



Optimization of bioactive compounds and sensory quality in thermosonicated black carrot juice: A study using response surface methodology, gradient boosting, and fuzzy logic

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ABSTRACT

This study investigates the optimization of bioactive components in thermosonicated black carrot juice using response surface methodology (RSM) and gradient boosting (GB) modeling techniques. Thermosonication, a combination of ultrasound and heat, was applied to enhance the nutritional quality of black carrot juice, which is rich in anthocyanins, phenolic compounds, and antioxidants. The study examined the effects of temperature, processing time, and ultrasonic amplitude on total carotenoid content (TCC), total anthocyanin content (TAC), ferric reducing antioxidant power (FRAP), and total phenolic content. RSM demonstrated higher prediction accuracy compared to GB, identifying optimal processing conditions at 48.68 °C, 11.15 minutes, and 82.62% amplitude. Thermosonication significantly increased total phenolic content to 414.28 mg GAE/L, surpassing traditional pasteurization. Sensory analysis, conducted via fuzzy logic, indicated improved sensory properties, including aroma, taste, and color, in thermosonicated samples. This study overcomes thermosonication as a promising method for improving both bioactive compounds and sensory quality in black carrot juice. **Chemical compounds:** Chlorogenic acid (PubChem CD:1794427); caffeic acid (PubChem CD: 689043); vanillin (PubChem CD: 1183); rutin (PubChem CD: 5280805); naringin (PubChem CD: 442428); rosmarinic acid (PubChem CD: 5281792); t-ferulic acid (PubChem CD: 445858); o- coumaric acid (PubChem CD: 637540); (PubChem CD: quercetin 5280459); 4-hydroxybenzoic acid (PubChem CD: 135).

1. Introduction

Carrots (*Daucus carota* L.) rank among the ten most widely cultivated crops globally, with an annual yield of 40–44 million tons and a cultivation area totaling 1.12 million hectares. (Bhandari et al., 2022; Luz

et al., 2023). Black carrot (*D. carota* L.) notable for its high anthocyanin source that contains up to 154 mg/g of anthocyanins, with 83% acylated (Mizgier et al., 2016), which exhibit antioxidant activity 10 to 35 times greater than that of other carrot varieties (Singh et al., 2018). The phenolic compounds in black carrots are linked to various health benefits,

Abbreviation: HPLC, high-performance liquid chromatography; TPC, total phenolic content; TAC, total anthocyanin content; TFC, total flavonoids content; DPPH, 2,2'-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; ND, not detected; RSM, Response Surface Methodology; GB, Gradient Boosting; CCD, Central Composite Design; MAE, Mean Absolute Error; RMSE, Root Mean Square Error; R², R-squared; AAD, absolute average deviation; TPTZ, 2,4,6-tri (2-pyridyl)-1,3,5-triazine; C- BCJ, Control black carrot juice; P- BCJ, Thermal pasteurized black carrot juice; TS-BCJ, thermosonication-treated black carrot juice..

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particularly due to their strong antioxidant and antimicrobial properties (Kamiloglu et al., 2018). In addition, studies on plants and plant extracts in animal and humans have shown positive healing effects (Choudhary & Tahir, 2023; Dalal et al., 2023; Khalifa et al., 2023; Saleh et al., 2023; Ya et al., 2023; Ghazy et al., 2023).

Ultrasonically assisted extraction is an emerging technique that effectively disrupts cell walls, facilitating the release of intracellular compounds (Choudhary & Tahir, 2023). Ultrasound treatment is recognized as an environmentally friendly, innovative, cost-effective, and scalable technology (Wen et al., 2018). In the fruit juice industry, ultrasound treatment is used for various processes, such as microbial and enzymatic inactivation, bioactive compounds extraction, homogenization, and degassing (Madanayake et al., 2021; Patist & Bates, 2008). It is often combined with other thermal or non-thermal methods to improve product quality, preserve bioactive compounds, and improve microbial inactivation (Dolas et al., 2019).

Ultrasound preserves the natural juice quality of fruits and vegetables through cavitation, where bubbles and microcurrents form and expand with ultrasonic waves (Qiu et al., 2024; Yıkmaş et al., 2022). Under high temperature and pressure, the collapsing bubbles effectively eliminate bacteria. Beyond its antibacterial effect, the rise in temperature improves juice quality through a beneficial thermal effect (Xie et al., 2024). Thermosonication, also known as an acoustic heat treatment, combines ultrasonic energy with heat to disrupt cellular structures, effectively inactivating pathogenic and spoilage microorganisms as well as endogenous enzymes while retaining the nutritional and sensory properties of fruits and vegetables (Abdulstar et al., 2023; Urango et al., 2022). Studies concluded that sonication of black carrot juice increases phenolic content (Hasheminya & Dehghannya, 2022; Kaur et al., 2023) while Erdal et al. (2022) reported a significant increase in p-coumaric acid and quercetin and Zou and Jiang (2016) reported a notable rise in total carotenoids in ultrasound-treated carrot juice (Erdal et al., 2022; Zou & Jiang, 2016).

RSM is a statistical and mathematical approach used for process optimization, involving experiment design, model creation, and the examination of how input variables system responses (Das et al., 2024). Boosting technologies are a distinct ensemble category, differing from by focusing on improving predictive performance. While bagging seeks to enhance diversity among base (weak) learners by resampling input data, boosting aims to incrementally improve prediction performance. Specifically, boosting involves an iterative process where base learners are successively trained to minimize the loss function, continuing until a desired threshold is achieved. This contrasts with bagging's primary goal of improving overall model accuracy through increased diversity among base learners within the ensemble (Natekin & Knoll, 2013).

A review of the literature revealed no studies have sufficiently optimized the bioactive components of thermosonication-treated black carrot juice using RSM and GB. This study aims to optimize the total carotenoid content (TCC), total anthocyanin content (TAC), and ferric-reducing antioxidant power (FRAP) of black carrot juice by integrating RSM and GB with the thermosonication. Additionally, the study utilized fuzzy logic technique to evaluate the sensory quality of black carrot juice.

2. Materials and methods

2.1. Preparation of black carrot juice

Black carrots (*D. carota* L. var. *atrorubens* Alef.) were sourced from a local market in Tekirdag, Türkiye. The carrots were cleaned and processed using a knife and a Waring Commercial Blender Model HGB2WTS3 (St. Louis, MO, USA) to extract the juice. Solid residues were filtered out using a two-layer muslin cloth. The freshly prepared control black carrot juice samples (C-BCJ) were labeled, stored at 4 °C prior to analysis, and tested in triplicate.

2.2. Thermosonication processing

In thermosonication treatment, 100 mL samples of black carrot juice were processed using a 200 W ultrasonic processor (UW 100, BANDELIN electronic GmbH & Co. KG, Berlin, Germany) operating at a frequency of 26 kHz. Thermosonication conditions studied encompassed temperature settings of 40, 45, 50, 55, and 60 °C, exposure times of 8, 10, 12, 14, and 16 minutes, and amplitudes varying from 60% to 100% in constant mode. An ice-water bath was employed to prevent overheating during the thermosonication treatment. All treatments were carried out in the dark in order to eliminate any possible interference from light. After thermosonication treatment, black carrot juice samples were immediately cooled in an ice bath. The samples were stored at -18 ± 1 °C until further analysis. Upon completion of the optimization process, the black carrot juice samples were identified as thermosonication-treated black carrot juice (TS-BCJ).

2.3. Thermal pasteurization

The collected black carrot juice samples were placed into 100 mL glass bottles and pasteurized using a water bath system (Wisd model WUC-D06H, Daihan, Wonju, Korea) at 85 ± 1 °C for 2 minutes. Following pasteurization, the samples were cooled to 20 ± 1 °C at room temperature. They were then labeled as pasteurized black carrot juice (P-BCJ) and stored at -20 ± 1 °C until required for analytical testing.

2.4. RSM modelling process

The impact of thermosonication treatment on TCC, TAC, and FRAP in black carrot juice was analyzed using RSM and GB. The independent variables included temperature (X_1 , 40–60 °C), time (X_2 , 8–16 minutes), and amplitude (X_3 , 60–100%). The response variables were TCC (mg/L), TAC (mg C3GE/L), and FRAP (mmol TE/L). For the RSM analysis, a Central Composite Design (CCD) was utilized, and the optimization of the thermosonication process was performed using Minitab software (version 19, Minitab Inc., State College, PA, USA). The CCD design outlined a total of 20 experiments, all of which were performed in triplicate. The results are summarized in Table 1. The models were developed using the following quadratic-polynomial equation (Equation 1):

$$y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^3 \sum_{j=1, j \neq i}^3 \beta_{ij} X_i X_j \quad (1)$$

The formula is defined as follows: β_i represents the coefficient of the first-order (linear) term, β_{ii} denotes the coefficient of the quadratic term, β_{ij} is the coefficient of the two-factor interaction term, and X_i and X_j are the independent variables.

2.5. Modelling procedure for GB

In this research, we have applied the GB technique to predict how thermosonication treatment affects the bioactive components of black carrot juice. The process began by preparing and normalizing experimental data, which included independent variables like temperature, time, and amplitude, as well as dependent variables such as TCC, TAC, and FRAP. Subsequently, we partitioned the dataset into training (80%) and testing (20%) subsets for model validation.

The GB model was built using Python's scikit-learn library, specifically the 'GradientBoostingRegressor' class. We fine-tuned key hyperparameters, including the number of estimators, learning rate, maximum depth, and minimum sample split, using grid search cross-validation to improve model performance. The optimal hyperparameters identified were 200 estimators, a learning rate of 0.1, a maximum depth of 4, and a minimum sample split of 2. During training,

Table 1
RSM and GB measured responses used in experimental design

Run	Independent variables			Dependent Variables								
	Temperature (°C -X ₁)	Time (min-X ₂)	Amplitude (%-X ₃)	TCC (mg/L)			TAC (mg C3GE/L)			FRAP (mmol TE/L)		
				Experimental data	RSM prediction	GB prediction	Experimental data	RSM prediction	GB prediction	Experimental data	RSM prediction	GB prediction
1	50	16	80	9.45	9.46	9.5	108.62	108.45	109.03	10.38	10.37	10.42
2	50	12	80	11.93	11.95	11.88	125.78	125.66	125.29	14.06	14.05	14.01
3	45	10	90	11.5	11.55	11.58	121.23	122.1	121.68	13.4	13.48	13.54
4	50	12	100	11	11.07	11.04	114.52	113.7	114.94	12.97	13.01	13.01
5	50	12	80	11.93	11.95	11.88	125.78	125.66	125.29	14.06	14.05	14.01
6	55	14	90	11.35	11.33	11.4	116.47	117.6	116.94	13.73	13.67	13.68
7	45	10	90	11.5	11.55	11.58	121.23	122.1	121.68	13.55	13.48	13.54
8	50	12	80	11.93	11.95	11.88	125.78	125.66	125.29	14.06	14.05	14.01
9	50	12	80	11.93	11.95	11.88	125.78	125.66	125.29	14.06	14.05	14.01
10	55	14	70	10.14	10.1	10.08	114.32	113.69	113.63	10.94	10.99	10.96
11	50	8	80	10.77	10.78	10.82	113.57	112.85	113.94	12.7	12.62	12.74
12	50	12	80	11.93	11.95	11.88	125.78	125.66	125.29	14.06	14.05	14.01
13	60	12	80	9.95	10.08	9.99	110.72	110.56	111.1	12.38	12.37	12.43
14	45	10	70	11.49	11.57	11.33	118.09	118.01	116.98	14.01	14.13	13.87
15	50	12	60	9.92	9.86	9.96	105.78	105.71	106.19	11.09	10.97	11.14
16	40	12	80	10.55	10.44	10.6	115.71	114.98	116.08	13.15	13.08	13.19
17	45	14	70	10.28	10.43	10.32	117.46	118.48	117.97	11.84	11.9	11.9
18	55	10	90	11.62	11.52	11.46	122.43	122.46	121.31	13.69	13.69	13.64
19	55	10	70	9.45	9.48	9.65	99.59	100.6	101.2	11.14	11.2	11.24
20	50	12	80	11.93	11.95	11.88	125.78	125.66	125.29	14.06	14.05	14.01
TS-BCJ (RSM optimization parameters)	48.68	11.15	82.62		12.07			126.26			14.29	
Experimental values (RSM)				12.82			129.55			13.48		
% Diference (RSM)				5.85			2.53			5.66		
TS-BCJ (GB optimization parameters)	49.62	11.08	84.62			11.93			125.73			14.06
Experimental values (GB)				12.77			130.08			13.42		
% Diference (GB)				6.57			3.34			4.55		

X₁— temperature; X₂— time; X₃ — amplitude; df—degrees of freedom; R²—coefficient of determination; p < 0.05. significant differences; p < 0.01. very significant differences; TCC: total carotenoids content; TAC: total anthocyanin content; FRAP: ferric-reducing antioxidant power; TS-BCJ: thermosonication-treated black carrot juice; RSM: Response Surface Methodology; GB: gradient boosting.

the GB model sequentially added weak learners (decision trees), each correcting the residual errors of its predecessors. This process continued until the specified number of estimators was reached or no significant improvement was observed.

GB is an ensemble technique that constructs models sequentially, with each new model attempting to correct the errors of the previous ones, typically using decision trees as base learners. The algorithm starts with a constant value that minimizes the loss function. During each iteration, it calculates residuals that represent the errors of the current model, fits a new decision tree to these residuals, and updates the existing model by incorporating the new tree, scaled according to the learning rate. This iterative process repeats for a predefined number of iterations, resulting in a final model comprising the initial model and all the weak learners. The performance of the GB model was assessed using metrics including Mean Absolute Error (MAE), Root Mean Square Error (RMSE), and R-squared (R^2), offering a thorough evaluation of its predictive accuracy.

2.6. RSM and GB comparison

To elucidate the performance of the models, the coefficient of determination (R^2), root mean square error (RMSE), and absolute average deviation (AAD) were compared between the RSM and GB models. The formulas for these metrics are provided below (Eqs. (2–4)).

$$R^2 = 1 - \frac{\sum_{i=1}^n (Y_{\text{Predicted}} - Y_{\text{Experimental}})^2}{\sum_{i=1}^n (Y_{\text{Average}} - Y_{\text{Experimental}})^2} \quad (2)$$

$$RMSE = \left(\frac{1}{n} \sum_{i=1}^n (Y_{\text{Predicted}} - Y_{\text{Experimental}})^2 \right)^{\frac{1}{2}} \quad (3)$$

$$\text{Mean Absolute Error (MAE)} : MAE = \frac{1}{n} \sum_{i=1}^n |Y_{\text{Predicted}} - Y_{\text{Experimental}}| \quad (4)$$

$$ADD = \left(\frac{1}{n} \sum_{i=1}^n \left| \frac{Y_{\text{Predicted}} - Y_{\text{Experimental}}}{Y_{\text{Experimental}}} \right| \right) * 100 \quad (5)$$

where n , Y_{Average} , $Y_{\text{Predicted}}$, and $Y_{\text{Experimental}}$, are number of data points, the average of data, the predicted value, and the experimental value, respectively. The validity and accuracy of the model were measured based on R^2 , AAD, and RMSE.

2.7. Total carotenoid content (TCC)

TCC was determined utilizing minor modifications to the spectroscopic methods used to analyse samples of black carrot juice (Martínez-Flores et al., 2015; Zhou et al., 2009). A 1 mL aliquot of black carrot juice was mixed with 5 mL methanol solution (1:2, v/v). The mixture was allowed to stand until phase separation occurred, after which the upper phase was carefully separated. 0.5 mL of saturated sodium chloride solution was added to this upper phase, and the mixture was shaken again. A small amount of sodium sulphate was added to the lower phase and the solution was centrifuged at 4000 rpm for 10 minutes. After centrifugation (GYROZEN, 1730 R, Korea) the upper phase was taken up again and 5 mL of methanol solution was added. The resulting mixture was analyzed with a UV-visible spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Victoria, Australia) at a wavelength of 450 nm. The absorbance values were compared against a calibration curve generated from β -carotene standard solutions, and the total carotenoid content in the juice was determined as mg β -carotene equivalent per liter.

2.8. Total phenolic compounds (TPC)

TPC was quantified according to the Folin-Ciocalteu method (Singleton & Rossi, 1965). For the TPC analysis, 2 mL of each sample was combined with 8 mL of 80% methanol. The mixture was then centrifuged at 4000 rpm for 20 minutes. A dilution factor of 5 was assumed, calculated as 10/2. Take 50 μ L of the supernatant and transfer it to a glass tube. Add 100 μ L Folin-Ciocalteu reagent and 1500 μ L deionized water. Mixed for 10 minutes. Following this incubation, 50 μ L of a 20% sodium carbonate (Na_2CO_3) solution was added. The mixture was allowed to react in the dark for 2 hours. The absorbance of the samples was then measured at 765 nm, using a blank for calibration. The results were reported as mg gallic acid equivalents per 100 mL of test solution.

2.9. Ferric-reducing antioxidant power (FRAP)

The FRAP assay was adapted to assess the total antioxidant activity (Thaipong et al., 2006). The working solution was prepared by mixing 50 mL acetate buffer (0.3 mol L^{-1} , pH 3.6), 5 mL 2,4,6-tri (2-pyridyl)-1,3,5-triazine (TPTZ) solution (0.01 mol L^{-1}) and 5 mL $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution (0.02 mol L^{-1}). Before use, the solution was stored at 37 °C. A 4.9 mL aliquot of the working solution was added to a 0.1 mL test sample and allowed to react at 37 °C for 10 minute. The absorbance at 593 nm was measured for the resulting colored product. Trolox was utilized as the standard, and the results were expressed as millimoles of Trolox equivalent (TE) per liter of black carrot juice.

2.10. Total anthocyanin content (TAC)

The pH difference method determined the TAC (Cemeroglu, 2010; Giusti & Wrolstad, 2001). The samples of black carrot juice were diluted 10 times and prepared for analysis. The dilutions were prepared using potassium chloride, a 0.4 M sodium acetate buffer (pH 4.5), along with a 0.025 M solution of potassium chloride (pH 1.0). The diluted samples were equilibrated for 15 minutes. Following this period, absorbance was measured at 515 and 700 nm using a spectrophotometer, with distilled water as the reference. For the analysis, 5 mL aliquots of black carrot juice were used. The samples were diluted to 10 g with pure water and thoroughly mixed by vortexing. Now diluted twofold, the samples were centrifuged at 4000 rpm for 10 minutes. Subsequently, 1 mL of the supernatant was removed, and the volume was adjusted to 5 mL using a 0.025 M potassium chloride solution (pH 1.0). Next, 1 mL of the clear supernatant was taken and diluted to 5 mL with a 0.4 M sodium acetate buffer (pH 4.5), achieving a tenfold dilution. The samples were then incubated for 30 minutes. After the incubation, absorbance measurements were taken at 515 nm and 700 nm. The level of monomeric anthocyanins was assessed using cyanidin-3-glucoside, the predominant anthocyanin in black carrot juice. The following equation has been used. The calculation was done using Formula 6.

$$TAC \text{ (mg/L)} = A(MW)(DF)1000/(\epsilon)(L) \quad (6)$$

TAC: Total anthocyanin content.

A: Absorbance difference (Measured at pH 1.0 and 4.5 absorbance difference).

MW: Molecular weight of anthocyanin (cyanidin-3-glucoside = 449.2 g/mol).

DF: Dilution factor.

ϵ : Molar absorption coefficient (26900).

L: Layer thickness of the absorbance measuring cuvette (cm).

The results are expressed in mg C3GE/L.

2.11. Analysis of phenolic compounds

Chromatography was conducted following the method of Portu et al. (2017) using an ACE Genix C-18 column (250 \times 4.6 mm; 5 μ m packing;

Agilent) (Portu et al., 2017). Polyphenols were analyzed using an Agilent 1260 chromatograph with a DAD detector. The flow rate was set to 0.80 mL/min, and the column temperature was maintained at 30 °C. Gradient elution was performed using eluents A and B. Solution A was water with 0.1% phosphoric acid, while Solution B was acetonitrile. The gradient elution profile was as follows: 17% B at 0 minutes, 15% at 7 minutes, 20% at 20 minutes, 24% at 25 minutes, 30% at 28 minutes, 40% at 30 minutes, 50% at 32 minutes, 70% at 36 minutes, and returning to 17% at 40 minutes. For phenolic analysis, the injection volume was 10 µL. The study was conducted at wavelengths of 280, 320, and 360 nm using a UV-Vis spectrophotometer, with results expressed

$$\text{TCC (mg/L)} = -12.39 + 0.326 X_1 + 1.191 X_2 + 0.2320 X_3 - 0.016892 X_1 X_1 - 0.11418 X_2 X_2 - 0.003697 X_3 X_3 + 0.04390 X_1 X_2 + 0.010228 X_1 X_3 - 0.01014 X_2 X_3 \quad (7)$$

$$\text{TAC (mg C3GE/L)} = -255.7 + 1.769 X_1 + 24.14 X_2 + 4.828 X_3 - 0.12889 X_1 X_1 - 0.9380 X_2 X_2 - 0.03988 X_3 X_3 + 0.3155 X_1 X_2 + 0.08891 X_1 X_3 - 0.2244 X_2 X_3 \quad (8)$$

$$\text{FRAP (mmol TE/L)} = 21.29 - 0.5691 X_1 + 0.840 X_2 + 0.0619 X_3 - 0.013233 X_1 X_1 - 0.15942 X_2 X_2 - 0.005150 X_3 X_3 + 0.05023 X_1 X_2 + 0.015680 X_1 X_3 + 0.00242 X_2 X_3 \quad (9)$$

as µg/mL of sample.

2.12. Sensory evaluation

The fuzzy logic technique was applied to analyze the observation data set obtained from linguistic sensory data. This method enables the objective assessment and measurement of sensory characteristics, thereby mitigating the subjective perception and rendering study outcomes more scientifically rigorous. Our study is based on the fuzzy logic approach, which was successfully applied (Xu et al., 2023). The assessors engaged in the sensory assessment process by assigning ratings to diverse attributes, including aroma, taste, color, and mouthfeel. All models have a three-digit random number code. It was evaluated by 36 trained panelists (13 male, 23 female). The six grades of Not Satisfactory, Fair, medium, Good, Very Good, and Excellent were used as the grade set for evaluating a set of 3 samples (C-BCJ, P-BCJ, TS-BCJ). Firstly, the weight set A was established after panelists rated the four factors (Aroma, Taste, Color, and Mouthfeel) based on their subjective feelings and experiences. Initially, each factor was assigned values ranging from 0 to 1 in ascending order of importance. Subsequently, fuzzy matrices R were computed based on the distribution of samples across four levels for each evaluation factor, as determined through sensory assessments conducted by panelists. After this, Fuzzy Sensory Scores were derived to facilitate a more precise differentiation of sensory quality disparities among the samples. These scores were computed by multiplying the R fuzzy matrices with the weight set and corresponding grade scores. The resultant score values were compared to discern sensory disparities concerning quality attributes, identifying the trial set exhibiting the highest score.

2.13. Statistical analysis

All experiments were performed in triplicate, and results are presented as mean values with standard deviation (SD). Data were analyzed using one-way ANOVA, and differences between means were determined with Tukey's honestly significant difference (HSD) test at a significance level of $p < 0.05$. Statistical analyses were carried out with SPSS 22.0 (SPSS Inc., Chicago, IL, USA), while three-dimensional RSM plots were created using Sigma Plot 12.0 (Systat Software, Inc., San Jose, CA, USA).

3. Results and discussion

3.1. Optimization of TCC, TAC, and FRAP

Table 1 presents the experimental and predicted results for the thermosonication treatments of black carrot juice samples, which varied in temperature, time, and amplitude levels, with respect to TCC, TAC, and FRAP values. After thermosonication, a second-order polynomial regression model was used to analyze the data. The equations for TCC, TAC, and FRAP are shown below (Equations (7)–(9)).

According to the formula, increases in the variables X_1 (°C), X_2 (min), and X_3 (%) positively influence the TCC (mg/L) and TAC (mg C3GE/L) values of black carrot juice, showing a directional positive effect. However, the quadratic effects of these independent variables were found to induce negative changes. It was noted that the FRAP (mmol TE/L) value of black carrot juice was positively influenced by the interaction effects of the variables X_1 (°C), X_2 (min), and X_3 (%). Table 2 displays the experimental and predicted values for TCC, TAC, and FRAP of TS-BCJ, across various temperatures, thermosonication treatment durations, and amplitude levels. The determination coefficient (R^2) and adjusted R^2 values obtained from the RSM are above 98%. The TS-BCJ values obtained using RSM were compared with those obtained using repeated measurements. The RSM modeling levels' R^2 values showed high correlations of 99.48%, 99.35%, and 99.77% (Table 2). Statistical significance was observed for both two-way and one-way modeling effects ($p < 0.05$). The ANOVA results indicated that the linear effects of the X_1 , X_2 , and X_3 parameters on all three responses were significant ($p < 0.05$). Both the two-way and one-way effects of the modeling were statistically significant ($p < 0.05$). TCC, TAC, and FRAP were highly influenced by the thermosonication treatment parameters.

Table 1 presents the experimental and predicted responses to thermosonication treatments as determined by RSM. RSM modeling was employed to examine the influence of the independent variables, namely temperature, time, and amplitude on TCC (mg/L), TAC (mg C3GE/L), and FRAP (mmol TE/L). The optimal levels of the independent variables identified by RSM were 48.68 °C for temperature, 11.15 minutes for time, and 82.62% for amplitude. Under these optimized conditions, TCC was found to be 12.07 mg/L, TAC 126.26 mg C3GE/L, and FRAP 14.29 mmol TE/L. The effect of thermosonication treatment on TCC (mg/L), TAC (mg C3GE/L), and FRAP (mmol TE/L) is illustrated through the response surface plots shown in Figure 1 (A–C). When analyzing the effects of time and amplitude, there was a general increase in TCC (mg/L), TAC (mg C3GE/L), and FRAP (mmol TE/L) values. Both models yielded effective results for TCC, TAC, and FRAP. However, RSM demonstrated higher prediction accuracy with smoother surface plots (Figure 1). On the other hand, GB emerged as a strong alternative for managing complex and nonlinear relationships. In conclusion, both models were effective in optimizing bioactive compounds, but RSM exhibited slightly superior performance. (See Fig. 2.)

Table 2
ANOVA in the regression model of the central test for the combination of tests

Source	DF	TCC (mg/L)		TAC (mg C3GE/L)		FRAP (mmol TE/L)	
		F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
Model	9	210.93	0.000	168.81	0.000	482.02	0.000
Linear	3	156.60	0.000	49.98	0.000	529.67	0.000
X ₁	1	15.24	0.003	24.49	0.001	65.33	0.000
X ₂	1	198.82	0.000	24.26	0.001	664.33	0.000
X ₃	1	163.76	0.000	79.57	0.000	546.87	0.000
Square	3	386.75	0.000	331.56	0.000	704.33	0.000
X ₁ X ₁	1	565.11	0.000	365.81	0.000	405.03	0.000
X ₂ X ₂	1	660.92	0.000	495.97	0.000	1504.77	0.000
X ₃ X ₃	1	433.14	0.000	560.27	0.000	981.59	0.000
2-Way Interaction	3	93.25	0.000	97.25	0.000	254.11	0.000
X ₁ X ₂	1	156.59	0.000	89.95	0.000	239.41	0.000
X ₁ X ₃	1	211.75	0.000	177.91	0.000	581.21	0.000
X ₂ X ₃	1	33.45	0.000	182.06	0.000	2.23	0.166
Error	10						
Lack-of-Fit	4					7.26	0.017
Pure Error	6						
Total	19						
R ²		99.48%		99.35%		99.77%	
Adj R ²		99.00%		98.76%		99.56%	
Pred R ²		94.62%		93.73%		97.89%	

X₁: temperature; X₂: time; X₃: amplitude; TCC: total carotenoids content; TAC: total anthocyanin content; FRAP: ferric reducing ability of plasma; df—degrees of freedom; R²—coefficient of determination. p < 0.05. significant differences; p < 0.01. very significant differences.

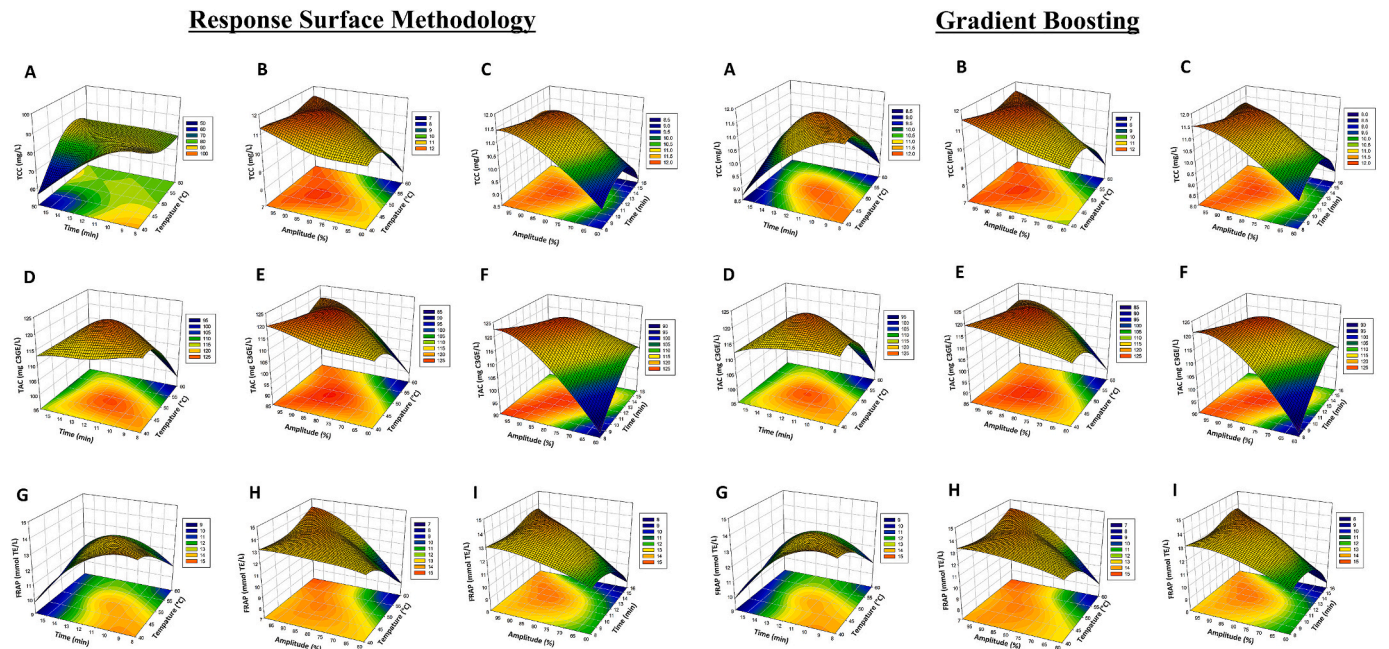


Figure 1. Response surface plots (3D) for TCC, TAC and FRAP as a function of significant interaction factors for RSM and GB

This study utilized both RSM and GB to optimize the bioactive components of black carrot juice treated with ultrasound. The comparative analysis of these two models reveals distinct advantages and performance characteristics that highlight their suitability for this optimization problem.

RSM utilizes a Central Composite Design (CCD) to systematically explore the effects of the independent variables—temperature, time, and amplitude—on the dependent variables, which include TCC, TAC, and FRAP. This method involves applying a second-order polynomial regression model to the experimental data, which helps in understanding the linear and quadratic interactions among the variables. The ANOVA results for RSM indicated highly significant models ($p < 0.05$) with R^2 values of 99.48% for TCC, 99.35% for TAC, and 99.77% for FRAP. These high R^2 values suggest that RSM effectively captures the

variability in the data, making it a robust tool for optimization. RSM provided slightly better predictive performance than GB, as evidenced by lower Root Mean Square Error (RMSE) and Absolute Average Deviation (AAD) values. Specifically, the RMSE for RSM was 0.065 for TCC, 0.597 for TAC, and 0.058 for FRAP, whereas the AAD values were 0.458%, 0.393%, and 0.360%, respectively. Additionally, the predicted values for the optimal conditions (48.68 °C, 11.15 minutes, and 82.62% amplitude) closely matched the experimental values, with a percentage difference of 5.85% for TCC, 2.53% for TAC, and 5.66% for FRAP.

Conversely, GB is an ensemble learning technique that builds models sequentially to correct the residuals of previous models. This method was applied using a grid search cross-validation to optimize key hyperparameters, identifying optimal conditions at 49.62 °C, 11.08 minutes, and 84.62% amplitude. The GB model also demonstrated high

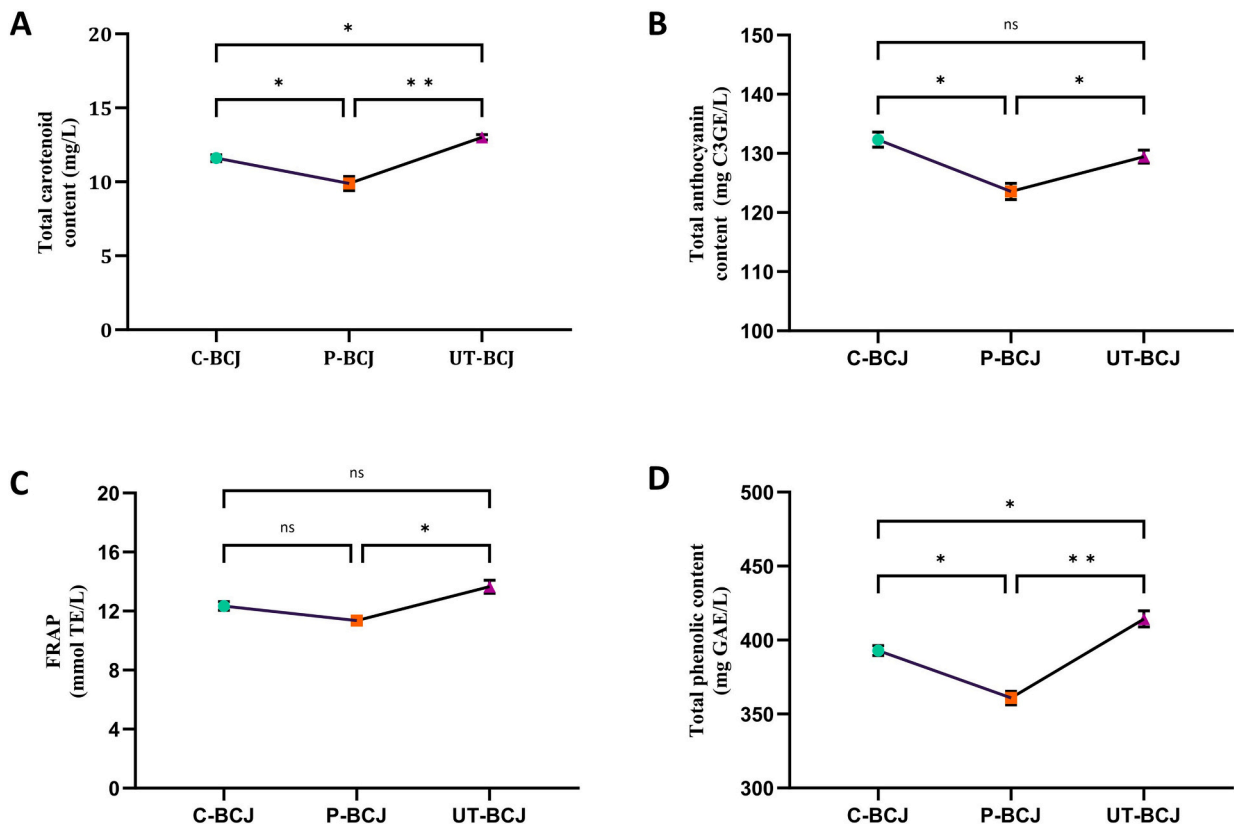


Figure 2. Results for total carotenoid content (A), Total anthocyanin content (B), FRAP (C), and Total phenolic content (D) of black carrot juices. Characters atop bars indicate statistically significant differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). n.s: non-significant.

predictive accuracy with R^2 values identical to RSM's for TCC, TAC, and FRAP. However, the RMSE values for GB were slightly higher at 0.083 for TCC, 0.664 for TAC, and 0.067 for FRAP, and the AAD values were 0.641%, 0.5106%, and 0.452%, respectively. While GB's predictive performance was slightly lower than RSM's, it still provided robust results and demonstrated its capability to handle complex, non-linear relationships between the variables. The percentage differences between the predicted and experimental values for the optimal conditions were 6.57% for TCC, 3.34% for TAC, and 4.55% for FRAP, comparable to RSM.

Both RSM and GB effectively optimize the bioactive components of ultrasound-treated black carrot juice. RSM has a slight edge in predictive accuracy and model fit, making it a more reliable choice for this specific dataset and experimental design. However, GB is also a powerful alternative, especially in scenarios where non-linear relationships are prevalent. Both models' high compatibility and accuracy underscore their utility in food science and bioactive component optimization. This detailed comparison highlights the nuanced strengths of each approach, providing a comprehensive understanding for researchers seeking to

apply these methodologies in similar optimization problems.

GB modeling was utilized to assess the impact of the independent variables, specifically temperature, time, and amplitude, on TCC (mg/L), TAC (mg C3GE/L), and FRAP (mmol TE/L). According to GB, the optimal settings for the independent variables were 49.62 °C for temperature, 11.08 minutes for duration, and 84.62% for amplitude. Under these optimized conditions, TCC was 11.93 mg/L, TAC 125.73 mg C3GE/L, and FRAP 14.06 mmol TE/L. The agreement between GB and RSM was strong, showing a high degree of alignment between the experimental results and the predicted outcomes. % Difference (GB) values are 6.57% for adjusted TCC (mg/L), 3.34% for TAC (mg C3GE/L) and 4.55% for FRAP (mmol TE/L). The % Difference (RSM) is 5.85% for TCC (mg/L), 2.53% for TAC (mg C3GE/L) and 5.66% for FRAP (mmol TE/L). Yıkmaş et al. (2024) observed a strong agreement between the experimental data and the RSM-predicted data in their study on gilaburu water, similar to the findings in our study (Yıkmaş et al., 2024).

3.2. RSM and GB model comparison

The table compares the performance of the RSM and GB models in predicting TCC, TAC, and FRAP (Table 3). The performance metrics used include Root Mean Square Error (RMSE), Coefficient of Determination (R^2), and Average Deviation of Differences (ADD%). (See Table 4.)

Looking at the RMSE values, RSM demonstrates lower error rates across all metrics compared to GB. For instance, the RMSE for TCC is 0.065 with RSM and 0.083 with GB. Similar patterns are observed for TAC and FRAP, indicating that RSM provides more accurate predictions. Both models exhibit high R^2 values of 0.99, indicating that they can explain 99% of the variability in the data. This shows that both models fit the data well. However, the lower RMSE and ADD values for RSM highlight its superior predictive accuracy.

The ADD values further confirm RSM's better performance. For TCC,

Table 3
Comparison of the performance of RSM and GB models in predicting TCC, TAC, and FRAP

Metrik	TCC (RSM)	TCC (GB)	TAC (RSM)	TAC (GB)	FRAP (RSM)	FRAP (GB)
RMSE	0.065	0.083	0.597	0.664	0.058	0.067
R^2	0.99	0.99	0.99	0.99	0.99	0.99
ADD (%)	0.458	0.641	0.393	0.5106	0.360	0.452

RMSE: root mean square error; AAD: absolute average deviation; R^2 : R-squared; GB: gradient boosting; TCC: total carotenoid content; TAC: total antioxidant capacity; FRAP: ferric reducing antioxidant power.

Table 4
Properties of Phenolic Compounds of C-BCJ, P-BCJ, and TS-BCJ Samples

Analyzes		Samples		
		C-BCJ	P-BCJ	TS-BCJ
Phenolic compounds (µg/mL)	Chlorogenic acid	89.75 ± 1.28 ^b	35.39 ± 1.46 ^a	149.26 ± 2.13 ^c
	Catechin hydrate	62.06 ± 0.89 ^c	10.10 ± 0.42 ^a	44.11 ± 0.63 ^b
	Caffeic acid	0.10 ± 0.00 ^a	1.90 ± 0.08 ^c	0.42 ± 0.01 ^b
	4-Hydroxybenzoic acid	n.d	n.d	0.15 ± 0.00
	Vanillin	0.76 ± 0.01 ^a	2.57 ± 0.11 ^b	2.47 ± 0.04 ^b
	p-Coumaric acid	0.11 ± 0.00 ^a	1.05 ± 0.04 ^b	1.01 ± 0.01 ^b
	Rutin	n.d	0.84 ± 0.03 ^a	0.80 ± 0.01 ^a
	t-Ferulic acid	3.65 ± 0.05 ^b	2.38 ± 0.10 ^a	3.95 ± 0.06 ^c
	Hydroxycinnamic acid	0.43 ± 0.01 ^b	0.30 ± 0.01 ^a	0.84 ± 0.01 ^c
	Naringin	19.09 ± 0.27 ^b	1.31 ± 0.05 ^a	n.d
	o-Coumaric acid	0.72 ± 0.01	n.d	n.d
	Rosmarinic acid	0.21 ± 0.00	n.d	n.d
	Salicylic acid	27.23 ± 0.39 ^b	n.d	17.50 ± 0.25 ^a
	Resveratrol	0.05 ± 0.00 ^a	0.08 ± 0.00 ^b	0.53 ± 0.01 ^c
	Quercetin	1.33 ± 0.02 ^b	0.76 ± 0.03 ^a	5.18 ± 0.07 ^c
	Total	205.49 ± 2.94 ^b	56.69 ± 2.33 ^a	226.22 ± 3.23 ^c

The results are the mean ± standard deviation (n = 3). The values marked with different letters within the line are significantly different from each other (p < 0.05). C- BCJ: Control black carrot juice; P- BCJ: Thermal pasteurized black carrot juice; TS-BCJ: thermosonication-treated black carrot juice; n.d: Not detected.

RSM shows an ADD of 0.458%, compared to GB's 0.641%. Similar trends are seen with TAC and FRAP, where RSM consistently outperforms GB. Overall, while both models show a good fit to the data, the RSM model consistently yields lower error rates and higher predictive accuracy than the GB model. This suggests that RSM is more reliable and precise for predicting TCC, TAC, and FRAP, making it a preferable choice for similar applications.

3.3. Bioactive compounds

In this study, the effects of different processing methods (control, thermal pasteurization, thermosonication) applied to black carrot juice samples on total carotenoid content, total anthocyanin content, The ferric reducing ability of plasm (FRAP) and total phenolic content were investigated. Total carotenoid content was measured as 13.01 mg/L in the thermosonicated black carrot juice (TS-BCJ) sample. This value is higher than 11.6 mg/L in the C-BCJ and 9.885 mg/L in the P-BCJ. The results indicate that these differences are significant (p < 0.05), demonstrating that thermosonication is effective in preserving and enhancing carotenoid levels. These discrepancies in results may be attributed to variations in the operating conditions.

Regarding anthocyanin content, the C-BCJ showed the highest value with 132.33 mg C3GE/L. While the TS-BCJ had a lower value with 129.44 mg C3GE/L, the pasteurized black carrot juice sample (P-BCJ) had the lowest anthocyanin content with 123.565 mg C3GE/L. These results show that pasteurization is not effective in preserving anthocyanins, but rather causes anthocyanin loss. According to the results, the differences in these components are highly significant (p < 0.001).

Sucheta and Yadav (2020) found anthocyanins, phenolics, and antioxidant activity at 297.9 mg/L, 1285.3 mg/L, and 37.6 µM/mL in Black carrot pomace. The results demonstrated that conventional heating yielded higher levels of total phenolics and anthocyanins and enhanced antioxidant activity compared to ultrasound treatment in black carrot pomace (Sucheta & Yadav, 2020).

When the FRAP values were examined, it was seen that the thermosonicated group (TS-BCJ) had the highest antioxidant capacity with 13.65 mmol TE/L. This value was significantly higher than the levels of 12.345 mmol TE/L in the C-BCJ and 11.35 mmol TE/L in the P-BCJ (p < 0.01). The results show that thermosonication is effective in increasing the antioxidant capacity. These findings follow the existing literature, which has demonstrated that ultrasound technology can enhance antioxidant activity (Bhat et al., 2011; Muzaffar et al., 2016). The elevated anthocyanin and phenolic concentrations observed in our study were significantly correlated with enhanced antioxidant activity. It is conceivable that additional extraction of phenolic compounds as a consequence of cavitation may result in an augmented antioxidant profile.

In terms of total phenolic content, the thermosonicated group (TS-BCJ) showed the highest value with 414.28 mg GAE/L, while the C-BCJ showed 392.905 mg GAE/L and the pasteurized group (P-BCJ) showed lower values with 360.905 mg GAE/L. These differences were significant according to the results (p < 0.001). This indicates that thermosonication is more effective in preserving phenolic compounds. Similarly, Kaur et al. (2023) found that there was a significant (P < 0.05) increase in the phenolic content of black carrot juice after sonication (364.39 ± 5.11 mg GAE/100mL to 420.43 ± 4.10 mg GAE/100mL) (Kaur et al., 2023). Additionally, Hasheminya et al. (2022) found that ultrasound treatment increased the phenolic content and antioxidant properties of black carrot juice (Hasheminya & Dehghannya, 2022).

The study results show that processing methods significantly affect the preservation and bioavailability of bioactive compounds in black carrot juice. The thermosonication process emerged as the most effective method for preserving and increasing carotenoids, anthocyanins, and antioxidant capacity. Pasteurization was remarkably ineffective in preserving anthocyanins and caused losses in these components. According to the results, the effects of these processing methods on bioactive compounds were demonstrated. It is suggested that thermosonication is a method that should be preferred, especially in preserving phenolic compounds and increasing their bioavailability.

3.4. Phenolic compounds

This study comprehensively evaluated the effects of different processing methods (C-BCJ, P-BCJ, and TS-BCJ) applied to black carrot juice samples on the diversity, preservation, and concentration of phenolic compounds. The findings revealed that thermosonication was the most effective treatment in terms of preservation and increasing the concentration of phenolic compounds. Additionally, the results revealed statistically significant differences (p < 0.05).

Chlorogenic acid concentration was determined at the highest level as 149.26 µg/mL in TS-BCJ sample. This value was statistically significantly higher than the levels of 89.75 µg/mL in the C-BCJ and 35.39 µg/mL in the P-BCJ (p < 0.05). Lopez-Martinez et al. (2022) found chlorogenic acid was the main phenolic group in ultrasound-treated carrots with turmeric addition (Lopez-Martinez et al., 2022). When total phenolic compound concentrations were examined, total phenolic content in TS-BCJ was measured as 226.22 µg/mL, which was statistically significantly higher than the values of 205.49 µg/mL in the control group and 56.69 µg/mL in the pasteurized group (p < 0.05). These data demonstrate the superiority of thermosonication process in terms of the preservation and enrichment of phenolic compounds.

Caffeic acid concentrations, although generally low, were found to be at the highest level in the thermally pasteurized group (P-BCJ) with 1.90 µg/mL, while this value was determined as 0.42 µg/mL in TS-BCJ

and 0.10 µg/mL in C-BCJ. These differences were also statistically significant. Similarly, in the literature, the levels of caffeic acid and coumaric acid in black carrots have been relatively low in comparison to the levels of chlorogenic acid (Anandhi et al., 2024; Blando et al., 2021). Quercetin concentration was determined as 5.18 µg/mL in TS-BCJ, 1.33 µg/mL in C-BCJ and 0.76 µg/mL in P-BCJ. Erdal et al. (2022) found that p-coumaric acid and quercetin levels increased significantly after ultrasound treatment, similar to the present study (Erdal et al., 2022). Vanillin has antimicrobial and antioxidant properties and is considered a potential food-preserving agent (Tomadoni et al., 2016). The amount of vanillin was determined as 2.57 µg/mL in P-BCJ, 2.47 µg/mL in TS-BCJ, and 0.76 µg/mL in C-BCJ. These differences in vanillin levels suggest that pasteurization may increase some phenolic compounds. While the highest value of t-Ferulic acid was measured as 3.95 µg/mL in TS-BCJ, it was measured as 3.65 µg/mL in C-BCJ and 2.38 µg/mL in P-BCJ. It has been expressed that the temperature and duration of the ultrasound influence the t-ferulic value (Cengiz et al., 2021). The amount of resveratrol was found as 0.53 µg/mL in TS-BCJ, 0.08 µg/mL in P-BCJ and 0.05 µg/mL in C-BCJ. Differences in these compounds showed significant differences in statistical analyses ($p < 0.05$). The positive effect of ultrasound on resveratrol is consistent with the literature (Dulger Altiner et al., 2024; Sales & Resurreccion, 2009).

These findings show that thermosonication is highly effective in preserving and increasing phenolic compounds in black carrot juice. Pasteurization was observed to have a negative effect, especially on chlorogenic acid, catechin hydrate, and total phenolic compound concentrations, leading to the loss of these compounds. It was concluded that thermosonication process could preserve the nutritional values of functional beverages such as black carrot juice by increasing the bioavailability of phenolic compounds. The reliability of these findings is increased by the statistical significance of the results at the $p < 0.05$ level. Therefore, thermosonication is recommended as a preferable technique in processing black carrot juice, a food rich in phenolic compounds (Anandhi et al., 2024; Blando et al., 2021; Erdal et al., 2022).

3.5. Fuzzy logic sensory

The study employed the fuzzy mathematical sensory evaluation method to assess how different processing techniques impact the sensory quality of black carrot juice. The Aroma, Taste, Color, and Mouthfeel factor set was evaluated. The evaluator group consisted of 36 panelists. Sensory evaluation scores were conducted using a fuzzy scorecard utilizing a 6-point sensory scale (similar to the study by Das et al., 2021) (Das et al., 2021) detailed in Table 5. Based on the evaluation results of the samples, each factor was normalized to create the fuzzy matrix R(R1-R3).

$$R1 = \begin{vmatrix} 0,00 & 0,00 & 0,11 & 0,67 & 0,22 & 0,00 \\ 0,00 & 0,00 & 0,03 & 0,36 & 0,53 & 0,08 \\ 0,00 & 0,00 & 0,17 & 0,08 & 0,58 & 0,17 \\ 0,00 & 0,00 & 0,50 & 0,11 & 0,36 & 0,03 \end{vmatrix}$$

$$R2 = \begin{vmatrix} 0,00 & 0,06 & 0,44 & 0,50 & 0,00 & 0,00 \\ 0,00 & 0,03 & 0,44 & 0,31 & 0,22 & 0,00 \\ 0,00 & 0,17 & 0,39 & 0,36 & 0,08 & 0,00 \\ 0,00 & 0,06 & 0,42 & 0,17 & 0,36 & 0,00 \end{vmatrix}$$

$$R3 = \begin{vmatrix} 0,00 & 0,00 & 0,06 & 0,72 & 0,06 & 0,17 \\ 0,00 & 0,00 & 0,17 & 0,25 & 0,47 & 0,11 \\ 0,00 & 0,00 & 0,00 & 0,25 & 0,72 & 0,03 \\ 0,00 & 0,00 & 0,19 & 0,39 & 0,42 & 0,00 \end{vmatrix}$$

Utilizing the fuzzy transformation principle $Y = X \times R$, matrix computations were calculated with the weight set $X = \{0.26, 0.25, 0.25, 0.24\}$. The X matrix showed that the rating result for the four quality attributes of black carrot juice was almost equally weighted across all

Table 5
Black carrot juices sensory evaluation score.

TREATMENTS	AROMA			TASTE			COLOR			MOUTHFEEL					
	Not satisfactory	Fair	Medium Good (GD)	Very Good (GD)	Excellent (EX)	Not satisfactory	Fair	Medium Good (GD)	Very Good (GD)	Excellent (EX)	Not satisfactory	Fair	Medium Good (GD)	Very Good (GD)	Excellent (EX)
C-BCJ	0	0	4	24	8	0	0	1	13	19	3	0	6	21	6
P-BCJ	0	2	16	18	0	0	0	1	16	11	8	0	6	14	3
TS-BCJ	0	0	2	26	2	6	0	0	6	9	17	4	0	9	26

C- BCJ: Control black carrot juice; P- BCJ: Thermal pasteurized black carrot juice; TS-BCJ: thermosonication-treated black carrot juice.

Table 6
Fuzzy Comprehension Evaluation Results

TRs Y_i	Score
C-BCJ $Y_1 = \{0.000, 0.000, 0.196, 0.312, 0.423, 0.069\}$	7.365
P-BCJ $Y_2 = \{0.000, 0.080, 0.423, 0.338, 0.161, 0.000\}$	6.596
TS-BCJ $Y_3 = \{0.000, 0.000, 0.104, 0.406, 0.414, 0.079\}$	7.487

C- BCJ: Control black carrot juice; P- BCJ: Thermal pasteurized black carrot juice; TS-BCJ: thermosonication-treated black carrot juice.

attributes. Although there were no extreme differences between the weights, similar to the (Xu et al., 2023) results, the overall ranking was as follows: Aroma > Taste > Color > Mouthfeel. The comprehensive evaluation outcomes Y1-Y6 were derived and documented in Table 6. Subsequently, the total fuzzy comprehensive evaluation scores, T1-T6, were determined as depicted in the same table.

The TS-BCJ group achieved the highest overall comprehensive score, followed by the C-BCJ treatment across varied conditions, exhibiting slight variations in sensory scores compared to TS-BCJ treatment samples. In contrast, the P-BCJ group obtained the lowest comprehensive score. As a result, the application of TS-BCJ demonstrated a predominantly favorable impact on black carrot juice, evidenced by its attainment of the highest aggregate score.

4. Conclusion

This study highlights the potential of thermosonication in optimizing the bioactive components and enhancing the sensory quality of black carrot juice. The effects of parameters such as temperature, processing time, and ultrasonic amplitude on TCC, TAC, FRAP, and total phenolic content were optimized using RSM and GB modeling techniques. The findings reveal that thermosonication significantly increases these bioactive components, demonstrating that this processing method enhances black carrot juice's nutritional value and health benefits while improving its sensory attributes. Moreover, sensory evaluations using fuzzy logic confirmed that thermosonication improves black carrot juice's aroma, taste, color, and texture. Thermosonication showed superior performance, particularly in preserving and increasing phenolic compounds, compared to traditional processing methods. These results suggest that thermosonication should be considered an effective method for producing functional beverages like black carrot juice. Future research could test these findings across a broader range of products, providing recommendations for the wider application of thermosonication in the food processing industry.

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CRediT authorship contribution statement

Seydi Yıkımsı: Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Melikenur Türköl:** Writing – original draft, Visualization, Resources, Methodology, Data curation, Conceptualization. **Ishak Pacal:** Writing – review & editing, Visualization, Supervision, Methodology. **Aylin Duman Altan:** Writing – review & editing, Validation, Software. **Nazlı Tokatlı:** Writing – review & editing, Visualization, Methodology. **Gholamreza Abdi:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Nazan Tokatlı Demirok:** Writing – review & editing, Validation. **Rana Muhammad Aadil:** Writing – review & editing.

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.

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