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Ultrasound technology in environmental sustainability: Vinegar production from black carrot pulp

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ORIGINAL ARTICLE

Abstract

In this study, vinegar obtained from untreated traditional black carrot pulp was compared with vinegar obtained from black carrot pulp subjected to thermal pasteurization and ultrasound treatment. Ultrasound treatment significantly improved the preservation and bioavailability of bioactive compounds, with higher total carotenoid content (TCC), total anthocyanin (TAC), and antioxidant (FRAP) values. It efficiently released bioactives from cell walls, enhancing bioavailability. RSM optimization revealed optimal conditions at 8 minutes processing time and 59.7% amplitude. However, ultrasound-treated vinegar (UT-BCV) was preferred in sensory analysis. Utilizing black carrot pulp supports sustainability, circular economy, and bioavailability goals.

Keywords: Black carrot pulp, Ultrasound treatment, Bioactive compounds, Sustainable food production, Vinegar production

Introduction

Economic growth, population growth, and globalization lead to structural transformations in consumption patterns on a global scale (Hoa *et al.*, 2023; Mariam *et al.*, 2023; Mohamed *et al.*, 2023; Nasaruddin *et al.*, 2024). These transformations increase the pressure on natural resources and pave the way for the emergence of serious problems that threaten environmental sustainability (Sharma *et al.*, 2018). The environmental impact of food waste has been a major concern for environmentalists for many years (Tahir *et al.*, 2024). Various studies have shown that the greenhouse gases emitted by food waste around the world have, in some cases, greater environmental impacts than coal-fired power plants (Kohli *et al.*, 2023). This situation has increased the need for innovative approaches to guarantee resource efficiency, reduce environmental impact, and create more sustainable production systems (Dabiah *et al.*, 2023; Muddassir *et al.*, 2024; Xue *et al.*, 2024). In particular, the recovery and utilization of food waste by the principles of circular economy offers important opportunities for both environmentally friendly waste management and functional food production (Noman and Azhar, 2023).

Organic by-products such as seeds, pulp, and peel obtained from agricultural wastes, especially from fruits and vegetables, are generally underutilized and discarded (Noman and Azhar, 2023). However, these organic wastes are potential sources of bioactive compounds that are beneficial for health. Recycling these wastes enrich and develop functional food products (Pattnaik *et al.*, 2021; Mejhed *et al.*, 2023). Fruit and vegetable by-products can be incorporated into food products, recycling fruit and vegetable waste and reducing the ecological burden. These products contain bioactive substances with antioxidant, antimicrobial, anti-fungal, anti-diabetic, anti-cancer, neurotransmitter, and anti-inflammatory properties (Ahmed *et al.*, 2020). To effectively preserve these compounds, the processing methods used need to be optimized appropriately. Traditional processing methods are generally known to cause losses in bioactive compounds. This necessitates the development of technologies such as ultrasound (Hasheminya and Dehghannya, 2022). Ultrasound treatment is widely recognized as an environmentally safe, innovative, inexpensive, rapidly evolving, and scalable technology (Wen *et al.*, 2018).

Ultrasound treatment preserves bioactive compounds and offers higher sensory quality than conventional methods (Lopez-Martinez *et al.*, 2022; Shen *et al.*, 2021; Tokatlı Demirok *et al.*, 2023; Yıkmış, 2019). However, the ultrasound process parameters must be correctly optimized to ensure that the best use is made of this technology. Response surface methodology (RSM) is a mathematical method for optimizing processes. It involves designing experiments, building models, and studying how inputs affect outputs (Das *et al.*, 2024). Modelling ultrasound parameters allows the ideal conditions for preserving and enhancing bioactive compounds to be determined.

In this context, processing a food by-product such as black carrot pulp can potentially provide an effective solution for both waste management and sustainable production models. The study focuses on modelling and optimizing the bioactive compounds of vinegar produced from black carrot pulp. The modeling approaches used in the study were employed to predict and optimize the effects of ultrasound parameters (duration, temperature, and power) on bioactive compounds. In addition, bioavailability and physicochemical and sensory properties of the obtained products were compared with conventional processing methods. This study also aims to increase the applicability of innovative technologies in the food industry by providing an important roadmap for sustainable production models.

Material and Methods

Materials

Samples of black carrots (*Daucus carota* L.) were collected from a commercial company in Türkiye. The black carrot pulp was obtained by juicing black carrots and separating the solid residue for use in vinegar production. The collected black carrot pulp was prepared for vinegar production by sorting and cleaning, followed by mixing with presterilized water and pine honey as a source of carbohydrate to support yeast and acetic acid bacteria. The mixture ratios by weight were black carrot pulp (10%), sterilized water (80%), and pine honey (10%). For the first fermentation, *Saccharomyces cerevisiae* yeast was inoculated at a rate of 0.4%, and fermentation was carried out at 28°C for 24 days. In the second fermentation, 5% acetic acid culture was added, and the process continued at 28°C for 50 days, reaching around 4% acetic acid content before termination. The cellulosic microorganism layer, known as the mother of vinegar, formed during fermentation, was removed, and the vinegar was filtered to complete the production. The first method refers to the so-called untreated vinegar (C-BCV). The second method refers to the vinegar produced by thermal pasteurization (P- BCV). The third method (UT- BCV) refers to vinegar which has been treated using the reaction surface method and the application of ultrasound.

Methods

Ultrasound processing

Samples of black carrot vinegar were subjected to different ultrasound parameters. The process was conducted on 100 mL of black carrot vinegar using a Hielscher Ultrasonics UP200St (Berlin, Germany). The frequency was 26 kHz, and the power was 200 W. Different amplitudes (40–80%) and treatment times (4–12 minutes) were used in continuous mode with an ice bath to prevent overheating. After ultrasonic treatment, the samples were cooled and stored at −18±1°C.

Thermal pasteurization

The black carrot vinegar samples were transferred to 100 mL glass bottles and pasteurized at 85±1°C for

2 minutes using a water bath system (Wisd model WUC-D06H, Daihan, Wonju, Korea). Following pasteurization, samples were cooled to room temperature and stored at −18±1°C until further analysis.

Ultrasound modeling procedure for RSM

TCC (mg/L), TAC (mg C_3GE/L), and FRAP (mmol TE/L) values of black carrot vinegar were optimized by RSM using coded parameters time (X_1: 4–12 minutes) and amplitude (X_2: 40–80%). A central composite design (CCD) was used with 13 experimental studies with two selected variables at five levels (−1.41 (very low), −1 (low), 0 (medium), +1 (high), and +1.41 (very high)). Minitab software (version 19, Minitab Inc., State College, PA, USA) was applied to optimize the ultrasound treatment. Each experiment was performed with three replications.

The equation models were developed utilizing the following quadratic polynomial formula:

$$y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{\substack{i=1 \\ i<j}}^{3} \sum_{j=1}^{3} \beta_{ij} X_i X_j \qquad (1)$$

The following formula definition is provided:

The linear equation coefficient (β_i) for the first order
The quadratic equation coefficient (β_{ii})
The coefficient for the interaction between two factors (β_{ij})

Total carotenoid content (TCC)

The total amount of carotenoids was determined utilizing minor modifications to the spectroscopic methods used to analyze samples of black carrot vinegar (Martínez-Flores *et al.*, 2015; Zhou *et al.*, 2009). A 1 mL aliquot of black carrot vinegar 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. Saturated sodium chloride solution (0.5 mL) was added to this upper phase, and the mixture was shaken again. A small amount of sodium sulfate was added to the lower phase, and the solution was centrifuged at 4000 rpm for 10 minutes. After centrifugation, the upper phase was removed and 5 mL of methanol solution was added. The resulting mixture was analyzed with a UV-visible spectrophotometer at a wavelength of 450 nm. The absorbance values were compared against a calibration curve generated from β-carotene standard solutions, and the TCC in the vinegar was determined as mg β-carotene equivalent per liter.

Total anthocyanin content (TAC)

The pH difference method determined the TAC (Giusti and Wrolstad, 2001; Cemeroğlu, 2010). The black carrot vinegar samples were diluted and prepared for analysis A 0.4 M sodium acetate buffer (pH 4.5) containing potassium chloride and a 0.025 M potassium chloride solution (pH 1.0) were used to prepare the dilutions. Samples were allowed to settle for 15 minutes before measuring absorbance at 515 and 700 nm using a spectrophotometer with distilled water as a reference. For the analysis, 5 mL aliquots of black carrot vinegar were used. A 10 g portion of each sample was diluted with pure water, mixed thoroughly, and centrifuged at 4000 rpm for 10 minutes. Supernatant (1 mL) was removed and adjusted to 5 mL in 0.025 M potassium chloride solution (pH 1.0). Next, 1 mL of the clear supernatant was removed and diluted 10 times with sodium acetate buffer. The samples were then incubated and the absorbance readings were taken. The level of monomeric anthocyanins was assessed using cyanidin-3-glucoside, the predominant anthocyanin in black carrot vinegar. The following equation has been used. The calculation was done using Formula 2.

$$\mathrm{TAC(mg/L)} = \frac{\mathrm{A(MW)(DF)1000}}{(\varepsilon)(\mathrm{L})} \qquad (2)$$

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 C_3GE/L.

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 $FeCl_3$-$6H_2O$ 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 minutes. 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 vinegar.

In vitro simulated gastrointestinal digestion analysis

An in vitro digestion model was used followed by dialysis according to the method of Minekus *et al.* (2014). The methodology consists of three sequential phases, including the oral (α-amylase, pH 7.0), gastric (pepsin, pH 3.0), and intestinal (pancreatin and fresh bile, pH 7.0) phases. Digestions and determinations of total phenolic compounds (TPC), TAC, TCC, and antioxidant activity

(FRAP) were performed after the gastric and intestinal phases and were determined in triplicate for each treatment and replicate.

Statistical analysis

The results of this study are expressed as the mean of three replicates ± standard error. A one-way analysis of variance (ANOVA) was carried out using SPSS software (version 22.0, SPSS Inc., Chicago, IL, USA). Tukey's test was used to compare the means of the samples. In addition, RSM was employed to optimize ultrasound parameters (time and amplitude) using Minitab software (version 19, Minitab Inc., State College, PA, USA).

Result and Discussion

Bioactive compound optimization

The experimental parameters were effectively optimized utilizing the RSM. This design was structured to evaluate the combined effects of linear interactions and quadratic influences of ultrasound-assisted variables, aiming to maximize the yield of bioactive compounds in black carrot vinegar. Table 1 presents the experimental data for various parameter combinations alongside the predicted outcomes for TCC (mg/ L), TAC (mg C_3GE/L), and FRAP (mmol TE/L) following ultrasound treatment. The results demonstrated that the optimal conditions were achieved at a treatment duration of 8 minutes and an amplitude of 59.7%.

The effects of two independent variables, duration and amplitude, on the TCC (mg/ L) (Equation 3), TAC (mg C_3GE/L) (Equation 4), and FRAP (mmol TE/L) (Equation 5) properties of black carrot vinegar are given below.

$$\mathrm{TCC}\left(\frac{\mathrm{mg}}{\mathrm{L}}\right) = -7.486 + 1.0172X_1 + 0.23894X_2 - 0.035010X_1X_1 - 0.001460X_2X_2 - 0.009051X_1X_2 \quad (3)$$

$$\mathrm{TAC}\left(\mathrm{mg}\frac{\mathrm{C3GE}}{\mathrm{L}}\right) = 19.36 - 0.200X_1 + 0.0631X_2 - 0.19907X_1X_1 - 0.004342X_2X_2 + 0.05759X_1X_2 \quad (4)$$

Table 1. Ultrasound RSM analysis of dependent and independent variables and TCC, TAC, FRAP results.

Run no.[a]	Independent variables		Dependent variables					
	Time (X_1)	Amplitude (X_2)	TCC (mg/L)		TAC (mg C_3GE/L)		FRAP (mmol TE/L)	
			Experimental data	RSM predicted	Experimental data	RSM predicted	Experimental data	RSM predicted
1	4	60	2.93	2.93	17.49	17.35	4.57	4.58
2	8	60	3.16	3.15	20.96	20.82	5.82	5.88
3	8	60	3.16	3.15	20.83	20.82	5.90	5.88
4	8	40	2.73	2.74	19.09	19.03	5.68	5.67
5	8	60	3.16	3.15	20.72	20.82	5.90	5.88
6	10	70	2.43	2.42	20.76	20.91	6.32	6.38
7	12	60	2.24	2.24	17.95	17.91	5.87	5.83
8	8	60	3.14	3.15	20.96	20.82	5.87	5.88
9	8	80	2.39	2.39	19.25	19.14	5.98	5.96
10	6	70	3.13	3.13	18.07	18.32	5.15	5.16
11	6	50	2.94	2.94	20.37	20.57	5.61	5.61
12	8	60	3.12	3.15	20.96	20.82	5.96	5.88
13	10	50	2.96	2.96	18.45	18.55	5.59	5.64
UT-BCV (RSM optimization parameters)	8 minutes	59.7 % amplitude	3.13		20.82		5.9	
Experimental values			3.23±0.07		22.19±0.49		6.14±0.13	
% Difference			3.19		6.58		4.06	

X_1: time; X_2: amplitude; RSM: response surface methodology; TCC: total carotenoid content; TAC: total anthocyanin content; C_3GE: cyanidin-3-glucoside equivalents; FRAP: ferric reducing antioxidant power; TE: trolox equivalents; UT-BCV: ultrasound-treated black carrot vinegar.

$$\text{FRAP}\left(\text{mmol}\frac{\text{TE}}{\text{L}}\right) = 8.076 - 0.0614\,X_1 - 0.0932\,X_2 - 0.04215\,X_1X_1 - 0.000155\,X_2X_2 + 0.01488\,X_1X_2 \quad (5)$$

When the equations are analyzed, it is seen that the increase in X_1 (time–minute) affects the TCC value positively, while it affects TAC and FRAP values negatively. The increase in X_2 (amplitude %) value was determined to positively affect TCC and TAC values and negatively affect FRAP value. It was observed that TCC, TAC, and FRAP findings of black carrot vinegar were negatively affected by the squared effects of X_1 and X_2 variables. Table 2 presents the optimization results, related R^2 values, ANOVA results, incompatibility evaluation, and regression coefficients for TCC (mg/ L), TAC (mg C_3GE/L), and FRAP (mmol TE/L) contents of black carrot vinegar.

Two key independent variables in the treatments applied to black carrot vinegar, namely, the treatment time (X_1) and the ultrasonic amplitude (X_2), play important roles in the extraction of bioactive compounds. As the processing time increases, more time is provided for the disintegration of cellular structures, which contributes to the increase of bioactive parameters such as TCC, TAC, and FRAP. Ultrasonic amplitude determines the energy density. Higher amplitude levels enable the physical disintegration of cells and a more efficient release of the bioactive compounds they contain. As a result of ultrasonic treatments applied to black carrot vinegar, it was determined that the TCC was significantly affected by the process time (X_1) and amplitude (X_2) parameters. With RSM optimization, the TCC value was predicted to be 3.13 mg/L at 8 minutes of processing time and 59.7% amplitude level, and this value was measured as 3.23±0.07 mg/L with a slight increase in experimental results. This supports the accuracy and reliability of the RSM model.

ANOVA analysis revealed that both procedure duration (X_1: F-value 2230.15, $p<0.01$) and amplitude (X_2: F-value 559.99, $p<0.01$) had highly significant effects on TCC. The R^2 value of the model was calculated as 99.91%, indicating that the model provided a firm fit. For example, at 4 minutes of treatment time and 60% amplitude level, the TCC value was 2.93 mg/L both experimentally and in the model prediction. When the treatment time was increased to 8 minutes, the experimental TCC value was 3.16 mg/L and the model prediction was 3.14 mg/L. Lopez-Martinez *et al.* (2022) observed that the TCC decreased as the ultrasound time increased, similar to our study, because of ultrasound treatment applied to turmeric-added mango and carrot drink mixture (Lopez-Martinez *et al.*, 2022). Studies on mango-based beverages (Mercado Mercado *et al.*, 2018) and guava juice (Campoli *et al.*, 2018)

Table 2. ANOVA in the regression model of the central combination test.

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	5	1638.71	0.000	120.91	0.000	170.36	0.000
Linear	2	1395.07	0.000	3.54	0.087	232.59	0.000
X_1	1	2230.15	0.000	6.82	0.035	441.54	0.000
X_2	1	559.99	0.000	0.27	0.622	23.64	0.002
Square	2	2290.62	0.000	222.94	0.000	126.95	0.000
X_1X_1	1	2818.45	0.000	415.04	0.000	244.10	0.000
X_2X_2	1	3061.64	0.000	123.41	0.000	2.06	0.195
Two-Way Interaction	1	822.17	0.000	151.61	0.000	132.68	0.000
X_1X_2	1	822.17	0.000	151.61	0.000	132.68	0.000
Error	7						
Lack-of-Fit	3	0.10	0.956	5.42	0.068	1.08	0.454
Pure Error	4						
Total	12						
R2		99.91%		98.86%		99.18%	
Adj. R^2		99.85%		98.04%		98.60%	
Pred. R^2		99.84%		93.09%		96.25%	

X_1: time; X_2: amplitude; DF: degrees of freedom; R^2: coefficient of determination; p<0.05: significant differences; p<0.01: very significant differences: TCC: total carotenoid content; TAC: total monomeric anthocyanin content; C_3GE: cyanidin-3-glucoside equivalents; FRAP: ferric reducing antioxidant power; TE: trolox equivalents.

found that the carotenoid content of fruit juices decreased as the duration and amplitude of ultrasound treatment increased. The authors suggested that the decrease in TCC could be explained by the effects of cavitation on their chemical structure, especially when dissolved in aqueous solutions (Campoli *et al.*, 2018; Rojas *et al.*, 2016). Atalar *et al.* (2020) applied ultrasound treatment to pasteurized rosehip (*Rosa canina* L.) nectar and concluded that the carotenoid content increased as the duration and amplitude values increased, contrary to our study (Atalar *et al.*, 2020). Although increasing the time increases the liberation of carotenoids, there is a risk of thermal degradation with excessively long processing times or high amplitude levels. Therefore, it is important to carefully optimize the process conditions. The effect of time and amplitude on TCC is shown in Figure 1. The TCC figure shows a positive correlation between treatment time (X_1) and amplitude (X_2) and carotenoid content. As the amplitude increases, so does the energy input. This facilitates the release of carotenoids from cellular structures. However, a plateau or a slight decrease was observed at very high combinations of processing time and amplitude because of the thermal degradation of carotenoids. The findings show that the highest TCC levels were obtained at medium-high amplitudes and medium times, indicating that the process conditions should be stabilized to improve carotenoid extraction efficiency.

The TAC in black carrot vinegar reflects the amount of naturally occurring anthocyanins and is significantly affected by the ultrasonic process parameters. At 4 minutes treatment time and 60% amplitude level, the TAC value was determined experimentally as 17.49 mg C_3GE/L, and the RSM estimate was 17.35 mg C_3GE/L. When the processing time was increased to 8 minutes, the TAC value increased to 20.96 mg C_3GE/L experimentally, and the RSM estimate increased to 20.81 mg C_3GE/L at the same amplitude. These results indicate that the increase in processing time promotes the liberation of anthocyanins. Similar to our study, Yıkmış *et al.* (2024) conducted a study on gilaburu juice and found an increase in the TAC value as the ultrasound time increased at the same amplitude values (Yıkmış *et al.*, 2024). However, if the amplitude level is increased excessively, anthocyanin degradation may occur, leading to a decrease in TAC levels. As a result of RSM optimization, the TAC value was predicted as 20.82 mg C_3GE/L, while the experimental results were 22.19±0.49 mg C_3GE/L. The agreement of the model with the experimental results showed that it provided reliable predictions with a difference of 6.58%. ANOVA analysis showed that treatment time (X_1: F-value 6.82, $p<0.05$) had a significant effect on TAC, but the effect of amplitude (X_2: $p>0.05$) was not significant. These findings emphasize that treatment time is critical in increasing anthocyanin content.

The effect of time and amplitude on TAC is shown in Figure 2. The TAC figure shows a positive correlation between treatment time (X_1), amplitude (X_2), and carotenoid content. As amplitude increases, energy input increases and carotenoids are more easily released from cellular structures. However, a plateau or a slight decrease was observed at very high combinations of processing time and amplitude because of the thermal degradation of carotenoids. In another study, similar to our study, increasing ultrasound intensity resulted in a decrease in the anthocyanin content of black carrot juice (Hasheminya and Dehghannya, 2022) and red grape juice (Masouleh *et al.*, 2022). The findings show that the highest TAC levels were obtained at medium-high amplitudes

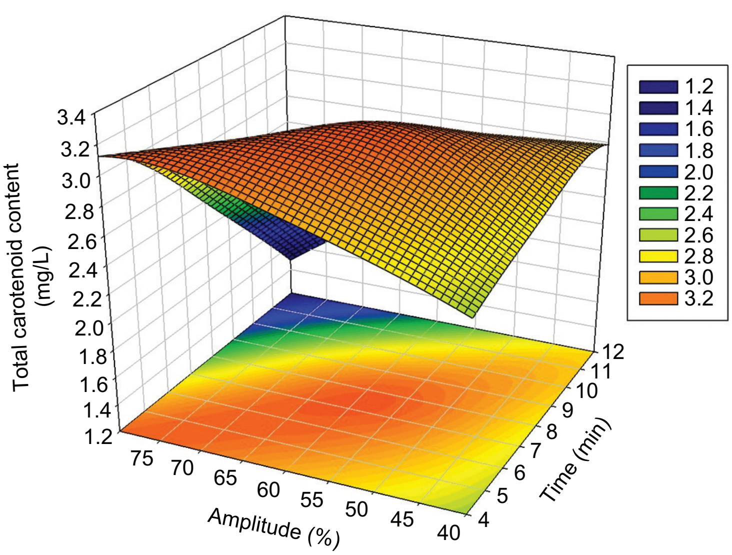

Figure 1. Effect of time and amplitude on total carotenoid content (TCC).

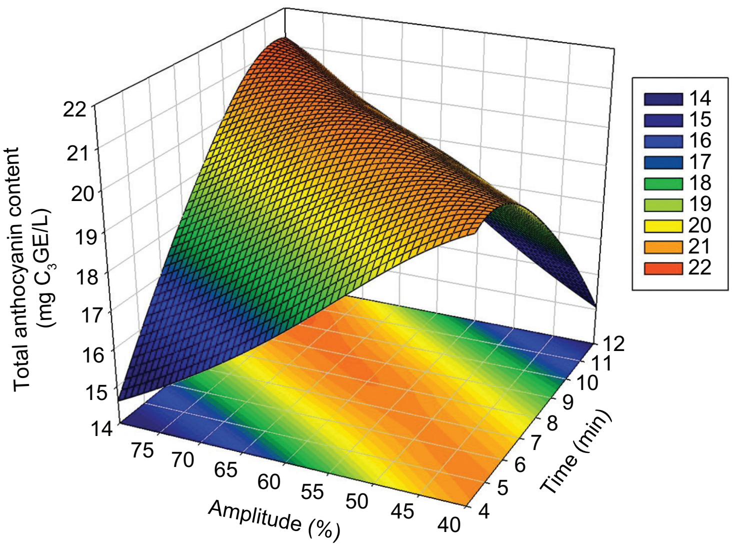

Figure 2. Impact of time and amplitude on total anthocyanin content (TAC).

and medium times, indicating that process conditions should be stabilized to improve carotenoid extraction efficiency.

The FRAP value, which expresses the antioxidant capacity of black carrot vinegar, is affected by ultrasonic process parameters and is associated with phenolic compounds. While the FRAP value was estimated as 5.9 mmol TE/L by RSM optimization, this value was found to be 6.14±0.13 mmol TE/L with a slight increase in experimental measurements. The R^2 value of the model was calculated as 99.18%, indicating that the model provides highly accurate predictions. ANOVA analysis showed that both treatment time (X_1: F-value 441.54, $p<0.01$) and amplitude (X_2: F-value 23.64, $p<0.01$) had significant effects on FRAP. When the treatment time was 4 minutes and the amplitude was 60%, the experimental FRAP value was 4.56 mmol TE/L, and the RSM estimate was 4.57 mmol TE/L. When the time was increased to 8 minutes, the experimental FRAP value increased to 5.82 mmol TE/L and the RSM estimate increased to 5.87 mmol TE/L. These results show that the antioxidant capacity increases with treatment time and amplitude. However, extreme processing conditions may cause oxidation of phenolic compounds, leading to decreases in the FRAP value. Therefore, it is essential to carefully select the process parameters to optimize the antioxidant capacity of black carrot vinegar.

The effect of time and amplitude on an FRAP is given in Figure 3. The FRAP figure shows that the antioxidant capacity increases with the increase in treatment time and amplitude. Li et al. (2022) reported that the FRAP value of red radish increased as the duration and power of ultrasound increased, similar to our study (Li *et al.*, 2022). It was observed that FRAP levels reached a maximum at medium times and high amplitude values; this may be attributed to the efficient release of phenolic compounds and other bioactive substances. However, extreme processing conditions may lead to oxidative degradation, resulting in a decrease in antioxidant activity. These results emphasize the importance of a balanced approach to increasing antioxidant capacity.

Ultrasound treatment parameters significantly affected TCC, TAC, and FRAP values of black carrot vinegar. Treatment time and amplitude, both alone and interactively (X_1X_2), were effective in liberating and preserving bioactive compounds. Minor differences were found between the optimum conditions determined by RSM (59.7% amplitude, 8 minutes treatment time) and experimental data, indicating that the model's predictive power was high. These results prove that ultrasonic treatment is an effective method to enhance the functional properties of black carrot vinegar. Bioactive compounds can be preserved by avoiding excessive duration and amplitude applications. This study provides valuable information to optimize both the nutritional and antioxidant properties of black carrot vinegar.

Bioactive compounds

C-BCV, P-BCV, and UT-BCV samples were compared in terms of their bioactive compounds. TPC, TCC, TAC, and FRAP values of bioactive compounds analyzed in black carrot vinegar are shown in Figure 4 (A–D). Phenolic compounds have an aromatic ring with one or more hydroxyl groups (Shah *et al.*, 2018). Ultrasound is an alternative nonthermal technology used for the

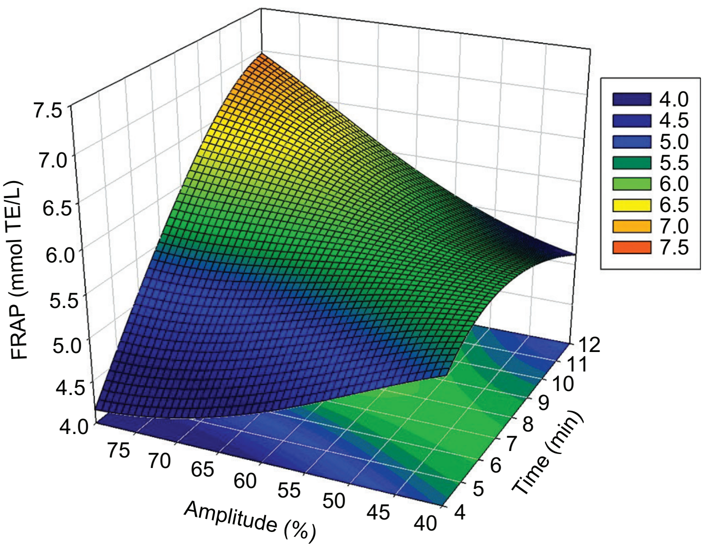

Figure 3. Influence of time and amplitude on antioxidant capacity (FRAP).

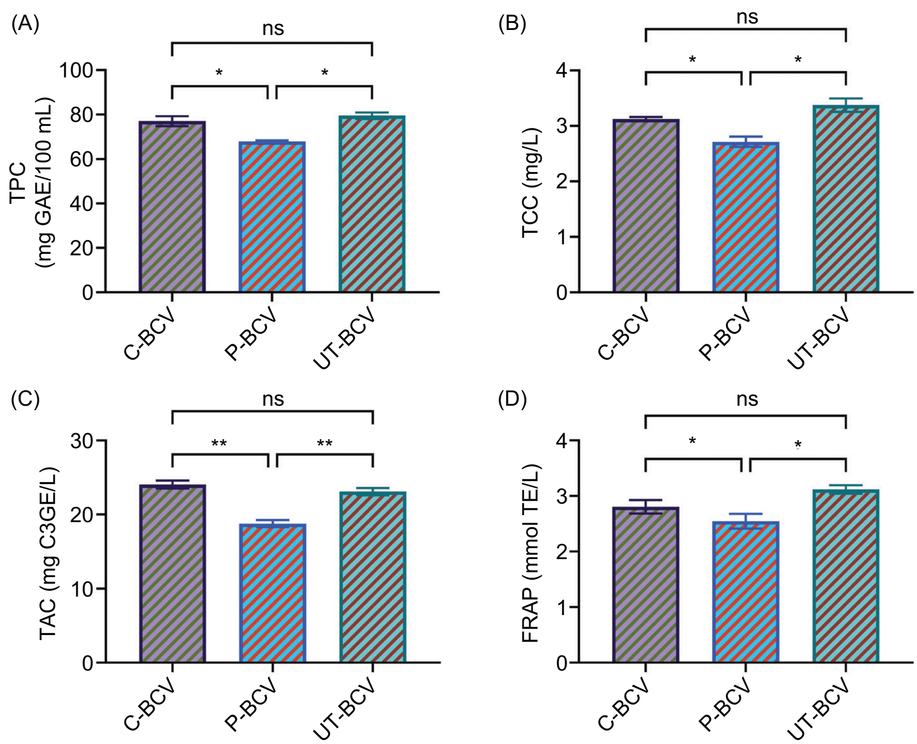

Figure 4. C-BCV (untreated black carrot vinegar), P-BCV (pasteurized black carrot vinegar), and UT-BCV (ultrasound-treated black carrot vinegar) samples were tested for TPC (A), TCC (B), TAC (C), and FRAP (D) values. Letters atop bars indicate statistically significant differences (ns: no significant; *p<0.05; **p<0.01, (n = 3±SD).

enrichment of bioactive compounds in foods and for food safety (Yıkmış, 2020; Yıkmış *et al.*, 2020). Ultrasound treatment enhanced the preservation and extraction of phenolic compounds, emphasizing the potential of the technology to improve functional properties in foods. The TPC results clearly demonstrated the effect of the treatments used in the production of black carrot vinegar on the preservation of phenolic compounds. UT-BCV had the highest TPC value of 79.52±1.46 mg GAE/100 mL, and there was no statistically significant difference between this value and C-BCV (p>0.05). Similarly, no significant changes in kiwifruit juice after exposure to ultrasound compared to fresh juice were reported by Bhutkar *et al.* (2024). However, P-BCV had a significantly lower phenolic content than the other groups with 67.89±0.48 mg GAE/100 mL ($p<0.01$). Türkol *et al.* (2024) concluded that thermal pasteurization decreased the TPC content of strawberry vinegar, and ultrasound treatment increased the TPC content (Türkol *et al.*, 2024). The increase of bioactive components with ultrasound treatment can be attributed to the breakage of cell walls under the influence of cavitation pressure and, thus, the release of forms bound to bioactive components (Aadil *et al.*, 2013). Studies on palm vinegar (Siddeeg *et al.*, 2019) and strawberry juice (Wang, Wang, *et al.*, 2019) also concluded that ultrasound treatment increased TPC content compared to nonultrasound treated samples. Another study on gilaburu juice found that thermal pasteurization decreased the total phenolic content, while ultrasound treatment increased it (Yıkmış *et al.*, 2024). Thermal degradation of phenolic compounds during the pasteurization process may be the main cause of these losses.

When carotenoid values were analyzed, UT-BCV had the highest TCC value with 3.38±0.12 mg/L. This group showed statistically significant differences compared to both C-BCV (3.13±0.04 mg/L) and P-BCV (2.72±0.09 mg/L) ($p<0.01$). Similar to our study, studies on pumpkin juice (Suo *et al.*, 2022), Cape gooseberry (Ordóñez-Santos *et al.*, 2017), and apple juice (Abid *et al.*, 2014) also showed a significant increase in carotenoid content in ultrasound-treated juice compared to the control group. In a study on different types of ultrasound-treated pumpkin juice, an increase in the carotenoid content of *C. moschata* juice was found as a result of ultrasound treatment. It is thought that the increase in carotenoid content after ultrasonic treatment may be because of the mechanical disruption of the cell wall and cell membrane structure by ultrasound and allowing the cell contents to flow out (Zhang *et al.*, 2024). These results are consistent with the results of ultrasonic treatment of grapefruit juice (Aadil *et al.*, 2015). The losses in the pasteurized sample of black carrot vinegar in our study reflect the sensitivity of carotenoids to thermal treatment and oxidative degradation. The high TCC values of UT-BCV indicate that ultrasound treatment is an effective alternative for the preservation of carotenoids. The significance level expressed by two asterisks statistically confirms that ultrasound technology is superior in terms of carotenoid content.

Anthocyanins are flavonoids that are commonly found in fruits and have strong antioxidant activity. Anthocyanins give fruits their purple, blue, or red color (Rybak and Wojdyło, 2023). Anthocyanin content showed significant changes according to the processing status of black carrot vinegar. C-BCV presented the highest value with 24.05±0.54 mg C_3GE/L, followed by UT-BCV with 23.11±0.49 mg C_3GE/L. While there was no statistically significant difference between these two groups ($p>0.05$), P-BCV had a significantly lower value than the other groups with 18.79±0.48 mg C_3GE/L ($p<0.01$). These results clearly indicate that anthocyanins undergo thermal degradation and oxidation during pasteurization. In contrast, ultrasound treatment minimized these losses by maintaining the stability of anthocyanins. The results show that ultrasound technology offers a significant advantage in the preservation of anthocyanins. Nguyen and Nguyen (2018) applied ultrasound treatment to mulberry juice and found that ultrasound treatment can increase the anthocyanin content of the sample (Nguyen and Nguyen, 2018). Thermal pasteurization of labu juice resulted in a 18.13% decrease in TAC, while ultrasound treatment increased it by 2.7%. The latter suggests that treatment duration, not temperature, affects TAC levels (Yıkmış *et al.*, 2024).

FRAP is based on absorbance changes in samples and is one of the most commonly used methods in the food industry and research (Wang, Vanga, *et al.*, 2019). FRAP values measuring antioxidant capacity showed that UT-BCV presented the highest value with 6.24±0.15 mmol TE/L. This group showed statistically significant differences compared to C-BCV (5.61±0.24 mmol TE/L) and P-BCV (5.09±0.27 mmol TE/L) ($p<0.01$). The lowest FRAP value of P-BCV can be explained by the thermal degradation of antioxidant compounds during the pasteurization process. The high FRAP value of UT-BCV indicates the positive effect of ultrasound treatment on the preservation of phenolic compounds and other antioxidant components. In a study that measured the effect of ultrasound treatment on the antioxidant capacity of kiwifruit juice, it was found that the total antioxidant capacity of kiwifruit juice was significantly increased after ultrasound treatment compared to untreated kiwifruit juice (Wang, Vanga, *et al.*, 2019). These increases are primarily attributed to the increase of antioxidant compounds (e.g., phenolic substances) after ultrasound treatment. Furthermore, the inactivation of some oxidation-related enzymes, such as polyphenol oxidase, is a consequence of the shear force occurring during the treatment, which contributes to the increase of the total antioxidant capacity of the fruit (Cheng *et al.*, 2007). In another study on strawberry juice, similar results to our study were obtained (Wang, Wang, *et al.*, 2019).

Sensory properties

This study evaluated the sensory characteristics of black carrot vinegar produced from black carrot pulp via different processing methods. Figure 5 shows the results of

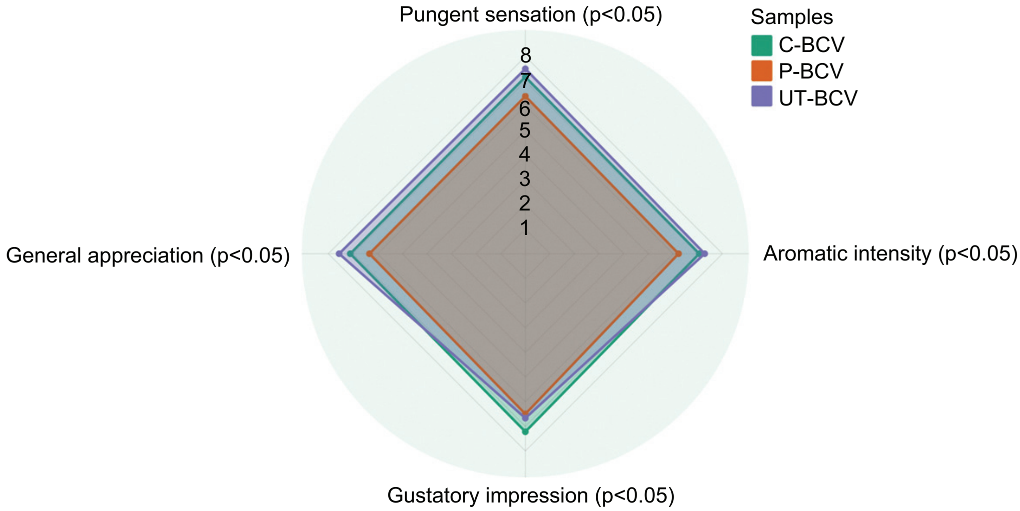

Figure 5. Results of the sensory analysis of the C-BCV, P-BCV, and UT-BCV. C-BCV: Untreated black carrot vinegar; P-BCV: Pasteurized black carrot vinegar; UT-BCV: Ultrasound-treated black carrot vinegar.

the sensory analysis of the vinegar samples. Statistically significant differences were observed in sensory parameters between C-BCV, P-BCV, and UT-BCV vinegar samples ($p<0.05$). In pungent sensation, the highest value was found in UT-BCV with 7.50, while this value was 7.17 for unprocessed vinegar and 6.39 for pasteurized vinegar. In a study conducted on Verjuice vinegar, similar to our study, the lowest pungent sensation value was found in pasteurized vinegar, while in contrast to our study, the highest pungent sensation value was found in untreated vinegar (Yıkmış *et al.*, 2020). In terms of aromatic intensity, the ultrasound-treated sample (UT-BCV, 7.28) had the highest score, followed by the untreated vinegar (C-BCV, 7.06), and the lowest score was obtained by the pasteurized vinegar (P-BCV, 6.22). In terms of gustatory impression, untreated vinegar (C-BCV, 7.22) presented the highest value, ultrasound treatment slightly decreased this value (6.67), while pasteurized vinegar showed the lowest value at 6.50. In the overall liking parameter, ultrasound-treated vinegar (UT-BCV, 7.56) was the most preferred product by consumers, while untreated vinegar (C-BCV, 7.11) ranked second with a high value in this parameter, and pasteurized vinegar (P-BCV, 6.33) received the lowest liking score. It can be inferred that the consumer appreciation of black carrot vinegar increased as a result of ultrasound treatment. In the study on the effect of ultrasound treatment on the sensory parameters of strawberry vinegar, a statistically significant increase was observed in pungent sensation, aromatic intensity, and general appreciation values as a result of ultrasound treatment ($p<0.05$) (Türkol *et al.*, 2024). In another study, similar to ours, the overall acceptability of ultrasonicated apple juices was higher than the pasteurized apple juice sample according to sensory evaluation results (Shen *et al.*, 2021).

Bioavailability

This study investigated the changes in the bioactive constituents of black carrot vinegar using heat treatment as a traditional method and ultrasound as an alternative technology during in vitro digestion. When predigestion samples are examined, it is seen that heat treatment causes a statistically significant decrease in all bioactive component contents ($p<0.05$) (Figure 6). Kamiloglu *et al.* (2016) studied changes in the bioactive components of processed black carrots and expressed that heat treatment processings lead to a conspicuous decrease in total phenolic content and total antioxidant activity. Exposure to high temperatures greatly reduces the stability of black carrot anthocyanins (Anandhi *et al.*, 2024; Kamiloglu *et al.*, 2018). On the other hand, in the predigestion step, all bioactive component contents of ultrasound-treated black carrot vinegar were statistically similar to the control sample.

When the change of bioactive components of black carrot vinegar samples during in vitro gastrointestinal digestion was examined, the amounts of bioactive components in all samples postdigestion decreased compared to undigested samples. The decrease in bioactive compounds after in vitro digestion has also been detected in related previous studies and is explained by the oxidation, degradation, or metabolism of polyphenols and anthocyanins to noncolored forms during digestion (Kamiloglu *et al.*, 2018; Toktaş *et al.*, 2018). However, in this study, decreasing of bioactive component content in pasteurized samples is statistically lower than in ultrasound-treated samples. UT-BCV also reached the highest values at all stages of digestion in terms of TPC, TCC, TAC, and FRAP antioxidant activity. Especially after intestinal digestion, the TPC value of UT-BCV (21.59±0.85 mg

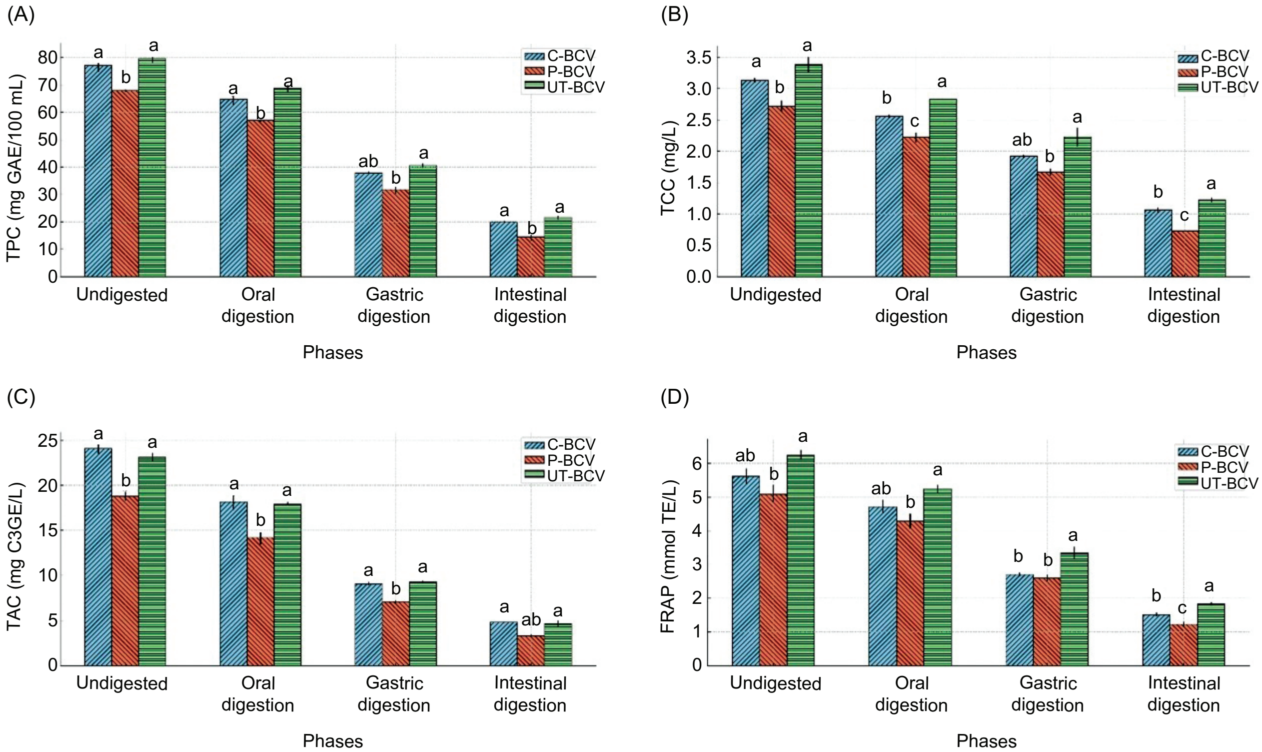

Figure 6. Changes in (A) the total phenolic content (mg GAE/100 mL), (B) total carotenoid content (mg/L), (C) total anthocyanin content (mg C_3GE/L), and (D) total antioxidant content-FRAP (mmol TE/L) of black carrot vinegar during in vitro gastrointestinal digestion.

GAE/100 mL) was found to be statistically similar to that of untreated vinegar (C-BCV, 19.87±0.46 mg GAE/100 mL) (p>0.05) but was significantly higher (p<0.05) than the values of pasteurized vinegar (P-BCV, 14.45±1.4 mg GAE/100 mL) (Figure 6). Similarly, while UT-BCV exhibits higher values than other groups in TCC and TACs, pasteurized samples have the lowest values in all parameters. Especially after intestinal digestion, the TCC value of UT-BCV (1.22±0.04 mg/L) showed a significant increase compared to C-BCV (1.06±0.04 mg/L) (p<0.05). In terms of TAC, UT-BCV showed better performance compared to pasteurized samples (p<0.05). These findings clearly show that ultrasound treatment contributes to the preservation of bioactive compounds and increases postdigestion bioavailability. Hasheminya and Dehghannya (2022) studied the ultrasound treatment of black carrot juice, and they found that ultrasound causes an increase in phenolic content and antioxidant activity, maintaining non-significant changes in total soluble solids, pH, viscosity, and turbidity of samples (Hasheminya and Dehghannya, 2022)

Recovery (%) results are important to evaluate how much bioactive compounds can be protected from digestion after different processes (Figure 7). In terms of TPC, the recovery rate of UT-BCV (27.15±0.57%) was found to be similar to C-BCV (25.78±1.35%) (p>0.05) but was significantly higher than P-BCV (21.28±1.91%) (p<0.05). According to the recovery of TCC, UT-BCV (36.17±2.51%) exhibited the highest protection rate compared to other processes. Similarly, TAC and FRAP recovery rates were statistically higher in UT-BCV (19.94±1.99% and 29.34±1.59%) than in P-BCV (17.62±1.28% and 23.87%). ±0.27) and at statistically similar levels to C-BCV (19.96±0.69% and 26.92±0.15%) (p>0.05). In the previous study, recovery of TPC for black carrot jam and marmalade after intestinal digestion was found in the range of 4.9–17.5% (Kamiloglu *et al.*, 2016; Toktaş *et al.*, 2018). The higher recovery values found in this study also show that processing black carrots as vinegar preserves bioactive components better during in vitro gastrointestinal digestion than processing it as marmalade and jam.

These findings indicate that ultrasound treatment increases the preservation of phenolic compounds, carotenoids, and antioxidants after digestion and provides higher bioavailability. Decreases in the amounts of bioactive components were observed in all groups throughout the digestion stages, but these decreases were significantly less in UT-BCV compared to the other treatments. For example, it has been determined that

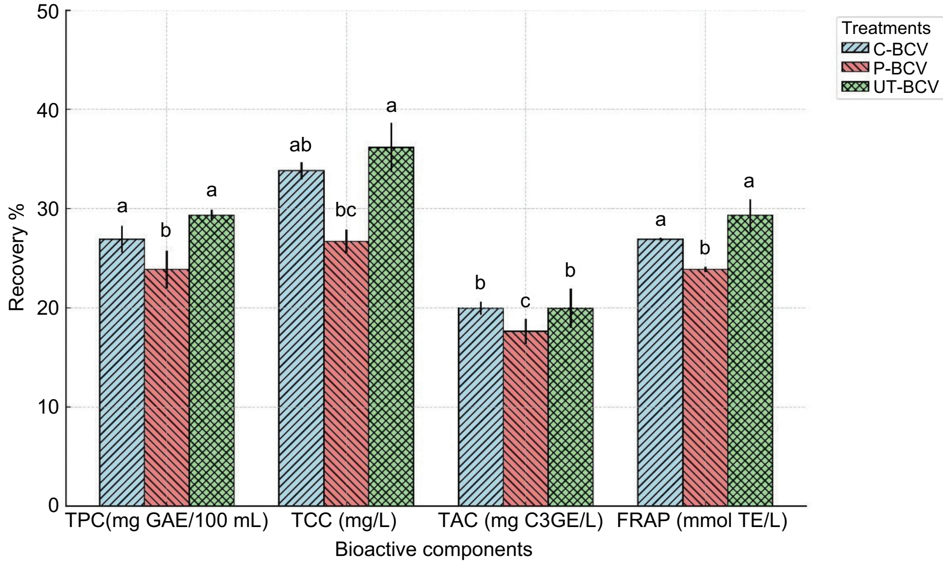

Figure 7. Recovery (%) values of bioactive compounds (TPC, TCC, TAC, and total antioxidant content-FRAP) at the end of the in vitro gastrointestinal digestion.

UT-BCV provides better protection in TPC, TCC, TAC, and FRAP values during intestinal digestion compared to other processes. This reveals that ultrasound treatment is an innovative technology that can optimize bioavailability compared to other processes, especially by increasing the digestion resistance of phenolic compounds. Turker and Dogan (2022) studied anthocyanin extraction techniques from black carrots and offered this technology to protect anthocyanins in food (Aslan Türker and Doğan, 2022).

The findings show that ultrasound processing is an effective method for preserving and increasing the bioavailability of bioactive components of functional foods such as black carrot vinegar. The positive effects observed, especially on phenolic compounds and antioxidant activities, show that this method can be used as an alternative to traditional processes such as pasteurization and improve the nutritional values of functional foods. Ultrasound technology, especially in beverage industries, also improves various quality characteristics (Hasheminya and Dehghannya, 2022). Therefore, ultrasound processing holds promise as an innovative approach.

Conclusion

The utilization of food waste is of great importance for sustainable food production and circular economy goals. The use of black carrot pulp in vinegar production offers an innovative approach to waste management and a solution that contributes to functional food production. In this study, it was determined that ultrasound technology provides superiority in terms of preservation of bioactive compounds, increasing bioavailability and improving sensory quality compared to conventional and thermal processing methods. The improvements in total carotenoid, TAC, and antioxidant capacity values showed that this technology provides a more effective release of bioactive components from cellular structures and increases bioavailability. In addition, the fact that ultrasound is an energy and environmentally friendly technology makes this method an advantageous option in terms of both economic and environmental sustainability. Stable results in physicochemical parameters and high consumer appreciation obtained in sensory analysis support the applicability of this technology in the production of functional products such as black carrot vinegar. In the future, studies focusing on utilizing different food wastes and scalable applications will contribute to disseminating sustainable production models in the food industry. This study provides a strong basis for the valorization of waste and the use of innovative technologies.

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Ethical Approval

This article does not contain any studies with human or animal subjects.

Authors Contributions

Melikenur Türkol did conceptualization, data curation, formal analysis, methodology, visualization, and writing–original draft. Seydi Yıkmış performed conceptualization, data curation, validation, visualization, methodology, resources, writing–review & editing, and supervision. Nazlı Tokatlı was involved in visualization, methodology, and writing–review & editing the original draft. Nihan Sağcan looked into methodology and writing–review & editing. Waseem Khadil was responsible for software, validation, visualization, and writing–review & editing. Suleiman A. Althawab did visualization and writing–review & editing. Tawfiq Alsulami Suleiman A. Althawab performed visualization and writing–review & editing. Abdullah Yinanç looked into writing–review & editing. Harun Aksu was concerned with writing–review & editing.

Conflicts of Interest

The authors do not have any conflicts of interest to report.

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