procedure was as follows: First, 2.9 mL of a 0.1 mM DPPH solution (prepared in ethanol) was combined with 0.1 mL of the broccoli juice sample. This mixture was thoroughly vortexed and then incubated in the dark at room temperature for 30 min. After incubation, the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (SP-UV/VIS-300SRB, Australia). The activity of the DPPH radical quenching system was then calculated according to Eq. 8.

$$DPPH\ radical\ scavenging\ activity\ (\%) = (A_0 - A_1)/A_0\ x\ 100 \quad (8)$$

A0 stands for the absorbance of the control sample, whereas A1 refers to the absorbance measured for the broccoli juice.

2.13. Total flavonoid content (TFC)

In this study, the total flavonoid content (TFC) was assessed using the colorimetric method (Zhishen et al., 1999). To assess the total flavonoid content, begin by adding 4 mL of distilled water and 0.3 mL of a 5 % sodium nitrite ($NaNO_2$) concentration solution to 1 mL of the diluted broccoli juice sample in each test tube. Allow the mixture to incubate for 5 min. Add 0.3 mL of 10 % aluminum chloride ($AlCl_3$) reagent, mix well, and leave for 6 min. Then, add 2 mL of 1 M sodium hydroxide (NaOH) to the mixture and adjust the final volume to 10 mL with distilled water. Measure the absorbance of the samples using a UV-VIS spectrophotometer (SP-UV/VIS-300SRB, Australia) at a wavelength of 510 nm. Perform the analyses in triplicate. The total flavonoid content is reported as catechin equivalents (mg CE/L).

2.14. Analysis of phenolic compounds

Chromatography was conducted on a C-18 (250 × 4.6 mm; 5 μm packing; Agilent) ACE genix column (Portu et al., 2017). The analysis of the polyphenols was carried out on an Agilent 1260 chromatograph equipped with a DAD. The sample flow rate was 0.80 mL/min. The column temperature was fixed at 30 °C. Gradient elution was performed using eluent A and eluent B. Solution A was water containing 0.1 % phosphoric acid. The gradient used was 17 % B (0 min), 15 % (7 min), 20 % (20 min), 24 % (25 min), 30 % (28 min), 40 % (30 min), 50 % (32 min), 70 % (36 min) and 17 % (40 min). The injection volume for phenolics was 10 μL. The study was carried out at 280, 320, and 360 nm. The UV–Vis spectrophotometer was used. The readings are expressed as μg/mL of sample.

2.15. ICP-OES analysis of minerals

Elemental analysis using ICP-OES was performed on the broccoli juice. All chemicals used were of analytical grade and were used without further purification. A solution of 65 % (v/v) nitric acid (HNO_3) from Sigma-Aldrich Corporation, St. Louis, MO, and 30 % (m/v) hydrogen peroxide (H_2O_2) from Merck, Darmstadt, Germany, was used for digestion. A multi-element standard solution from Merck (Darmstadt,

Table 3
The statistical defect values are described in the following section.*

Parameter	Equation	Eqn. Num.	Expected Value	Ref.
Correlation coefficient	$R^2 = 1 - \frac{\sum_{i=1}^{n}(Y_{Predicted} - Y_{Experimental})^2}{\sum_{i=1}^{n}(Y_{Average} - Y_{Experimental})^2}$	(1)	Close to 1	(Onu et al., 2022)
Root means square error	$RMSE = \left(\frac{1}{n}\sum_{i=1}^{n}(Y_{Predicted} - Y_{Experimental})^2\right)^{\frac{1}{2}}$	(2)	Close to 0	(Onu et al., 2022)
Mean absolute percentage error	$MAPE = \left(\frac{1}{n}\sum_{i=1}^{n}\left\lvert\frac{Y_{Experimental} - Y_{Predicted}}{Y_{Experimental}}\right\rvert\right)*100$	(3)	Less than %10	(Lewis, 1982)

* Where n. $Y_{Average}$. $Y_{Predicted}$. and $Y_{Experimental}$ are a number of data points. Are the predicted and experimental scores, respectively.

Germany), catalog number 1.11355, was used for the analysis. Sample digestion was carried out with a microwave digestion system (Anton Paar Multiwave Go Model, USA). A 0.5 mL aliquot of broccoli juice was transferred to Teflon digestion vessels. To each vessel, 6 mL of 65 % nitric acid (HNO_3) and 1 mL of 35 % hydrogen peroxide (H_2O_2) were added. The microwave digestion protocol included: (i) heat at 120 °C for 5 min, then (ii) increase the temperature to 200 °C and maintain for 10 min. After digestion, the vessels were allowed to cool. The resulting colorless solutions were then transferred to polypropylene tubes and diluted with 5 % nitric acid (HNO_3) if necessary, especially for concentrated element analysis. An ICP-OES instrument was used to determine the concentrations of Fe, K, Mg, Ca, Na, and Zn. The following analysis spectra were employed: Fe at 259.9 nm, K at 766.5 nm, Mg at 279.5 nm, Ca at 317.9 nm, Zn at 213.8 nm, and Na at 589.5 nm (Sezer et al., 2019).

2.16. Statistical analysis

All tests were performed in triplicate and the results are presented as mean with standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA), with differences between means assessed using Tukey's her mentally significant deviation (HSD) test at a significant value of $p < 0.05$. Statistical analysis was done using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). Three-dimensional RSM plots were generated using Sigma Plot 12.0 statistical analysis software (Systat Software Inc., San Jose, California, USA).

3. Results and discussion

3.1. Total chlorophyll and ascorbic acid optimization

Experimental and calculated results of ascorbic acid and total chlorophyll for broccoli samples treated with ultrasound at several levels and periods are shown in Table 4. The coefficient of determination (R^2) exceeds 99 %, demonstrating that the model effectively validates the experimental results. The impact of the two independent variables, amplitude and duration, on the total chlorophyll (as described in Eq. 9) and ascorbic acid (as detailed in Eq. 10) properties of broccoli is illustrated in the following equations.

Table 4
Corrected p-values of linear, interaction and quadratic terms of RSM regression factors of total chlorophyll and ascorbic acid answers as a result of ultrasound.

Source	DF	Total chlorophyll (mg/100 mL) F-Value	P-Value	AA (mg/100 mL) F-Value	P-Value
Model	5	147.87	0.000	237.27	0.000
Linear	2	201.71	0.000	323.18	0.000
X_1	1	371.47	0.000	599.26	0.000
X_2	1	31.96	0.001	47.10	0.000
Square	2	123.48	0.000	204.27	0.000
X_1X_1	1	28.38	0.001	56.40	0.000
X_2X_2	1	246.12	0.000	405.03	0.000
2-Way Interaction	1	88.94	0.000	131.46	0.000
X_1X_2	1	88.94	0.000	131.46	0.000
Error	7				
Lack-of-Fit	3	0.48	0.713	1.37	0.373
Pure Error	4				
Total	12				
R^2		99.06 %		99.41 %	
Adj. R^2		98.39 %		98.99 %	
Pred. R^2		96.47 %		96.56 %	

*X_1: time; X_2: amplitude; DF: degrees of freedom; R^2—coefficient of determination; AA: Ascorbic acid; p <0.05, significant differences; p <0.01, very significant differences

$$Total\ chlorophyll = -32,33 + 2,585\ X_1 + 0,8771\ X_2 - 0,03503\ X_1X_1 - 0,004126\ X_2X_2 - 0,02968\ X_1X_2 \quad (9)$$

$$Ascorbic\ acid = -210,8 + 16,67\ X_1 + 5,730\ X2 - 0,2547\ X_1X_1 - 0,02731X_2X_2 - 0,1862\ X_1X_2 \quad (10)$$

According to the equations, the total chlorophyll and ascorbic acids in broccoli juice had a linear positive effect when the variables X_1 (time) and X_2 (amplitude) were increased. This increase in the total chlorophyll content of broccoli juice can be attributed to acoustic and hydro-cavitation, where microbubbles form, expand and collapse abruptly in the solvent. Ultrasound treatment increases the accessibility of solid particles to the solvent, alters the interior architecture of the plant cell, intensifies mass transfer rates and thereby increases extraction efficiency (Anticona et al., 2020; Raghavendra et al., 2022). Pooja et al. (2023) similarly concluded in their research on pea pods that the total chlorophyll value increases with the duration of ultrasound treatment, akin to the findings of our study (Pooja et al., 2023). Dadan et al. (2021) noted in their study that applying ultrasound to basil plants reduced the amount of chlorophyll a (Dadan et al., 2021). The second-order effects of both variables X_1 and X_2 and the interaction between those two negatively affect the total chlorophyll and ascorbic acid levels of broccoli juice. Ascorbate is extremely sensitive too light and too different conditions of processing. Its degradation mechanism can be either aerobic or anaerobic (Vieira et al., 2000). Ultrasound processing subjects the juice to air, and oxidation processes are enhanced as the processing time increases. This is likely to increase the depletion of ascorbic acid. In their study, Ordóñez-Santos et al. (2017) provided evidence that ascorbic acid was significantly degraded in ultrasound-treated gooseberry juice compared to the untreated study sample (Ordóñez-Santos et al., 2017). Nevertheless, our study found that increasing the duration and amplitude of the ultrasound treatment positively affected the ascorbic acid level in broccoli juice. Similarly, in a strawberry juice study, Wang et al. (2019) reported that all ultrasound treatments (4, 8, 12, 16 min) increased ascorbate content in strawberry juice specimens compared to controls (Wang et al., 2019). Increase in ascorbic acid in fruit juices treated with ultrasound may be attributed to the rupturing of the cell wall by mechanical means. As a result, more ascorbic acid is released into the juice, although some of it may be oxidised. This increase in ascorbic acid may also be due to the removal of dissolved oxygen during ultrasound treatment by the cavitation process (Cheng et al., 2007).

Table 1 shows the experimental and predicted responses and results for samples following ultrasound treatment using RSM and ANFIS. RSM modeling was carried out in order to determine the optimal values of the independent variables for their effects on the total ascorbic acid and on the chlorophyll content. ANOVA was applied to evaluate the statistical significance of the model ($p < 0.05$). The model's fitness was further assessed using lack of fit tests, R2, and adjusted R2 coefficients, in conjunction with the ANOVA findings. Time (X1) and amplitude (X2) were identified as the independent variables. The ultrasound parameters were optimised using a numerical optimization approach. The goal was to maximize the values of ascorbic acid (mg/100 mL) and total chlorophyll (mg/100 mL). For the broccoli samples, the ANOVA for the total chlorophyll and the ascorbic acid showed that the model was highly significant ($p < 0.05$) with a high coefficient of determination (R2) (Table 4). This suggests that for total chlorophyll and ascorbic acid in broccoli, there is a strong correlation between the measured and predicted data. According to the results of the ANOVA, the linear effects of parameters X1 and X2 on both responses are significant ($p < 0.05$). This shows that total chlorophyll and ascorbic acid content are strongly influenced by the ultrasound parameters.

As a conclusion of the optimisation, the ultrasound treated for 4 min and with an amplitude of 91.1 % gave the best results for the values of total chlorophyll and ascorbic acid. Therefore, total chlorophyll was determined to be 12.29 mg/100 mL and ascorbic acid was determined to be 79.38 mg/100 mL for UT-BJ by RSM and total chlorophyll was obtained to be 12.5 mg/100 mL and ascorbic acid was determined to be 75.70 mg/100 mL for UT-BJ by ANFIS (Table 1). For both methods, the prediction values from the modeling results were quite consistent with the experimental results. The ANFIS technique yielded a more consistent prediction of the optimization parameters.

Fig. 1 compares the effects of ultrasound amplitude and duration on ascorbic acid and total chlorophyll levels using two different modeling techniques (RSM and ANFIS). RSM results generally show smoother and more predictable surfaces. ANFIS models more flexible and complex relationships, resulting in sharper surface changes. The amplitude and duration of the increase in chlorophyll and ascorbic acid levels can be seen in both the RSM and ANFIS plots. The more intricate predictions ANFIS offers indicate that this model better captures flexible and complex relationships.

The calculated and measured values of total chlorophyll and ascorbic acid for broccoli samples thermosonicated at different times (X_1), amplitudes (X_2) and temperatures (X_3) are given in Table 5. The coefficient of determination (R^2) is over 99 %, indicating that the model is in good agreement with the experimental results. The effects of the three independent variables (time, amplitude, and temperature) on the total chlorophyll (Eq. 11) and ascorbic acid (Eq. 12) properties of broccoli are shown in the equations below.

Table 5
Correlated *p*-values of the linear, interaction and quadratic terms of the regression coefficients obtained by RSM of the responses for total chlorophyll and ascorbic acid as a result of thermosonication.

		Total chlorophyll (mg/100 mL)		AA (mg/100 mL)	
Source	DF	F-Value	P-Value	F-Value	P-Value
Model	9	164.15	0.000	115.13	0.000
Linear	3	112.30	0.000	173.68	0.000
X_1	1	255.82	0.000	454.33	0.000
X_2	1	79.33	0.000	58.00	0.000
X_3	1	1.75	0.215	8.72	0.014
Square	3	269.47	0.000	146.91	0.000
X_1X_1	1	409.20	0.000	79.79	0.000
X_2X_2	1	546.62	0.000	136.33	0.000
X_3X_3	1	12.25	0.006	141.55	0.000
2-Way Interaction	3	110.67	0.000	24.80	0.000
X_1X_2	1	5.74	0.038	2.82	0.124
X_1X_3	1	43.17	0.000	35.08	0.000
X_2X_3	1	283.09	0.000	36.51	0.000
Error	10				
Lack-of-Fit	5	0.92	0.534	4.67	0.058
Pure Error	5				
Total	19				
R^2		99.33 %		99.04 %	
Adj. R^2		98.72 %		98.18 %	
Pred. R^2		97.09 %		93.39 %	

*X_1: time; X_2: amplitude; DF: degrees of freedom; R^2—coefficient of determination; AA: Ascorbic acid; $p < 0.05$, significant differences; $p < 0.01$, very significant differences.

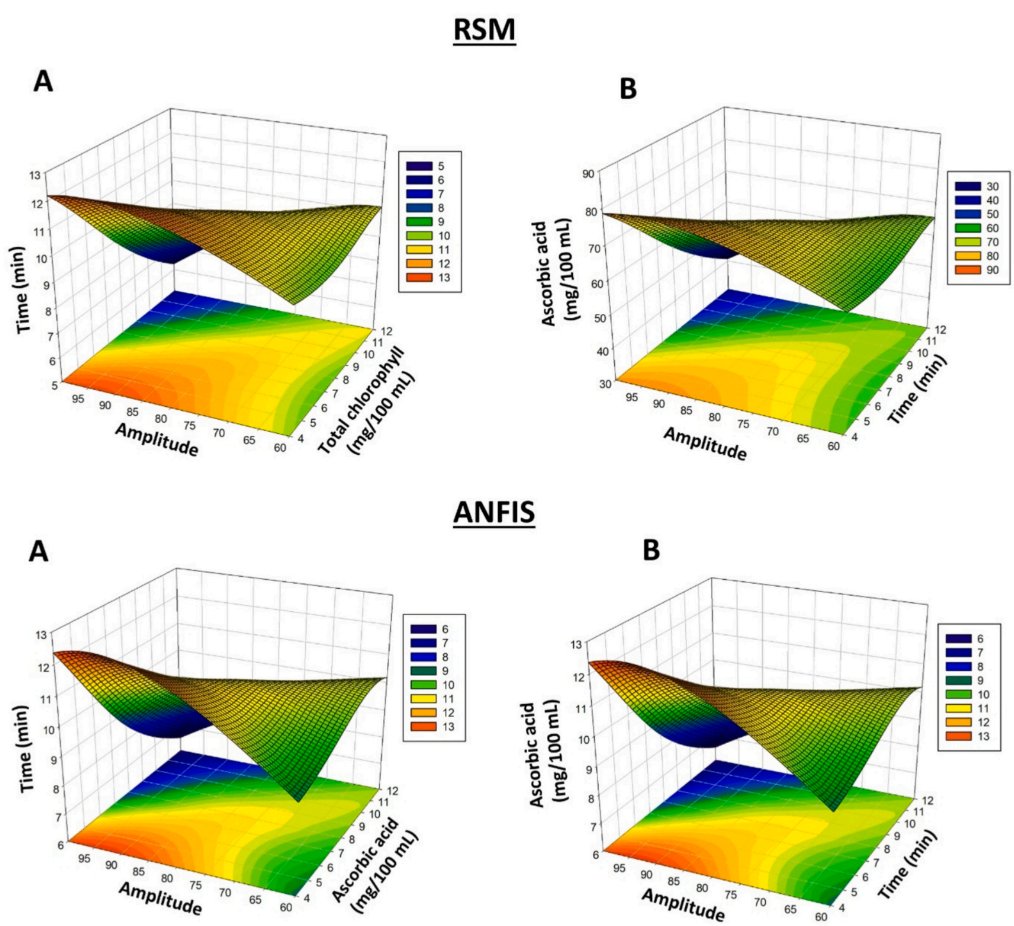

Fig. 1. Response surface plots (3D) for ascorbic acid (mg/100 mL) and total chlorophyll (mg/100 mL) and amounts of ultrasound treated broccoli juice as a function of the significant factors for RSM and ANFIS.

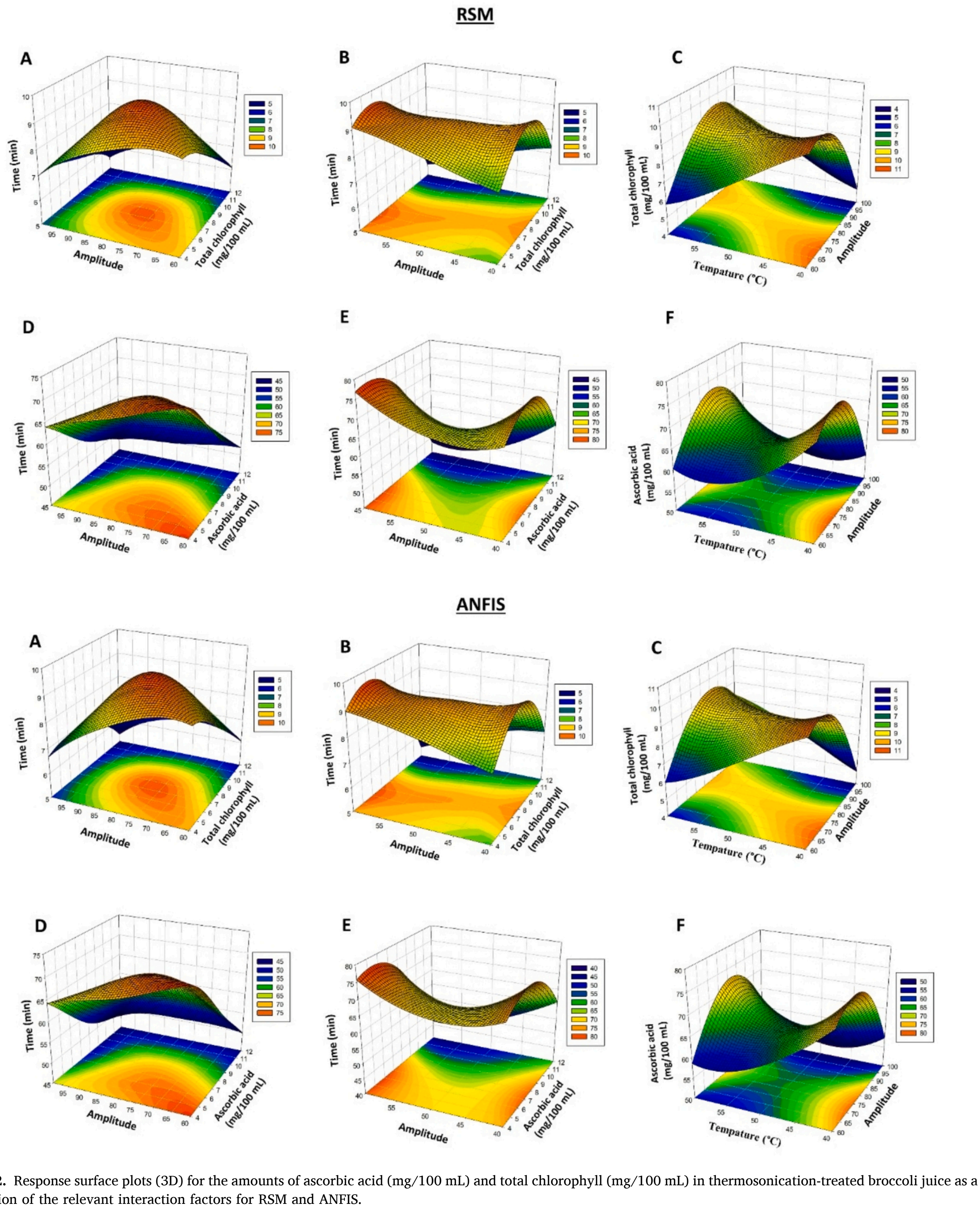

Fig. 2. Response surface plots (3D) for the amounts of ascorbic acid (mg/100 mL) and total chlorophyll (mg/100 mL) in thermosonication-treated broccoli juice as a function of the relevant interaction factors for RSM and ANFIS.

$$Total\ chlorophyll = 11,33 + 2,842\ X_1 + 0,1207\ X_2 - 0,627\ X_3 - 0,12815\ X_1X_1 - 0,005925\ X_2X_2 - 0,00355\ X_3X_3 + 0,00538\ X_1X_2 - 0,02951\ X_1X_3 + 0,015117\ X_2X_3 \quad (11)$$

$$Ascorbic\ acid = 250,5 + 11,07\ X_1 + 1,024\ X_2 - 10,034\ X_3 - 0,3927\ X_1X_1 - 0,02053\ X_2X_2 + 0,08368\ X_3X_3 + 0,0262\ X_1X_2 - 0,1846\ X_1X_3 + 0,03767\ X_2X_3 \quad (12)$$

The results show that increasing X_1 (time) and X_2 (amplitude) has a linear positive effect on total chlorophyll and ascorbic acids in broccoli juice, whereas increasing X_3 (temperature) has a decreasing effect on both. The response of the RSM and ANFIS to the thermosonication treatment was determined experimentally and by prediction. To evaluate the effects of the independent variables on the total chlorophyll and the ascorbic acid, the RSM modeling was carried out and the optimal values were determined. Statistical significance of the model ($p < 0.05$) was assessed using ANOVA. As indicators of model fit, the goodness of fit test, R^2 and adjusted R^2 coefficients were evaluated in conjunction with the ANOVA results. Time (X_1), amplitude (X_2), and temperature (X_3) were determined as the independent variables. A numerical optimization approach was used to optimize the thermosensation parameters. ANOVA for broccoli samples indicated that model was highly significant ($p < 0.05$) with a high coefficient of determination (R^2) for total chlorophyll and ascorbic acid (Table 5). Result is indicative of a high level of correspondence as between experimental data and prediction data for the total chlorophyll and ascorbic acid content of broccoli. This shows that ascorbic acid is strongly influenced by the thermosonication parameters. The linear effects of the parameters X_1 and X_2 on the chlorophyll response are significant ($p < 0.05$), while the linear effect of X_3 on the chlorophyll respond is not so significant ($p > 0.05$). ANOVA analysis showed that the significant linear impact of parameters X_1 and X_2 on the chlorophyll yield was significant ($p < 0.05$), while the linear impact of parameter X_3 on the chlorophyll yield was not significant ($p > 0.05$) in a study conducted by Dulger Altıner et al. (2024) on parsley juice using thermosonication (Dulger Altıner et al., 2024).

As shown in Eq. (12), there was a linear positive effect on ascorbic acid levels as broccoli juice was thermally treated for longer periods. Similarly, Yıkmış et al. (2022) found that increasing the duration of thermosonication had a linear and positive effect on the ascorbic acid value in freshly squeezed pomegranate juice (Yıkmış et al., 2022).

After optimization, the thermosonication treatment of 6.9 min, 66 amplitude and 40 °C temperature yielded the best performance for total chlorophyll and ascorbic acid levels. The total chlorophyll and ascorbic acid content of UT-BJ were 10.46 mg/100 mL and 79.27 mg/100 mL, respectively, using the RSM and 10.4 mg/100 mL and 75.7 mg/100 mL, respectively, using the ANFIS (Table 2). Although these values are quite in agreement with the experiments, the ANFIS method shows less deviation in the estimation of the optimisation parameters.

Fig. 2 compares the RSM and ANFIS approaches to modeling the impact of ultrasound amplitude, duration, and temperature parameters on the total chlorophyll and ascorbic acid levels. Both models demonstrate that increased amplitude and duration positively affect chlorophyll and ascorbic acid levels. While RSM presents more predictable and regular surfaces, the ANFIS model reflects the effects of these parameters with more intricate variations and dynamic surface structures. Particularly when examining the effect of temperature, it is evident that the ANFIS model captures more complex interactions, whereas RSM provides a simpler structure. This highlights ANFIS's ability to better capture nonlinear relationships in multivariate systems by offering a more flexible modeling approach.

3.2. Comparison of RSM and ANFIS models

Although the RSM method seems superior based on R^2, RMSE, and MAPE values according to the ultrasonication experiment results, it does not provide superiority according to the thermosonication experiment results (Table 6). According to the thermosonication experiment results, the RMSE value of the RSM method is closer to 0, while the MAPE value is farther from 0. In this case, the necessity of using more than one parameter in measuring the model performance arises. In addition, although the model performance of the RSM method in the ultrasonic environment seems slightly superior, the ANFIS method is superior under all conditions in optimum parameter estimation since the RSM

Table 7
Phenolic properties of C-BJ, P-BJ, UT-BJ and TS-BJ samples.

Phenolic Compounds (μg/mL)	Samples			
	C-BJ	P-BJ	UT-BJ	TS-BJ
Gallic acid	47.36 ± 2.35[a]	34.39 ± 1.65[b]	49.43 ± 0.47[a]	49.01 ± 0.18[a]
Protocatechuic acid	0.02 ± 0.01[b]	0.02 ± 0.01[b]	0.07 ± 0.00[a]	0.02 ± 0.00[b]
Hydroxybenzoic acid	0.70 ± 0.41[a]	0.67 ± 0.04[a]	0.44 ± 0.02[a]	1.04 ± 0.05[a]
Vanillic acid	0.52 ± 0.01[a]	0.23 ± 0.01[b]	0.53 ± 0.03[a]	0.53 ± 0.05[a]
Gentisic acid	0.15 ± 0.20[ab]	0.43 ± 0.01[a]	0.00 ± 0.00	0.30 ± 0.04[ab]
P-coumaric acid	0.39 ± 0.05[a]	0.43 ± 0.04[a]	0.40 ± 0.04[a]	0.48 ± 0.03[a]
Naringin	3.84 ± 0.61[ab]	2.78 ± 0.04[b]	3.67 ± 0.15[ab]	4.57 ± 0.04[a]
O-coumaric acid	n.d	n.d	n.d	n.d
Coumarin	0.09 ± 0.13[a]	0.13 ± 0.02[a]	0.04 ± 0.00[a]	0.00 ± 0.00
Quercetin	0.95 ± 0.18[ab]	1.29 ± 0.03[a]	1.25 ± 0.04[ab]	0.90 ± 0.02[b]
Trans-cinnamic acid	0.46 ± 0.02[a]	0.18 ± 0.01[b]	0.47 ± 0.05[a]	0.53 ± 0.02[a]
Hesperidin	0.10 ± 0.05[a]	0.11 ± 0.01[a]	0.12 ± 0.00[a]	0.07 ± 0.01[a]
Alirazin	0.71 ± 0.05[a]	0.56 ± 0.02[b]	0.65 ± 0.04[ab]	0.72 ± 0.01[a]
Total	55.29 ± 1.45[a]	41.20 ± 1.63[b]	57.04 ± 0.17[a]	58.14 ± 0.29[a]

*C-BJ: Untreated broccoli juice; P-BJ: thermal pasteurized broccoli juice; UT-BJ: ultrasound-treated broccoli juice; TS-BJ: thermosonication-treated broccoli juice. Mean ± standard deviation. Letters of different types indicate significant differences between results within the lines ($p < 0.05$). n.d.: not detected.

Table 6
Comparison of the RSM and ANFIS performance in predicting AA (mg/100 mL) and total chlorophyll (mg/100 mL) using ultrasound and thermosonication.

Parameters	Ultrosound				Thermosonication			
	Total chlorophyll (mg/100 mL)		AA (mg/100 mL)		Total chlorophyll (mg/100 mL)		AA (mg/100 mL)	
	RSM	ANFIS	RSM	ANFIS	RSM	ANFIS	RSM	ANFIS
R^2	0.9907	0.9744	0.9941	0.9930	0.9933	0.9932	0.9904	0.9959
RMSE	0.0920	0.1701	0.4875	0.5520	0.0915	0.0937	0.7752	0.5488
MAPE	0.0535	0.0973	0.0470	0.0505	0.0092	0.0072	0.0704	0.0315

*RMSE: root mean square error; MAPE: mean absolute percentage error R^2: R-squared; AA: ascorbic acid.

method examines only linear relationships, unlike the ANFIS method (Table 1, Table 2).

3.3. Phenolic compounds

Phenolic compounds, widely present in plant-based foods, have attracted growing interest for their potential health benefits (Amna et al., 2023). Found mainly in fruits, legumes, vegetables, tea and coffee, phenolics significantly contribute to the organoleptic properties of plant foods. Simple phenols (e.g. caffeic acid), flavonoids (the most diverse and widespread class, e.g. catechin, quercetin), stilbenes (e.g. resveratrol), lignans and tannins are the main classes of phenols in foods. Flavonoids constitute the main polyphenols in human diets, with the most common flavonoids being quercetin, catechin, naringenin, cyanidin-glucoside, and daidzein. Phenolic acids may be divided into two classes: benzoic acid derivatives (e.g. gallic acid) and cinnamic acid derivatives (e.g. p-coumaric and ferulic acids) (Alara et al., 2021; Delgado et al., 2019). In this research, the effects of different processing methods P-BJ, UT-BJ, and TS-BJ on the phenolic compound content in broccoli juice were quantitatively evaluated. The results in Table 7 indicate how the concentrations of various phenolic compounds change with different processing methods compared to C-BJ.

In this study, 12 different phenolic compounds, coded as C-BJ, P-BJ, UT-BJ and TS-BJ, were analyzed in broccoli juice samples. Gallic acid has been found to be a major phenolic compound in the juice of broccoli, together with significant amounts of naringin derivatives. Gallic acid concentration in C-BJ sample was 47.36 ± 2.35 μg/mL. However, in the P-BJ sample, this value then decreased by 27.3 % to 34.39 ± 1.65 μg/mL ($p < 0.05$). In the UT-BJ and TS-BJ samples, the gallic acid content increased to 49.43 ± 0.74 μg/mL and 49.01 ± 0.18 μg/mL, correspondingly, keeping the gallic acid levels comparable to the untreated sample. Naringin content then decreased by 27.6 % in the P-BJ sample to a concentration of 2.78 ± 0.40 μg/mL. On the other hand, naringin levels increased to 3.67 ± 0.15 μg/mL and 4.57 ± 0.04 μg/mL in UT-BJ and TS-BJ samples, correspondingly ($p < 0.05$).

For total phenols, the total phenol content in C-BJ sample was found to be 55.29 ± 1.45 μg/mL. The broccoli sample showed a 25.48 % reduction to 41.20 ± 1.65 μg/mL, indicating that heat pasteurization substantially reduced the total phenolic content of broccoli juice. Ultrasonication and thermosonication processes increased the total phenolic amount. For example, in UT-BJ sample, total phenolic content increased by 38.62 % to 57.04 ± 0.17 μg/mL, and in TS-BJ sample, it increased by 1.85 % to 58.14 ± 0.29 μg/mL. This shows that the processing methods led to an increase in the total phenolic substances compared to the unprocessed ones ($p < 0.05$). In addition, thermal pasteurization decreased the number of specific fragments such as protocatechuic acid and hydroxybenzoic acid, while ultrasonication and thermosonication increased these volume amounts. These data reveal that ultrasonication and thermosonication processes are effective methods in preserving and increasing the number of phenolic compounds. Similarly, research by Xie et al. (2022) on grape pomace showed that the yield of anthocyanins obtained by ultrasound extraction was significantly higher compared to non-ultrasound extraction methods, except for peoniflorin pigm-3-glucoside ($P < 0.05$) (Xie et al., 2022). In addition, ultrasound-assisted extraction improved the extraction yield of polyphenols and flavonoids by 19 % and 40 %, respectively, and showed greater antioxidant activity than traditional extraction methods, according to a study by Santos et al. (2023) on blackberry pomace (S. S. Santos et al., 2023). Phenolic compounds such as chlorogenic acid (3.47 ± 0.06 mg/g), kaempferol (1.39 ± 0.02 mg/g), quercetin hydrate (1.92 ± 0.01 mg/g) were reported to be obtained in high amounts by the ultrasound-assisted water extraction method in a study conducted on mulberry leaves (Algan Cavuldak et al., 2019). In contrast, in the present study, the amount of quercetin was found to be 0.95 ± 0.18 μg/ml in the C-BJ sample. Higher amounts were found in other samples such as P-BJ (35.79 % increase) and UT-BJ (31.58 % increase). However, it was observed that the amount of quercetin in the TS-BJ sample was reduced by 5.26 % in comparison with the C-BJ sample.

These findings highlight that the stability of phenolic compounds varies with different processing methods (Geraldi et al., 2021; Mäkilä et al., 2017; Salazar-Orbea et al., 2023). The concentration of phenolic compounds was found to be significantly reduced by thermal pasteurization. In contrast, ultrasonication and thermosonication methods were effective in maintaining and, in some cases, increasing the levels of phenolic compounds. The effectiveness of ultrasonication and thermosonication in improving the extraction and stability of phenolic compounds was demonstrated by the increase in compounds such as gallic acid and naringin. Many studies, like ours, show that individual phenolic compounds were retained in heat-treated fruit juices over control or thermally treated fruit juices (Cheng et al., 2020; Santhirasegaram et al., 2015). This increase in total phenolic content suggests that these processing techniques may be beneficial in the preparation of these functional foods, contributing to the preservation and enhancement of their nutritional value. In conclusion, there is promising potential for the preservation and enrichment of phenolic compounds in broccoli juice production using alternative processing techniques, including sonication and thermal sonication. In order to assess the practical implications of these findings, future studies focusing on the integration of these methods into large-scale production processes and the evaluation of consumer acceptance will be essential.

3.4. Bioactive compounds

Due to its content of antioxidant and anticarcinogenic compounds, broccoli has many health benefits (Mahn & Reyes, 2012). The amount and effectiveness of these compounds may vary depending on processing methods. This study examined the total chlorophyll, antioxidant capacity (DPPH % inhibition), ascorbic acid, and total phenolic content of the C-BJ, P-BJ, UT-BJ, and TS-BJ samples. Food colorants, especially those found on fruits and vegetables, consist of different chemical compounds that have different color, stability, dissolution, and sensitivities to the presence of other substances in the environment (Nowacka et al., 2021).

UT-BJ with 12.55 mg/100 mL has the greatest total chlorophyll content, according to Fig. 3. Ultrasound treatment of hemp (*Cannabis sativa L.*) seed oil was found to have a linear effect on the color index, while extraction time had a quadratic effect, in a study by Esmaeilzadeh Kenari and Dehghan (2020) (Esmaeilzadeh Kenari & Dehghan, 2020). Color is used to assess the aesthetic value and perceived desirability of food. The color intensity and visual appearance of many foods have been improved by ultrasound treatment. This is due to the transient high local temperatures and pressures generated by the acoustic cavity. These can trigger specific reactions that accelerate the generation of certain more stable types of pigment (Kutlu et al., 2022). C-BJ exhibited 9.54 mg/100 mL, TS-BJ showed 10.18 mg/100 mL, and P-BJ had 8.67 mg/100 mL chlorophyll content. These results indicate that ultrasound treatment effectively increases chlorophyll levels, whereas thermal processing leads to chlorophyll degradation. Chlorophyll is highly vulnerable to heat damage (Hu et al., 2020). Thermosonication treatment did not cause chlorophyll degradation compared to untreated broccoli juice. Statistical analyses revealed that P-BJ had significantly lower chlorophyll content than all other groups ($p < 0.01$). Reduced chlorophyll was detected following the thermal treatment administered to broccoli juice.

C-BJ showed the highest antioxidant capacity with 63.085 % DPPH inhibition as shown in Fig. 3B. P-BJ showed 60.07 %, UT-BJ 65.47 %, and TS-BJ 66.275 % inhibition. These results indicate that thermosonication effectively preserved antioxidant capacity, while thermal processing led to a significant reduction. No statistically substantial different was detected between C-BJ and TS-BJ, while P-BJ and UT-BJ showed significantly lower inhibition (*$p < 0.001$). Similarly, also observed an apparent reduction in DPPH in pasteurized parsley juices in comparison to the control sample. The sensitivity of these compounds to

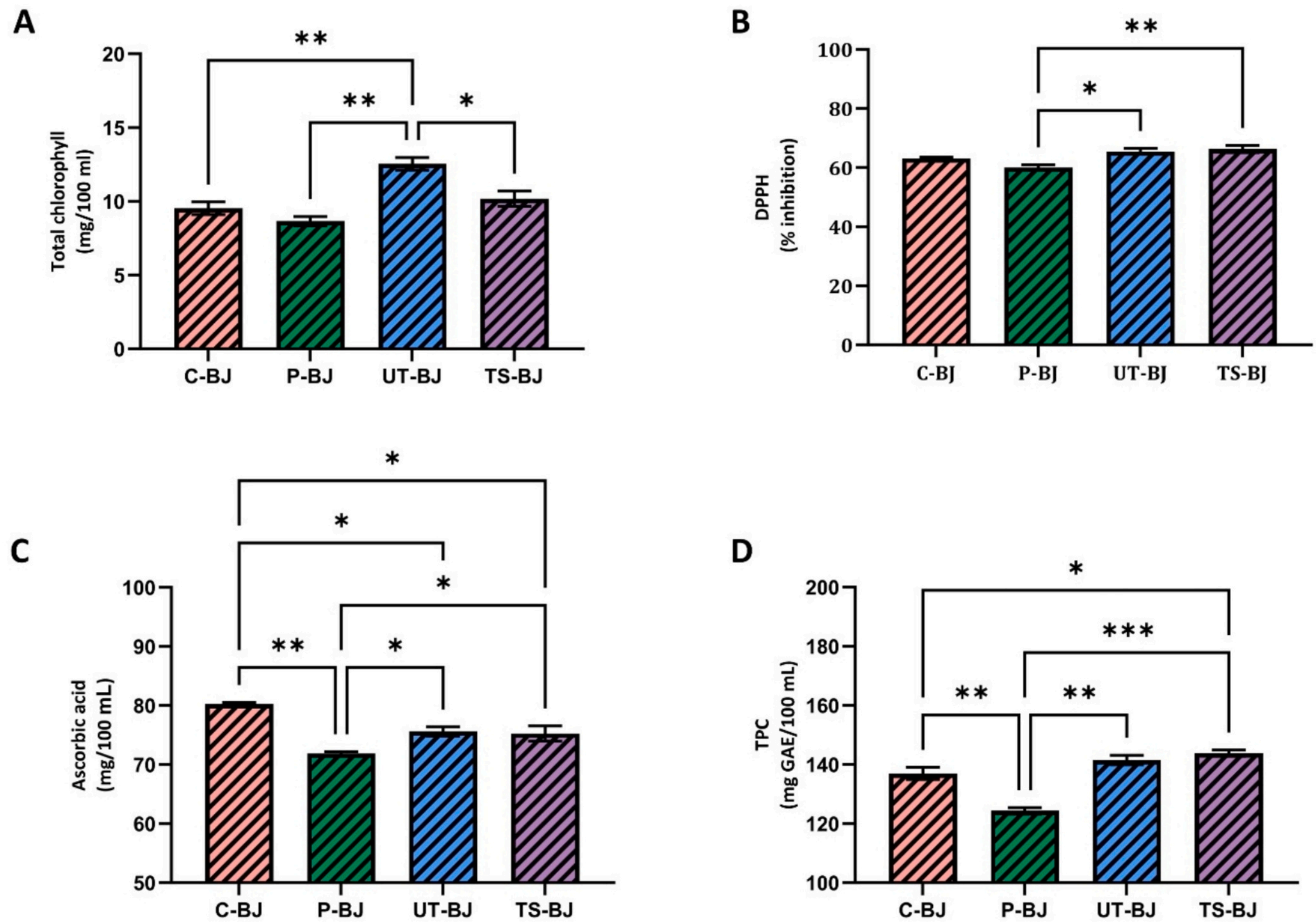

Fig. 3. Total chlorophyll (A), DPPH (B), ascorbic acid (C) and total phenolic content (D) results of broccoli juice samples. C-BJ: Control broccoli juice; P-BJ: Pasteurized treated broccoli juice; UT-BJ: Ultrasound-treated broccoli juice; TS-BJ: Thermosonication-treated broccoli juice n.d: Not detected Letters superimposed indicate statistically significant differences. Analyses used two replicates (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

thermal processing may explain the marked reduction in DPPH levels in pasteurized parsley juice (Dulger Altıner et al., 2024). Sensitivity to heat treatment may have caused the decrease in DPPH in broccoli juice.

According to the data in Fig. 3C, C-BJ exhibited the highest ascorbic acid (vitamin C) content, with 80.29 mg/100 mL. P-BJ and UT-BJ broccoli juices showed ascorbic acid contents of 71.88 mg/100 mL and 75.63 mg/100 mL, respectively. The thermosonication process minimized this loss, with 75.28 mg/100 mL. These findings suggest that thermal processing may lead to ascorbic acid degradation, while ultrasound and thermosonication treatments can help reduce this loss. Statistically, the P-BJ group had significantly lower ascorbic acid levels than all other groups ($p < 0.05$).

As indicated in Fig. 3D, P-BJ and UT-BJ showed significant increases in total phenolic content. P-BJ had 124.36 mg GAE/100 mL, while UT-BJ contained 141.56 mg GAE/100 mL of total phenolic compounds. Similarly, Alongi et al. (2019) reported a 7.5-fold increase of total phenolic content in apple juice after ultrasound treatment when tested against a control group (Alongi et al., 2019). Regardless of the processing time, ultrasound treatment did not affect the physical parameters or antioxidant properties of calcots in the research by Zudaire et al. (2019) on calcots. However, thermal processing was observed to produce a notable change in TPC (Zudaire et al., 2019). Similarly, no significant changes in kiwi juice after exposure to ultrasound compared to fresh fruit juice were reported (Bhutkar et al., 2024). The effect of ultrasound on TPC levels has been documented to be dependent on application duration and temperature (Nayak et al., 2020). Wang et al. (2019) reported an increase in the amount of TPC after ultrasound treatment of strawberry juice, in comparison to the control group (Wang et al., 2019). These conclusions are in line with those of our research. C-BJ and TS-BJ have phenolic compounds of 136.99 mg GAE/100 mL and 143.87 mg GAE/100 mL, respectively. These findings suggest that certain processing methods can enhance the release or extraction of phenolic compounds. Bhutkar et al. (2024) concluded that thermosonication applied to fresh kiwi juice likely slightly improved the total phenolic content, possibly due to the longer processing time. This increase in total phenolics was reported to be related to the liberation of compounds due to cell wall breakdown by cavitation during thermosonication (Bhutkar et al., 2024). In general, the phenolic content is found in a soluble form in the tissues of the plant. High energy ultrasonic processing can cause the breakdown of cell walls and vacuoles in plant tissues, which can potentially lead to an increase in the total phenolic content of fruit juices (Hoque et al., 2024). Improvements in extractability associated with thermosonication and the release of the bound phenolic acid forms due to cavitation effects also contributed to these increases (Nayak et al., 2020). Likewise, Shokri et al. (2022) reported that the total phenolic content of untreated broccoli puree increased significantly after thermosonication treatment (Shokri et al., 2022). In a similar study, significantly higher levels of TPC and DPPH were observed in ultrasonically treated tangerine juice as against pasteurized tangerine juice. This is in line with the results of our study, which found that (Priyadarshini & Priyadarshini, 2018). Bioactive compounds have been further preserved using ultrasonic treatment in food processing, especially in fruit juices. Ultrasound's ability to generate high-impact shock waves and shear forces. This promotes enhanced enzymatic protein degradation (Mintah et al., 2019). Statistical analysis showed that the P-BJ and UT-BJ groups had significantly higher total phenolic content than all other groups ($p < 0.001$).

The frequency range of 20 kHz–100 kHz used during food processing enables the extraction of bioactive substances. Ultrasound application increases the efficiency of extraction of bioactive compounds from different parts of plants, fruits, and vegetables by supporting the interface transfer of molecules (Jadhav et al., 2021). Different fruit and vegetable juice matrices also affect the ultrasound process. Sonication has been reported to increase the TPC content as it generates hydroxyl radicals and increases DPPH activity (Roobab et al., 2023). Similarly, an increase in ascorbic acid and phenolic compound release due to cavitation was reported in black carrot juice treated with ultrasound (US) (at 50 % amplitude for 4, 8 and 12 min) (Hasheminya & Dehghannya, 2022). In our study, after optimization, the thermosonication treatment (at 66 % amplitude for 6.9 min, 40 °C temperature) yielded the best performance for total chlorophyll and ascorbic acid levels.

In a study, the yield of bioactive substances increased from 88 % to 94 % in an optimal 39.5 min 54.8 °C ultrasonication treatment applied to extract bioactive substances from *Croton heliotropiifolius Kunth* leaves (Cheila et al., 2020). Similar results were reported for the ultrasound-assisted extraction of phenolic compounds from mango peels, where the TPC content increased by 630 % on average (Martínez-Ramos et al., 2020). It was reported that higher TPC content can be obtained in juice obtained from Araticum (*Annona crassiflora*) fruit when ultrasound with a processing time longer than 8 min and low power density (<80 W) is applied (Santos et al., 2024). Wang et al. (2021) reported that the loss of ascorbic acid content of fresh mango juice fermented with ultraviolet-assisted ultrasound during storage for 30 days decreased from 89.47 % to 66.46 %. This was explained by the fact that fresh mango juice is sensitive to temperature, pH, sugar, minerals and enzymes, which affects the stability of ascorbic acid during processing and storage (Wang et al., 2021).

Yıkmış et al. (2025) applied a thermosonication process in black carrot juice and reported optimum processing conditions of 48.68 °C, 11.15 min and 82.62 % amplitude in RSM results. Similar to our study, the thermosonication process increased the TPC content (thermosonication group: 414.28 mg/GAE/L, pasteurized group (P-BCJ) 360.905 mg GAE/L). The anthocyanin content was reported as 123.565 mg C_3Ge/L in the pasteurized sample and 129.44 mg C_3Ge/L in the thermosonicated sample and similar to our study, it was determined that pasteurization was not effective in anthocyanin preservation and caused loss (Yıkmış et al., 2025). In the probe ultrasonically assisted extraction of phenolic compounds from *Punica granatum* peel, optimum process conditions at

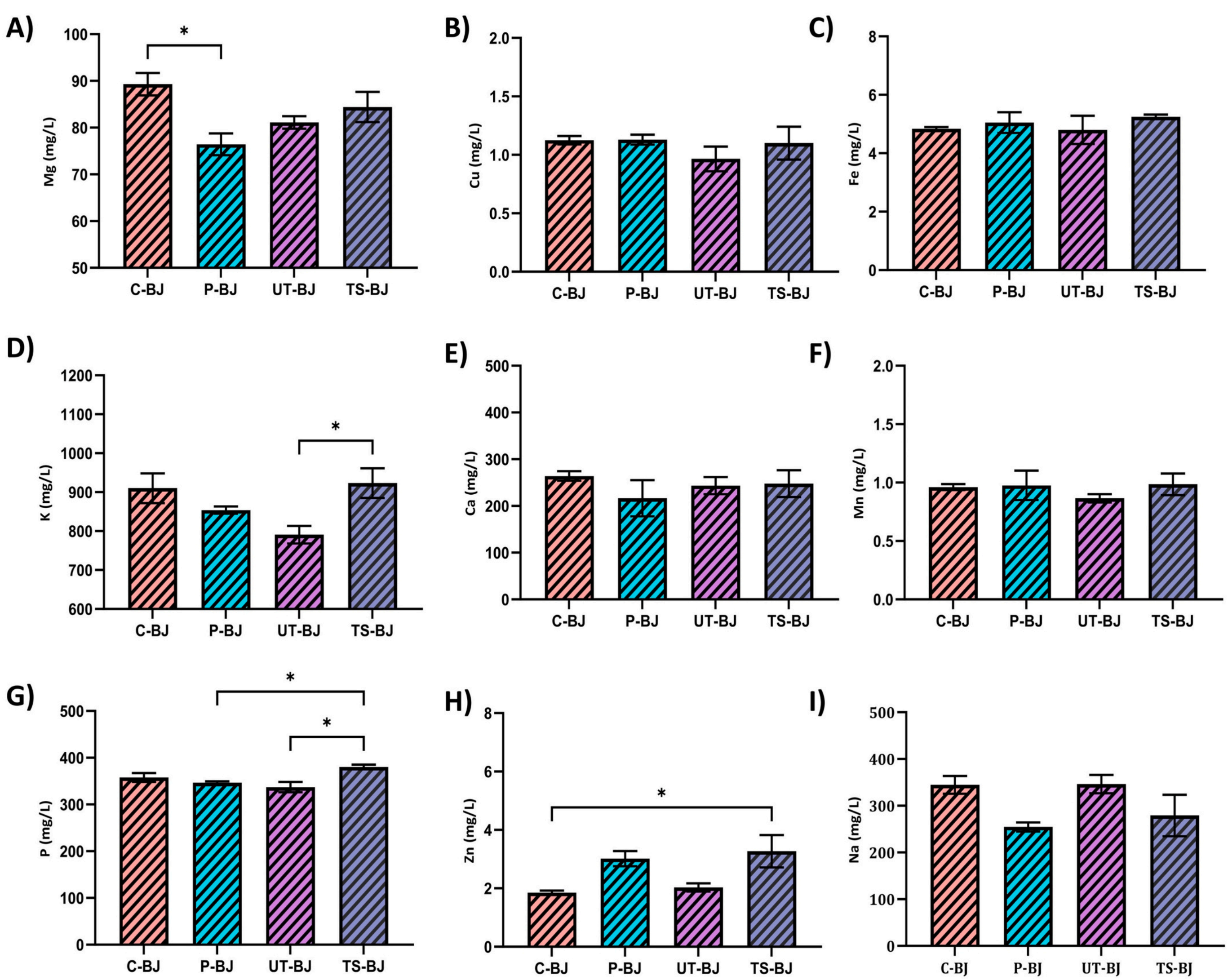

Fig. 4. Results for Mg (A), Cu (B), Fe (C), K (D), Ca (E), Mn (F), P (G), Zn (H), and Na (I) minerals of C-BJ, P-BJ, UT-BJ and TS-BJ samples. C-BJ: Control broccoli juice; P-BJ: Pasteurized-treated broccoli juice; UT-BJ: Ultrasound-treated broccoli juice; TS-BJ: Thermosonication-treated broccoli juice n.d: Not detected. Letters at the top of the bars indicate significant differences ($n = 3 \pm$ SD).

15.12 min extraction time and 30 % amplitude resulted in 42.45 % yield and TPC was reported as 354.67 mg GAE/g (Foujdar et al., 2020).

When ultrasonic energy propagates in the liquid, cavitation bubbles are formed due to pressure changes, collapse of the bubbles with the sound wave, and high temperature-pressure zones are formed. As seen in the results of the studies, this causes the release of bioactive compounds found in plant tissues and cell walls (Foujdar et al., 2020; Santos et al., 2024; Yıkmış et al., 2025). With the increasing cavitation pressure with ultrasound (US) application, phenolic compounds increase by breaking the cell wall, and the increase of free hydroxyl radicals increases antioxidant activity (Roobab et al., 2023). When US application is combined with other techniques, it increases the stability of bioactive compounds as in thermosonication application. In a study with combined treatment, it was reported that the TPC content of Custard apple juice with thermosonication treatment (20 kHz, 67.84 W, 0–40 min at 30 °C) increased from 70.9 to 81.7 mg GAE/g (Nikoo & Gavlighi, 2022). The positive content increase in bioactive compounds in thermosonicated broccoli juice samples is also observed in our study results.

3.5. Analysis of minerals

Broccoli (*Brassica oleracea* L. *var.*) is high in minerals, vitamins and bioactive components, and has high nutritional value among similar vegetable groups (Sardar et al., 2022). Potassium (427–644 mg/100 g), magnesium (66 mg/100 g), calcium (90 mg/100 g) and iron (7.3 mg/100 g) have been reported as the mineral content of broccoli (Mandelová & Totušek, 2006). It has been suggested that vegetable and fruit juices made from broccoli will garner increased interest in the future (Houška et al., 2006). The mineral analysis results of the broccoli juices used in this study are presented in Fig. 4. The C-BJ, P-BJ, UT-BJ and TS-BJ samples were found to contain 9 minerals. Statistically significance differences ($p < 0.05$) were observed between the concentrations of magnesium (Mg), potassium (K), phosphorus (P) and zinc (Zn) in the pasteurized, optimally ultrasound treated and optimally thermosonic treated broccoli juices compared to the untreated control sample. The calcium (Ca), copper (Cu), iron (Fe), manganese (Mn), and sodium (Na) contents of the broccoli juices were found to be similar ($p \geq 0.05$). The most abundant minerals in the broccoli juices were identified as K>P>Na>Ca>Mg>Fe>Zn>Cu>Mn. Potassium (K), sodium (Na), calcium (Ca), and magnesium (Mg) are critically important for the maintenance of bone and dental structure, nerve transmission, enzyme activity, blood pressure regulation, and fluid-electrolyte balance in humans (Shokunbi et al., 2023). According to the analysis results, the highest contents of K (923.385 mg/L), P (380.265 mg/L), Fe (5.25 mg/L), and Zn (3.27 mg/L) were found in the T-BJ sample. Similar to our study, research conducted on different vegetable juices reported the highest results for the macro minerals Na and K, and the micro minerals Zn and Mn (El-Shatanovi et al., 2012). In our study, after thermosonication treatment, the Zn content in broccoli juice increased 1.5 times in the TS-BJ sample (3.27 mg/L) compared to the C-BJ sample (1.85 mg/L). Increases were also observed in the contents of P, Zn, Fe, K and Mn. Similar results were observed in a study of broccoli juice treated with non-thermal technology and pulsed electric fields. The mineral contents of iron (214.2 %), manganese (117.7 %) and zinc (148.4 %) were increased (Sánchez-Vega et al., 2020). It has been shown that thermal treatment of fruit and vegetable juices has a negative effect on the color and flavour of the product and reduces its nutritional properties, so ultrasound applications, a type of green technology, are preferable as they retain more components in the product (Zhang et al., 2024). It has been emphasized that these technologies, along with other non-thermal processing techniques, enhance the nutritional content of foods (Lan et al., 2023). Therefore, in order to preserve and improve the quality of fresh fruit juices, thermosonication and ultrasound are considered alternatives to thermal processing. Similar results have been observed in studies on the juice of posotia (*Vitex negundo*) (Das et al., 2023), pineapple juice (Hoque et al., 2024), wheat plantlet juice (Ahmed et al., 2021), tomato juice (Zhang et al., 2019) and orange-fleshed sweet potato juice (Rios-Romero et al., 2021). The effect of ultrasound varies depending on the fruit and vegetable tissue, food matrix, and processing conditions (Lan et al., 2023). Literature research into the mineral composition of broccoli juice is scarce (Sánchez-Vega et al., 2020) with most research focusing on bioactive compounds, total phenol content, and antioxidant capacity (Saavedra-Leos et al., 2021; Sánchez-Vega et al., 2015).

4. Conclusion

Broccoli juice is an essential health drink because it contains nutritious ingredients and bioactive compounds such as vitamins, fibre, phenolics and glucosinolates. These components contribute to its beneficial health effects. This investigation shows the promise of ultrasound and thermosonication as innovative non-thermal processing techniques for preserving bioactive compounds and increasing mineral content in broccoli juice. RSM and ANFIS models enabled precise optimization of processing parameters, with significant improvements in chlorophyll, ascorbic acid and phenolic content. The highest levels of potassium (K), phosphorus (P), iron (Fe), and zinc (Zn) in broccoli juices were achieved with the thermosonication method. While thermosonication significantly increases mineral content, pasteurization results in a depletion of certain mineral contents. To preserve the chlorophyll and antioxidant capacity of broccoli juice, ultrasound and thermosonication treatments were found to be effective. In particular, the thermosonication process enhanced chlorophyll and total phenolic content, thereby preserving bioactive components. In contrast, thermal processing was observed to cause a noticeable reduction in chlorophyll and ascorbic acid content. For total phenolics, ultrasound and thermosonication increased the release of bioactive compounds and improved total phenolics. These results show that ultrasound and thermosonication may serve as an effective, sustainable and scalable alternative to traditional thermal processes. They may also offer improved preservation and enrichment of nutritional value.

Ultrasound technology is preferred by producers as a new processing method to improve functional properties and quality in fruit juices, providing long shelf life in cold storage and providing innovative products for consumers. It is important to provide the necessary lines and equipment for the applicability of US and combined applications to large-scale enterprises in the future in terms of optimizing the sensory and nutritional properties of the products and enabling the development of healthier products and increasing the bioavailability of some bioactive components. However, our study was conducted at laboratory scale and scale-up studies are needed for industrial applications. Additionally, some manufacturing factors such as temperature, pH and pressure were not evaluated. Future research should include optimization of these variables, shelf-life studies and consumer acceptance.

CRediT authorship contribution statement

Seydi Yıkmış: Software, Resources, Data curation, Conceptualization. **Melikenur Türkol:** Writing – original draft, Software, Resources, Data curation. **Dilek Dülger Altıner:** Writing – original draft, Software. **Aylin Duman Altan:** Software, Resources, Methodology, Data curation. **Kübra Sağlam:** Writing – original draft, Validation, Resources. **Gholamreza Abdi:** Writing – review & editing, Writing – original draft, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Nazlı Tokatlı:** Writing – original draft, Software, Resources, Methodology. **Güler Çelik:** Writing – original draft, Software, Resources, Methodology, Investigation. **Rana Muhammad Aadil:** Writing – review & editing, Writing – original draft, Methodology.

Ethical approval

No human or animal studies are included in this article.

Funding

The authors declare no financial support.

Declaration of competing interest

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

Data availability

Data will be made available on request.

References

Abduallah, A. M., Ahmed, A. E., Bajaber, M. A., & Alalwiat, A. A. (2023). Evaluation of the Antidiabetic Effects of Methanolic Extracts of Neem (*Azadirachta indica*) Seeds on the Streptozotocin-induced Wistar Rats. *Pakistan Veterinary Journal, 43*(4), 792–798. https://doi.org/10.29261/pakvetj/2023.106

Abdulstar, A. R., Altemimi, A. B., & Al-Hilphy, A. R. (2023). Exploring the Power of Thermosonication: A Comprehensive Review of Its Applications and Impact in the Food Industry. *Foods, 12*(7), Article 1459. https://doi.org/10.3390/FOODS12071459

Ahmed, Z., Faisal Manzoor, M., Hussain, A., Hanif, M., Zia-ud-Din, & Zeng, X. A. (2021). Study the impact of ultra-sonication and pulsed electric field on the quality of wheat plantlet juice through FTIR and SERS. *Ultrasonics Sonochemistry, 76*(April), 105648. https://doi.org/10.1016/j.ultsonch.2021.105648

Alara, O. R., Abdurahman, N. H., & Ukaegbu, C. I. (2021). Extraction of phenolic compounds: A review. *Current Research in Food Science, 4*(December 2020), 200–214. https://doi.org/10.1016/j.crfs.2021.03.011

Algan Cavuldak, Ö., Vural, N., Akay, M. A., & Anlı, R. E. (2019). Optimization of ultrasound-assisted water extraction conditions for the extraction of phenolic compounds from black mulberry leaves (*Morus nigra* L.). *Journal of Food Process Engineering, 42*(5), 1–15. https://doi.org/10.1111/jfpe.13132

Alongi, M., Verardo, G., Gorassini, A., Lemos, M. A., Hungerford, G., Cortella, G., & Anese, M. (2019). Phenolic content and potential bioactivity of apple juice as affected by thermal and ultrasound pasteurization. *Food & Function, 10*(11), 7366–7377. https://doi.org/10.1039/C9FO01762C

Amna, D., Islam, M. R., Farooq, A., & Munawar, I. (2023). Unveiling the functional implications and complex interplay between bound phenolic compounds and phenolics in food: a comprehensive review. *Agrobiological Records, 13*, 70–81. https://doi.org/10.47278/journal.abr/2023.027

Anticona, M., Blesa, J., Frigola, A., & Esteve, M. J. (2020). High biological value compounds extraction from citruswaste with non-conventional methods. *Foods, 9*(6). https://doi.org/10.3390/foods9060811

Awad, T. S., Moharram, H. A., Shaltout, O. E., Asker, D., & Youssef, M. M. (2012). Applications of ultrasound in analysis, processing and quality control of food: A review. *Food Research International, 48*(2), 410–427. https://doi.org/10.1016/j.foodres.2012.05.004

Bhutkar, S., Brandão, T. R. S., Silva, C. L. M., & Miller, F. A. (2024). Application of Ultrasound Treatments in the Processing and Production of High-Quality and Safe-to-Drink Kiwi Juice. *Foods, 13*(2), 328. https://doi.org/10.3390/FOODS13020328/S1

Breig, S. J. M., & Luti, K. J. K. (2021). Response surface methodology: A review on its applications and challenges in microbial cultures. *Materials Today Proceedings, 42*, 2277–2284. https://doi.org/10.1016/j.matpr.2020.12.316

Cheila, C. B., dos Anjos, G. L., Nóbrega, R. S. A., da Magaton, S. A., de Miranda, F. M., & de Dias, S. F. (2020). Greener ultrasound-assisted extraction of bioactive phenolic compounds in *Croton heliotropiifolius* Kunth leaves. *Microchemical Journal, 159*(June). https://doi.org/10.1016/j.microc.2020.105525

Cheng, C., Xiang Jia, M., Gui, Y., & Ma, Y. (2020). Comparison of the effects of novel processing technologies and conventional thermal pasteurisation on the nutritional quality and aroma of Mandarin (Citrus unshiu) juice. *Innovative Food Science & Emerging Technologies, 64*, Article 102425. https://doi.org/10.1016/J.IFSET.2020.102425

Cheng, L. H., Soh, C. Y., Liew, S. C., & Teh, F. F. (2007). Effects of sonication and carbonation on guava juice quality. *Food Chemistry, 104*(4), 1396–1401. https://doi.org/10.1016/j.foodchem.2007.02.001

Choudhary, A. N., & Tahir, F. (2023). Fruıt peel extracted polyphenols through ultrasonıc assısted extractıon: a revıew. *Agrobiological Records, 14*, 50–58. https://doi.org/10.47278/journal.abr/2023.038

Çöl, B. G., Akhan, M., Sancar, B.Ç., Türkol, M., Yıkmış, S., & Hecer, C. (2023). Effect of Thermosonication on Amino Acids, Phenolic Compounds, Sensory Properties and Microbial Quality in Freshly Squeezed Verjuice. *Foods, 12*(11), 2167. https://doi.org/10.3390/FOODS12112167

Dadan, M., Tylewicz, U., Tappi, S., Rybak, K., Witrowa-Rajchert, D., & Rosa, M. D. (2021). Effect of ultrasound, steaming, and dipping on bioactive compound contents and antioxidant capacity of basil and parsley. *Polish Journal Of Food And Nutrition Sciences, 71*(3), 311–321. https://doi.org/10.31883/pjfns/141430

Dalal, D., Kunte, S., Oblureddy, V. T., & Anjali, A. K. (2023). Comparative Evaluation of Antimicrobial Efficacy of German Chamomile Extract, Tea Tree Oil and Chlorhexidine as Root Canal Irrigants against E-faecalis and *Streptococcus Mutans*-An In Vitro Study. *International Journal of Agriculture and Biosciences, 12*(4), 252–256. https://doi.org/10.47278/journal.ijab/2023.072

Das, P. S., Nayak, P. K., Senthilnathan, K., Das, P., Kesavan, R., & krishnan.. (2023). Optimization of thermosonication treatments to improve the quality parameters of posotia (*Vitex negundo*) juice. *Journal of Agriculture and Food Research, 14*(October), Article 100811. https://doi.org/10.1016/j.jafr.2023.100811

Delgado, A. M., Issaoui, M., & Chammem, N. (2019). Analysis of main and healthy Phenolic compounds in foods. *Journal of AOAC International, 102*(5), 1356–1364. https://doi.org/10.5740/jaoacint.19-0128

Dulger Altıner, D., Yıkmış, S., Şimşek, M. A., Türkol, M., Tokatlı Demirok, N., & Celik, G. (2024). Impact of Thermosonication Treatment on Parsley Juice: Particle Swarm Algorithm (PSO), Multiple Linear Regression (MLR), and Response Surface Methodology (RSM). *ACS Omega*. https://doi.org/10.1021/acsomega.4c02749

El-Shatanovi, G. A. T. A., Ashoush, I. S., Ahmed, E. K., & Ali, S. A. (2012). Antiatherogenic properties of vegetable juice rich in antioxidants in cholesterol-fed rats. *Annals of Agricultural Sciences, 57*(2), 167–173. https://doi.org/10.1016/J.AOAS.2012.08.011

Esmaeilzadeh Kenari, R., & Dehghan, B. (2020). Optimization of ultrasound-assisted solvent extraction of hemp (*Cannabis sativa* L.) seed oil using RSM: Evaluation of oxidative stability and physicochemical properties of oil. *Food Science & Nutrition, 8*(9), 4976–4986. https://doi.org/10.1002/fsn3.1796

Foujdar, R., Bera, M. B., & Chopra, H. K. (2020). Optimization of process variables of probe ultrasonic-assisted extraction of phenolic compounds from the peel of *Punica granatum* Var. Bhagwa and it's chemical and bioactivity characterization. *Journal of Food Processing and Preservation, 44*(1), 1–16. https://doi.org/10.1111/jfpp.14317

Geraldi, M. V., Betim Cazarin, C. B., Dias-Audibert, F. L., Pereira, G. A., Carvalho, G. G., Kabuki, D. Y., … Maróstica Júnior, M. R. (2021). Influence of high isostatic pressure and thermal pasteurization on chemical composition, color, antioxidant properties and sensory evaluation of jabuticaba juice. *Lwt, 139*(August 2020). https://doi.org/10.1016/j.lwt.2020.110548

Grajeda-Iglesias, C., Salas, E., Barouh, N., Baréa, B., Panya, A., & Figueroa-Espinoza, M. C. (2016). Antioxidant activity of protocatechuates evaluated by DPPH, ORAC, and CAT methods. *Food Chemistry, 194*, 749–757. https://doi.org/10.1016/j.foodchem.2015.07.119

Guo, L., Yang, R., Wang, Z., Guo, Q., & Gu, Z. (2014). Glucoraphanin, sulforaphane and myrosinase activity in germinating broccoli sprouts as affected by growth temperature and plant organs. *Journal of Functional Foods, 9*(1), 70–77. https://doi.org/10.1016/j.jff.2014.04.015

Hasheminya, S. M., & Dehghannya, J. (2022). Non-thermal processing of black carrot juice using ultrasound: Intensification of bioactive compounds and microbiological quality. *International Journal of Food Science & Technology, 57*(9), 5848–5858. https://doi.org/10.1111/IJFS.15901

Hiscox, J. D., & Israelstam, G. F. (2011). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany, 57*(12), 1332–1334. https://doi.org/10.1139/B79-163

Hoque, M., Talukdar, S., Roy, K. R., Hossain, M. A., & Zzaman, W. (2024). Sonication and thermal treatment of pineapple juice: Comparative assessment of the physicochemical properties, antioxidant activities and microbial inactivation. *Food Science and Technology International, 30*(1), 37–48. https://doi.org/10.1007/s11947-011-0746-9

Houška, M., Strohalm, J., Kocurová, K., Totušek, J., Lefnerová, D., Tříska, J., Vrchotová, N., Fiedrleová, V., Holasova, M., Gabrovská, D., & Paulíčková, I. (2006). High pressure and foods-fruit/vegetable juices. *Journal of Food Engineering, 77*(3), 386–398. https://doi.org/10.1016/j.jfoodeng.2005.07.003

Hu, S., Ding, Y., & Zhu, C. (2020). Sensitivity and Responses of Chloroplasts to Heat Stress in Plants. *Frontiers in Plant Science, 11*(April), 1–11. https://doi.org/10.3389/fpls.2020.00375

Jadhav, H. B., Annapure, U. S., & Deshmukh, R. R. (2021). Non-thermal Technologies for Food Processing. *Frontiers in Nutrition, 8*(June), 1–14. https://doi.org/10.3389/fnut.2021.657090

Karaboga, D., Ebubekir, K., & Kaya, B. E. (2019). Adaptive network based fuzzy inference system (ANFIS) training approaches: a comprehensive survey. *Artificial Intelligence Review, 52*, 2263–2293. https://doi.org/10.1007/s10462-017-9610-2

Kurilich, A. C., Tsau, G. J., Brown, A., Howard, L., Klein, B. P., Jeffery, E. H., … Juvik, J. A. (1999). Carotene, tocopherol, and ascorbate contents in subspecies of *Brassica oleracea*. *Journal of Agricultural and Food Chemistry, 47*(4), 1576–1581. https://doi.org/10.1021/jf9810158

Kutlu, N., Pandiselvam, R., Kamiloglu, A., Saka, I., Sruthi, N. U., Kothakota, A., … Maerescu, C. M. (2022). Impact of ultrasonication applications on color profile of foods. *Ultrasonics Sonochemistry, 89*(June), Article 106109. https://doi.org/10.1016/j.ultsonch.2022.106109

Lan, T., Wang, J., Bao, S., Zhao, Q., Sun, X., Fang, Y., Ma, T., & Liu, S. (2023). Effects and impacts of technical processing units on the nutrients and functional components of fruit and vegetable juice. *Food Research International, 168*(April), Article 112784. https://doi.org/10.1016/j.foodres.2023.112784

Lewis, C. D. (1982). *Industrial and business forecasting methods : a practical guide to exponential smoothing and curve fitting (D. Colin (red))*. Butterworth Scientific.

Li, Y., Zhang, T., Korkaya, H., Liu, S., Lee, H. F., Newman, B., … Sun, D. (2010). Sulforaphane, a dietary component of broccoli/broccoli sprouts, inhibits breast cancer stem cells. *Clinical Cancer Research, 16*(9), 2580–2590. https://doi.org/10.1158/1078-0432.CCR-09-2937

Li, Y., Zhang, T., Schwartz, S. J., & Sun, D. (2011). Sulforaphane potentiates the efficacy of 17-allylamino 17- demethoxygeldanamycin against pancreatic cancer through enhanced abrogation of Hsp90 chaperone function. *Nutrition and Cancer, 63*(7), 1151–1159. https://doi.org/10.1080/01635581.2011.596645

Mahn, A., & Reyes, A. (2012). An overview of health-promoting compounds of broccoli (*Brassica oleracea var. italica*) and the effect of processing. *Food Science and Technology International, 18*(6), 503–514. https://doi.org/10.1177/1082013211433073

Mäkilä, L., Laaksonen, O., Kallio, H., & Yang, B. (2017). Effect of processing technologies and storage conditions on stability of black currant juices with special focus on phenolic compounds and sensory properties. *Food Chemistry, 221*, 422–430. https://doi.org/10.1016/j.foodchem.2016.10.079

Mandelová, L., & Totušek, J. (2006). Chemoprotective effects of broccoli juice treated with high pressure. *Czech Journal of Food Sciences, 24*(1), 19–25. https://doi.org/10.17221/3289-cjfs

Martínez-Ramos, T., Benedito-Fort, J., Watson, N. J., Ruiz-López, I. I., Che-Galicia, G., & Corona-Jiménez, E. (2020). 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 and Bioproducts Processing, 122*, 41–54. https://doi.org/10.1016/j.fbp.2020.03.011

Miao, H., Zeng, W., Zhao, M., Wang, J., & Wang, Q. (2020). Effect of melatonin treatment on visual quality and health-promoting properties of broccoli florets under room temperature. *Food Chemistry, 319*(February), Article 126498. https://doi.org/10.1016/j.foodchem.2020.126498

Mintah, B. K., He, R., Dabbour, M., Golly, M. K., Agyekum, A. A., & Ma, H. (2019). Effect of sonication pretreatment parameters and their optimization on the antioxidant activity of Hermitia illucens larvae meal protein hydrolysates. *Journal of Food Processing and Preservation, 43*(9), 1–12. https://doi.org/10.1111/jfpp.14093

Nayak, P. K., Basumatary, B., Chandrasekar, C. M., Seth, D., & Kesavan, R. K. (2020). Impact of thermosonication and pasteurization on total phenolic contents, total flavonoid contents, antioxidant activity, and vitamin C levels of elephant apple (*Dillenia indica*) juice. *Journal of Food Process Engineering, 43*(8), 13447. https://doi.org/10.1111/JFPE.13447

Nikoo, M., & Gavlighi, H. A. (2022). Natural antioxidants and flavorings for clean label foods. In *The age of clean label foods (bll 73–102)*. Springer. https://doi.org/10.3390/foods11172613.

Nowacka, M., Dadan, M., Janowicz, M., Wiktor, A., Witrowa-Rajchert, D., Mandal, R., Pratap-Singh, A., & Janiszewska-Turak, E. (2021). Effect of nonthermal treatments on selected natural food pigments and color changes in plant material. *Comprehensive Reviews in Food Science and Food Safety, 20*(5), 5097–5144. https://doi.org/10.1111/1541-4337.12824

Oladunjoye, A. O., Adeboyejo, F. O., Okekunbi, T. A., & Aderibigbe, O. R. (2021). Effect of thermosonication on quality attributes of hog plum (*Spondias mombin* L.) juice. *Ultrasonics Sonochemistry, 70*, Article 105316. https://doi.org/10.1016/J.ULTSONCH.2020.105316

Oliveira, G. A. R., Guimarães, J. T., Ramos, G. L. P. A., Esmerino, E. A., Pimentel, T. C., Neto, R. P. C., … Cruz, A. G. (2022). Benefits of thermosonication in orange juice whey drink processing. *Innovative Food Science & Emerging Technologies, 75*, Article 102876. https://doi.org/10.1016/J.IFSET.2021.102876

Onu, C. E., Igbokwe, P. K., Nwabanne, J. T., & Ohale, P. E. (2022). ANFIS, ANN, and RSM modeling of moisture content reduction of cocoyam slices. *Journal of Food Processing and Preservation, 46*(1), Article e16032. https://doi.org/10.1111/JFPP.16032

Ordóñez-Santos, L. E., Martínez-Girón, J., & Arias-Jaramillo, M. E. (2017). Effect of ultrasound treatment on visual color, vitamin C, total phenols, and carotenoids content in Cape gooseberry juice. *Food Chemistry, 233*, 96–100. https://doi.org/10.1016/j.foodchem.2017.04.114

Ordóñez-Santos, L. E., & Vázquez-Riascos, A. (2010). Effect of processing and storage time on the vitamin C and lycopene contents of nectar of pink guava (*Psidium guajava* L.). *Archivos Latinoamericanos de Nutrición, 60*(3), 280–284.

Plumb, G. W., Price, K. R., Rhodes, M. J. C., & Williamson, G. (1997). Antioxidant properties of the major polyphenolic compounds in broccoli. *Free Radical Research, 27*(4), 429–435. https://doi.org/10.3109/10715769709065782

Pooja, B. K., Sethi, S., Joshi, A., Varghese, E., Kaur, C., Kumar, R., & Shridhar.. (2023). Ultrasound-Assisted Extraction of Chlorophyll from Pea Pod Waste: Optimization, Kinetics, and Stability Study. *Food Analytical Methods, 16*(8), 1358–1369. https://doi.org/10.1007/s12161-023-02502-8

Portu, J., López, R., Santamaría, P., & Garde-Cerdán, T. (2017). Elicitation with methyl jasmonate supported by precursor feeding with phenylalanine: Effect on Garnacha grape phenolic content. *Food Chemistry, 237*, 416–422. https://doi.org/10.1016/J.FOODCHEM.2017.05.126

Priyadarshini, A., & Priyadarshini, A. (2018). Market Dimensions of the Fruit Juice Industry. *Fruit Juices*, 15–32.

Raghavendra, H. R., Sethi, S., Bhowmik, A., Varghese, E., & Joshi, A. (2022). Phenolics from potato peel and its extraction intensification usinresponse surface methodology and genetic algorithm approach. *Indian Journal of Horticulture, 79*(1), 83–90. https://doi.org/10.5958/0974-0112.2022.00013.5

Rios-Romero, E. A., Ochoa-Martínez, L. A., Bello-Pérez, L. A., Morales-Castro, J., Quintero-Ramos, A., & Gallegos-Infante, J. A. (2021). Effect of ultrasound and steam treatments on bioaccessibility of β-carotene and physicochemical parameters in orange-fleshed sweet potato juice. *Heliyon, 7*(4). https://doi.org/10.1016/j.heliyon.2021.e06632, 0–5.

Roobab, U., Abida, A., Madni, G. M., Ranjha, M. M. A. N., Zeng, X. A., Mousavi Khaneghah, A., & Aadil, R. M. (2023). An updated overview of ultrasound-based interventions on bioactive compounds and quality of fruit juices. *Journal of Agriculture and Food Research, 14*(September), Article 100864. https://doi.org/10.1016/j.jafr.2023.100864

Saavedra-Leos, M. Z., Leyva-Porras, C., Toxqui-Terán, A., & Espinosa-Solis, V. (2021). Physicochemical properties and antioxidant activity of spray-dry broccoli (*Brassica oleracea* var Italica) stalk and floret juice powders. *Molecules, 26*(7). https://doi.org/10.3390/molecules26071973

Salazar-Orbea, G. L., García-Villalba, R., Bernal, M. J., Hernández, A., Tomás-Barberán, F. A., & Sánchez-Siles, L. M. (2023). Stability of phenolic compounds in apple and strawberry: Effect of different processing techniques in industrial set up. *Food Chemistry, 401*(July 2022). https://doi.org/10.1016/j.foodchem.2022.134099

Sánchez-Vega, R., Elez-Martínez, P., & Martín-Belloso, O. (2015). Influence of high-intensity pulsed electric field processing parameters on antioxidant compounds of broccoli juice. *Innovative Food Science and Emerging Technologies, 29*, 70–77. https://doi.org/10.1016/j.ifset.2014.12.002

Sánchez-Vega, R., Garde-Cerdán, T., Rodríguez-Roque, M. J., Elez-Martínez, P., & Martín-Belloso, O. (2020). High-intensity pulsed electric fields or thermal treatment of broccoli juice: the effects of processing on minerals and free amino acids. *European Food Research and Technology, 246*(3), 539–548. https://doi.org/10.1007/s00217-019-03420-y

Santhirasegaram, V., Razali, Z., George, D. S., & Somasundram, C. (2015). Effects of Thermal and Non-thermal Processing on Phenolic Compounds, Antioxidant Activity and Sensory Attributes of Chokanan Mango (*Mangifera indica* L.) Juice. *Food and Bioprocess Technology, 8*(11), 2256–2267. https://doi.org/10.1007/s11947-015-1576-y

Santos, J. C. C., Correa, J. L. G., Furtado, M. L. B., de Morais, L. C., Borges, S. V., de Oliveira, C. R., … de Oliveira, L. F. (2024). Influence of intensity ultrasound on rheological properties and bioactive compounds of araticum (Annona crassiflora) juice. *Ultrasonics Sonochemistry, 105*(April), Article 106868. https://doi.org/10.1016/j.ultsonch.2024.106868

Santos, S. S., de Magalhães, F. S., Paraíso, C. M., Ogawa, C. Y. L., Sato, F., de Santos Junior, O. O., … Reis, M. H. M. (2023). Enhanced conditions for anthocyanin extraction from blackberry pomace under ultrasound irradiation. *Journal of Food Process Engineering, 46*(6), Article e14077. https://doi.org/10.3390/app14020821

Sardar, H., Irshad, M., Anjum, M. A., Hussain, S., Ali, S., Ahmed, R., & Ejaz, S. (2022). Foliar application of micronutrients improves the growth, yield, mineral contents, and nutritional quality of broccoli (*Brassica oleracea* L.). *Turkish Journal of Agriculture and Forestry, 46*(6), 791–801. https://doi.org/10.55730/1300-011X.3043

Sezer, B., Apaydin, H., Bilge, G., & Boyaci, I. H. (2019). Detection of *Pistacia vera* adulteration by using laser induced breakdown spectroscopy. *Journal of the Science of Food and Agriculture, 99*(5), 2236–2242. https://doi.org/10.1002/JSFA.9418

Shokri, S., Jegasothy, H., Hliang, M. M., Augustin, M. A., & Terefe, N. S. (2022). Thermosonication of Broccoli Florets Prior to Fermentation Increases Bioactive Components in Fermented Broccoli Puree. *Fermentation, 8*(5), 1–14. https://doi.org/10.3390/fermentation8050236

Shokunbi, O. S., Adepoju, O. T., Ramaite, I. D. I., Shokunbi, O. S., Mojapelo, P. E. L., & Akinyele, I. O. (2023). Potassium, sodium, calcium and magnesium levels of commonly consumed foods and estimates of dietary intakes of selected Nigerian adults. *Heliyon, 9*(3), Article e13729. https://doi.org/10.1016/j.heliyon.2023.e13729

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture, 16*(3), 144–158.

Tahir, F., Fatima, F., Fatima, R., & Ali, E. (2024). Fruit Peel Extracted Polyphenols Through Ultrasonic Assisted Extraction: a Review. *Agrobiological Records, 15*, 1–12. https://doi.org/10.47278/journal.abr/2023.043

Tang, L., Paonessa, J. D., Zhang, Y., Ambrosone, C. B., & McCann, S. E. (2013). Total isothiocyanate yield from raw cruciferous vegetables commonly consumed in the United States. *Journal of Functional Foods, 5*(4), 1996–2001. https://doi.org/10.1016/j.jff.2013.07.011

Thomas, M., Badr, A., Desjardins, Y., Gosselin, A., & Angers, P. (2018). Characterization of industrial broccoli discards (*Brassica oleracea var. italica*) for their glucosinolate, polyphenol and flavonoid contents using UPLC MS/MS and spectrophotometric methods. *Food Chemistry, 245*(July 2017), 1204–1211. https://doi.org/10.1016/j.foodchem.2017.11.021

Tokatlı Demirok, N., Yıkmış, S., Duman Altan, A., & Apaydın, H. (2024). Optimization of ultrasound-treated horsetail-fortified traditional apple vinegar using RSM and ANFIS modeling: bioactive and sensory properties. *Journal of Food Measurement and Characterization, 18*(1), 256–271. https://doi.org/10.1007/S11694-023-02156-4/FIGURES/7

Tse, G., & Eslick, G. D. (2014). Cruciferous vegetables and risk of colorectal neoplasms: A systematic review and meta-analysis. *Nutrition and Cancer, 66*(1), 128–139. https://doi.org/10.1080/01635581.2014.852686

Turan, I., Canbolat, D., Demir, S., Kerimoglu, G., Colak, F., Alemdar, N. T., … Aliyazicioglu, Y. (2023). An Investigation of the Protective Effect of *Rhododendron luteum* Extract on Cisplatin-Induced DNA Damage and Nephrotoxicity and Biochemical Parameters in Rats. *Pakistan Veterinary Journal, 43*(3), 442–448. https://doi.org/10.29261/pakvetj/2023.047

Urango, A. C. M., Strieder, M. M., Silva, E. K., & Meireles, M. A. A. (2022). Impact of Thermosonication Processing on Food Quality and Safety: a Review. *Food and Bioprocess Technology, 15*(8), 1700–1728. https://doi.org/10.1007/S11947-022-02760-0

Vieira, M. C., Teixeira, A. A., & Silva, C. L. M. (2000). Mathematical modeling of the thermal degradation kinetics of vitamin C in Cupuacu (*Theobroma grandiflorum*) nectar. *Journal of Food Engineering, 43*(1), 1–7. https://doi.org/10.1016/S0260-8774(99)00121-1

Wang, J., Wang, J., Ye, J., Vanga, S. K., & Raghavan, V. (2019). Influence of high-intensity ultrasound on bioactive compounds of strawberry juice: Profiles of ascorbic acid, phenolics, antioxidant activity and microstructure. *Food Control, 96*(September 2018), 128–136. https://doi.org/10.1016/j.foodcont.2018.09.007

Wang, J., Xie, B., & Sun, Z. (2021). Quality parameters and bioactive compound bioaccessibility changes in probiotics fermented mango juice using ultraviolet-assisted ultrasonic pre-treatment during cold storage. *Lwt, 137*, Article 110438. https://doi.org/10.1016/j.lwt.2020.110438

Wen, C., Zhang, J., Zhang, H., Dzah, C. S., Zandile, M., Duan, Y., … Luo, X. (2018). Advances in ultrasound assisted extraction of bioactive compounds from cash crops – A review. *Ultrasonics Sonochemistry, 48*(June), 538–549. https://doi.org/10.1016/j.ultsonch.2018.07.018

Xie, G., Shen, J., Luo, J., Li, D., Tao, Y., Song, C., & Han, Y. (2022). Simultaneous extraction and preliminary purification of polyphenols from grape pomace using an aqueous two-phase system exposed to ultrasound irradiation: Process characterization and simulation. *Frontiers in Nutrition, 9*. https://doi.org/10.3389/fnut.2022.993475

Yıkmış, S., Ozer, H., Levent, Okan, B., Çöl, G., Erdal, B., & Yıkmış, S. (2022). Effect of thermosonication and thermal treatments on antidiabetic, antihypertensive, mineral elements and in vitro bioaccessibility of bioactive compounds in freshly squeezed pomegranate juice. *Journal of Food Measurement and Characterization, 2022*, 1–19. https://doi.org/10.1007/S11694-022-01402-5

Yıkmış, S., Tokatlı Demirok, N., Levent, O., & Apaydın, D. (2023). Impact of thermal pasteurization and thermosonication treatments on black grape juice (*Vitis vinifera* L): ICP-OES, GC–MS/MS and HPLC analyses. *Heliyon, 9*(9). https://doi.org/10.1016/j.heliyon.2023.e19314

Yıkmış, S., Türkol, M., Pacal, I., Duman Altan, A., Tokatlı, N., Abdi, G., … Aadil, R. M. (2025). Optimization of bioactive compounds and sensory quality in thermosonicated black carrot juice: A study using response surface methodology, gradient boosting, and fuzzy logic. *Food Chemistry: X, 25*(December 2024). https://doi.org/10.1016/j.fochx.2024.102096

Zhang, J., Cheng, J., Li, Z., Weng, M., Zhang, X., Tang, X., & Pan, Y. (2024). Effects of ultra-high pressure, thermal pasteurization, and ultra-high temperature sterilization on color and nutritional components of freshly-squeezed lettuce juice. *Food Chemistry, 435*(April 2023), Article 137524. https://doi.org/10.1016/j.foodchem.2023.137524

Zhang, W., Yu, Y., Xie, F., Gu, X., Wu, J., & Wang, Z. (2019). High pressure homogenization versus ultrasound treatment of tomato juice: Effects on stability and in vitro bioaccessibility of carotenoids. *LWT, 116*, Article 108597. https://doi.org/10.1016/J.LWT.2019.108597

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64*(4), 555–559. https://doi.org/10.1016/S0308-8146(98)00102-2

Zudaire, L., Lafarga, T., Viñas, I., Abadias, M., Brunton, N., & Aguiló-Aguayo, I. (2019). Effect of Ultrasound Pre-Treatment on the Physical, Microbiological, and Antioxidant Properties of Calçots. *Food and Bioprocess Technology, 12*(3), 387–394. https://doi.org/10.1007/s11947-018-2217-z