

## Optimization of Ultrasound Treatment of Beverage from Mango and Carrot with Added Turmeric Using Response Surface Methodology

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The effect of ultrasound treatment (UT) on a beverage from mango pulp and carrot juice with added turmeric powder on total soluble phenolic content (TSP), total carotenoid content (TC) and antioxidant capacity (AOC) was evaluated. Response surface methodology (RSM) was applied to obtain the optimal formulation of the beverage. The AOC was assigned as a response variable in addition to TSP and TC. Mathematical modeling showed that the formulation with 35% (v/v) of mango pulp, 10% (v/v) of carrot juice, and 0.7% (w/v) of turmeric powder, yielded the highest values of TSP, TC, and AOC. The beverages were subjected to different ultrasound conditions with varying exposure times (ET), sonication amplitudes (SA), and pulse cycles (PC) to obtain the highest values for response variables. Statistical modeling showed that a UT at 21 min ET, 100% SA, and 0.7 s PC, increased TSP, Trolox equivalent antioxidant capacity (TEAC), and ferric reducing antioxidant power (FRAP) by 15.5%, 45.1%, and 15.9%, respectively. Seven phenolic acids, three curcuminoids, five flavonoids, and a xanthonoid were identified in the beverages. The quantities of 3,4-dihydroxybenzoic acid, gallic acid, chlorogenic acids, (+)-catechin, quercetin, kaempferol, (-)-gallic catechin gallate, and mangiferin were higher in the UT beverage compared to the control, suggesting their release from cell-wall structures as a result of UT.

### ABBREVIATIONS

UT, Ultrasound treatment; TSP, total soluble phenolic content; TC, total carotenoid content; AOC, antioxidant capacity, RSM, response surface methodology; ET, exposure time; SA, sonication amplitudes, PC, pulse cycles; TEAC, Trolox equivalent antioxidant capacity; ABTS, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), FRAP, ferric reducing antioxidant power, TPTZ, 2,4,6-tripyridyl-s-triazine; GAE, gallic acid equivalent; TE, Trolox equivalent.

### INTRODUCTION

Several epidemiological studies have demonstrated the relationship between consuming beverages from vegetable sources and the prevention of diseases related to oxidative

stress, such as cardiovascular and neurodegenerative diseases and some types of cancer [Fujiki *et al.*, 2017; Wu *et al.*, 2016; Zhou *et al.*, 2015]. The main compounds to which these effects are attributed are phenolics and carotenoids. Consuming fruit and vegetable beverages can result in an increased intake of these compounds. Blending different fruit and vegetables increases the content and variety of bioactive compounds in beverages [Manna *et al.*, 2017]. Furthermore, it has been reported that combining compounds of different chemical characteristics, such as phenolic compounds and carotenoids, can possibly enhance their antioxidant properties [Zhao *et al.*, 2014]. To further maximize compound diversity and enhance organoleptic characteristics of beverages, the use of spices, like ginger or turmeric, and extracts thereof in their preparation has been investigated [Bhardwaj & Pandey, 2011]. In Mexico, mango and carrot are consumed

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by most of the population, consequently, their bioactive compound profile has been studied. Pacheco-Ordaz *et al.* [2018] established that mango contains chlorogenic acid, gallic acid, vanillic acid, protocatechuic acid,  $\alpha$ - and  $\beta$ -carotene, lutein, and zeaxanthin. Flavonoids, like quercetin 3-isobutyrate, quercetin 4'-*O*- $\beta$ -D-glucopyranoside-3,7-dimethyl ether, and quercetin, one gallotannin (theogallin) and 2 xanthenes (mangiferin and mangiferin isomer) have also been identified in mango pulp [Barrón-García *et al.*, 2022]. On the other hand,  $\beta$ -carotene, apigenin, rutin, chlorogenic acid, and protocatechuic acid have been reported in carrots [Šeregelj *et al.*, 2020]. Bisdemethoxycurcumin, demethoxycurcumin, curcumin, (+)-catechin, quercetin, gallic acid, and *p*-coumaric acid have been identified in turmeric [Pal *et al.*, 2020]. These molecules are known for their health benefits, which are related to the activation of different intracellular signaling pathways, immunomodulation, and cardioprotective properties [Palafox-Carlos *et al.*, 2012]. Due to the reported properties of mango and carrot, in conjunction with the trend of adding spices to beverages, the use of powdered turmeric rhizomes is proposed due to their anti-inflammatory and antimutagenic properties [Deogade & Ghate, 2015].

Different investigations have been carried out where ultrasound treatments (UT) have been applied to fruits such as *Annona muricata* [Aguilar-Hernández *et al.*, 2019, 2020; Nolasco-González *et al.*, 2022]; juices like red and yellow watermelon juice [Yıkımsı, 2020], black carrot juice [Hasheminya & Dehghannya, 2022], pear juice [Saeduddin *et al.*, 2016], Cape gooseberry juice [Ordóñez-Santos *et al.*, 2017]; and beverages like grape-based beverage [Ahmad *et al.*, 2020], carrot and grape beverage [Nadeem *et al.*, 2018], and mango-based beverage [Mercado-Mercado *et al.*, 2018]. These studies reported significant increases of bioactive compounds, like phenolics, carotenoids, anthocyanins or curcuminoids, in sonicated samples, as compared to the control, suggesting that UT is suitable to retain or enhance the content of bioactive compounds present in juices or beverages.

The aim of this study was to optimize UT parameters, like exposure time (ET), pulse cycle (PC) and sonication amplitude (SA), using response surface methodology (RSM), to obtain a beverage from mango, carrot and turmeric with the highest total soluble phenolic content (TSP), total carotenoid content (TC), and antioxidant capacity (AOC). Changes to the profile of antioxidants in the beverages as a result of UT were reported as well.

## MATERIALS AND METHODS

### Chemical and reagents

The Folin–Ciocalteu reagent, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Trolox (6-hydroxy-2,3,7,8-tetramethylchroman-2-carboxylic acid), TPTZ (2,4,6-tripyridyl-s-triazine), ferulic acid, as well as high-performance liquid chromatography (HPLC)-grade phenolic standards: *p*-coumaric acid, (+)-catechin hydrate, gallic acid, quercetin dihydrate, 3-hydroxybenzoic acid, and chlorogenic acid, were purchased from Sigma-Aldrich (St. Louis, MO, USA). The rest of the reagents used were obtained from J.T. Baker (Phillipsburg, NJ, USA). All solvents were of analytical grade.

### Beverage preparation

Mango cv. Ataulfo and carrot cv. Early Gold, both of commercial ripeness, were acquired from local suppliers in Hermosillo, Mexico. Turmeric powder (food grade) was purchased from Proquimerce enterprise (Jalisco, Mexico). Mangoes and carrots were washed with tap water and disinfected with 0.05 mL/L sodium hypochlorite for 5 min. Mango peel was manually removed using a stainless-steel knife, and the flesh was cut into 3 mm cubes. Carrots were cut into sections about 3 to 5 mm thick and were subjected to a juice extraction process using a commercial juice extractor (Moulinex model 140–1–03, Naucalpan, State of Mexico, Mexico). Mango pulp, carrot juice, turmeric powder, and purified water were mixed at a 35:10:0.7:54.3 (v/v/w/v) ratio and blended using a commercial blender (Oster®, Milwaukee, WI, USA). Beverage formulation was based on previous experiments in the Laboratory of Antioxidants and Functional Foods, Center for Research in Food and Development, A.C. (Hermosillo, Sonora, Mexico). The contents of mango, carrots, and turmeric were based in reference to the Codex Alimentarius [2005] for the preparation of juices ( $\geq 45\%$  fruit content) and for turmeric ( $\leq 0.7\%$ ). Experiments were performed in triplicate (3 separate beverages were made).

### Ultrasound treatment of beverage

UT was carried out according to the method of Wei *et al.* [2014] with some modifications. An ultrasonic processor (UP400S, Hielscher, Teltow, Germany) was used for sonication. The beverage (50 mL) was processed at a constant frequency of 24 kHz. Energy input was controlled by setting the maximum amplitude at 175  $\mu\text{m}$  and acoustic power density of 300 W/cm<sup>2</sup> of the sonicator probe (H7 Tip 7). Constant temperature of 25°C was maintained by circulating cold water through a jacketed vessel. The probe was immersed 2 cm into the beverage, and sonication started immediately. Exposure time (ET, 10–30 min), sonication amplitude (SA, 30–100%), and pulse cycles (PC, 0.4–0.8 s) varied. PC values were equal to acoustic power time in s, and the difference to 1 s as pause time (*e.g.*, 0.6 s indicates a power discharge of 0.6 s, and a pause of 0.4 s). Nine treatments (T1 to T9) were performed immediately after the beverage was prepared (Table 1). After UT, the beverages were filtered through filter paper (Whatman No. 1) under vacuum. In parallel, untreated beverages were also filtered. The filtrate was collected in a flask and used for the determination of TSP, TC, and AOC. Beverage samples (control and UT) were kept in sterilized airtight bottles and stored at 4°C until further analysis.

### Experimental design

A fractional 3<sup>3-1</sup> factorial design and RSM were used to establish the optimum conditions for UT. Factors were considered at different levels to determine which combination yielded the maximum TSP, TC, and AOC values. Exposure time ( $X_{ET}$ , min), pulse cycle ( $X_{PC}$ , s), and sonication amplitude ( $X_{SA}$ , %) were taken as design factors. The experimental design used, with three factors at three different levels:  $X_{ET}$  (10, 20 or 30 min),  $X_{PC}$  (0.4, 0.6 or 0.8 s), and  $X_{SA}$  (30, 65

TABLE 1. Levels of experimental design factors used to optimize ultrasound treatment of beverage from mango and carrot with added turmeric and levels of response variables: total soluble phenolic content (TSP), total carotenoid content (TC), Trolox equivalent antioxidant capacity (TEAC), and ferric reducing antioxidant power (FRAP).

Treatment	Factor			Response variable			
	X <sub>ET</sub> (min)	X <sub>PC</sub> (s)	X <sub>SA</sub> (%)	TSP (mg GAE/mL)	TC (μg β-carotene E/mL)	TEAC (mmol TE/mL)	FRAP (mmol TE/mL)
Control	–	–	–	5.21±0.11 <sup>i</sup>	26.78±1.19 <sup>f</sup>	100.16±1.19 <sup>h</sup>	0.59±0.03 <sup>g</sup>
T 1	10	0.4	30	5.81±0.06 <sup>h</sup>	42.87±1.03 <sup>c</sup>	171.49±3.02 <sup>d</sup>	0.68±0.00 <sup>e</sup>
T 2	10	0.6	65	7.95±0.03 <sup>de</sup>	42.09±1.93 <sup>c</sup>	173.23±2.13 <sup>c</sup>	0.83±0.04 <sup>cd</sup>
T 3	10	0.8	100	8.04±0.09 <sup>d</sup>	34.72±0.69 <sup>d</sup>	173.85±1.22 <sup>bc</sup>	0.90±0.02 <sup>b</sup>
T 4	20	0.4	30	6.36±0.05 <sup>f</sup>	43.25±1.66 <sup>c</sup>	168.09±1.31 <sup>c</sup>	0.62±0.03 <sup>g</sup>
T 5	20	0.6	65	6.17±0.04 <sup>g</sup>	53.71±1.54 <sup>a</sup>	161.25±0.71 <sup>g</sup>	0.77±0.03 <sup>d</sup>
T 6	20	0.8	100	8.79±0.10 <sup>a</sup>	30.21±0.04 <sup>e</sup>	195.84±2.09 <sup>a</sup>	0.87±0.01 <sup>c</sup>
T 7	30	0.4	30	7.41±0.13 <sup>e</sup>	34.00±1.97 <sup>d</sup>	176.09±2.64 <sup>b</sup>	0.64±0.02 <sup>e</sup>
T 8	30	0.6	65	8.28±0.05 <sup>bc</sup>	48.87±2.47 <sup>b</sup>	165.48±2.21 <sup>f</sup>	0.82±0.01 <sup>cd</sup>
T 9	30	0.8	100	8.43±0.02 <sup>b</sup>	27.93±0.84 <sup>f</sup>	172.32±2.58 <sup>d</sup>	0.97±0.02 <sup>a</sup>

Values are shown as mean ± standard deviation of three independent experiments. X<sub>ET</sub>, exposure time; X<sub>PC</sub>, pulse cycle; X<sub>SA</sub>, sonication amplitude; GAE, gallic acid equivalents; TE, Trolox equivalents. Different letters in the same column indicate significant differences ( $p < 0.05$ ).

or 100%), is shown in Table 1. TSP (mg gallic acid equivalents (GAE)/mL), TC (μg β-carotene equivalents/mL) and AOC (mmol Trolox equivalents (TE)/mL) were defined as response variables.

To predict the behavior of response variables, the second-order polynomial equation used in the response surface was as described in equation (1) [Savasari et al., 2015; Yirsaw et al., 2016]:

$$Y = \beta_0 + \sum_{i=1}^B \beta_i X_i + \sum_{i=1}^B \sum_{i \neq j}^B \beta_{ij} X_i X_j + E \quad (1)$$

where: Y is the predicted variable (TSP, TC or AOC), X<sub>i</sub> represents coded values for the factors (X<sub>ET</sub>, X<sub>PC</sub>, and X<sub>SA</sub>), β<sub>0</sub> is a constant, β<sub>i</sub> is the main effect coefficient for each variable, and β<sub>ij</sub> is the interaction effect coefficient. Model adequacy was evaluated using the F ratio. Lack of fit test was used to determine significant interactions in the model and the coefficient of determination (R<sup>2</sup> and R<sup>2</sup> adjusted) represented at a 5% level of significance.

### Optimization and validation of conditions

The numerical optimization technique was adapted to optimize the process conditions to provide maximum TSP, TC, and AOC. The nature of the optimal condition (peak or saddle point response) was also evaluated by transforming the developed regression model into the conical form, and the values were computed using the statistical software Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA). The experiment under optimal conditions was carried out in triplicate to determine the validity of the optimized parameters. The average values of the experiments were compared with the predicted values of the optimized conditions to determine the accuracy of the optimized conditions.

### Extracts from the beverage preparation

An aliquot of 30 mL was taken from the beverages (control and UT), lyophilized (Labconco, model 77520 Series, Kansas City, KS, USA), and preserved at –20°C for future analyses. Extraction was performed using methanol solutions (80%, v/v). First, 1 g of the lyophilized material was placed in a tube and 20 mL of the solvent was added. Subsequently, it was placed for 30 min in a sonicator (Bransonic, model 2510R-DTH, Connecticut, CT, USA) and centrifuged at 18,407×g for 15 min at 4°C. The supernatant was recovered, the residue was washed twice with 10 mL of the same methanolic solution and centrifuged again. The combined supernatants were filtered through Whatman No. 1 paper and the volume of filtrate was adjusted to 30 mL with the 80% (v/v) methanolic solution. The extracts were kept at –35°C. They were later used to determine TSP, TC, Trolox equivalent antioxidant capacity (TEAC), and ferric reducing antioxidant power (FRAP).

### Determination of total soluble phenolic content

The TSP was determined using the Folin-Ciocalteu method [Alvarez-Parrilla et al., 2011]. Briefly, 250 μL of the extract or standard solutions were mixed with 1 mL of a sodium carbonate solution (75 g/L) and 1250 μL of the Folin-Ciocalteu reagent (10%, v/v), and incubated for 30 min at 25°C. After reaction, the mixtures were moved to a 96-well microplate and absorbance was measured at 750 nm using a microplate reader (Synergy HT, Bio-Tek, Winooski, VT, USA). The results were calculated using a gallic acid standard curve (from 0.02 to 0.4 mg/mL), and the content of total soluble phenolics of beverage was expressed as mg of gallic acid equivalents per mL of beverage (mg GAE/mL).

### Determination of total carotenoid content

The TC was measured as reported by Davis et al. [2007]. One milliliter of beverage was added to 5 mL of a tert-butylated

hydroxytoluene solution in acetone (0.05%, w/v), 5 mL of 95% (v/v) ethanol, and 10 mL of *n*-hexane. The mixture was stirred at 180 rpm on an orbital shaker (ORBi-Shaker, Benchmark, Menlo Park, CA, USA) for 15 min; then, 3 mL of distilled water (5°C) were added to induce phase separation and recover the hydrophobic phase in which carotenoids are dissolved. The collected phase was centrifuged at  $18,407\times g$  for 15 min at 4°C and the absorbance of the supernatant was read at 450 nm. TC was expressed as  $\mu\text{g}$   $\beta$ -carotene equivalents per mL of beverage ( $\mu\text{g}$   $\beta$ -carotene E/mL) using the equation:

$$TC = \frac{(A \times V \times 10^4)}{(A_{1\text{cm}}^{1\%} \times w)} \quad (2)$$

where: TC is the content of total carotenoids, A is absorbance value, V is the recovered volume of the hydrophobic phase,  $A_{1\text{cm}}^{1\%}$  is the specific absorbance of  $\beta$ -carotene (2500), and w is sample weight (g).

### Determination of antioxidant capacity

The AOC of beverages was evaluated as TEAC determined by the ABTS assay and as FRAP. The method of Re *et al.* [1999] was used to carry out the ABTS assay. ABTS (7 mM) was dissolved in a 2.45 mM potassium persulfate solution and stored in the dark for 16 h to generate ABTS<sup>•+</sup>. Prior to use, the ABTS<sup>•+</sup> solution was diluted with a phosphate buffer to an absorbance of  $0.70 \pm 0.02$  at 734 nm. The extract or a Trolox standard solution (20  $\mu\text{L}$ ) was mixed with 255  $\mu\text{L}$  of the ABTS<sup>•+</sup> solution and incubated (30°C for 7 min) in a 96-well microplate. The absorbance was measured at 734 nm using a microplate reader (Synergy HT, Bio-Tek). The Trolox curve from 0.05–1.00 mmol/g was used to calculate the results, which were expressed as mmol of Trolox equivalents per mL of beverage (mmol TE/mL).

The FRAP of beverages was determined according to the Benzie & Strain [1996] method. The FRAP solution contained 10:1:1 (v/v/v) sodium acetate buffer (0.3 M, pH 3.6), 10 mM TPTZ, and 20 mM ferric chloride hexahydrate. The solution was warmed to 37°C before mixing with the samples. For the reaction, 24  $\mu\text{L}$  of the extract or Trolox standard were added to a 96-well microplate and mixed with 180  $\mu\text{L}$  of a FRAP solution. Absorbance was measured at 595 nm after 30 min, a Trolox curve from 0.05–1.00 mmol/g was used to calculate the results, which were expressed as mmol of Trolox equivalents per mL of beverage (mmol TE/mL).

### Phenolic profile analysis

The identification and quantification of phenolic compounds was carried out using HPLC with a diode array detector (DAD) and liquid chromatography-mass spectrometry (LC-MS) techniques. An Agilent 1260 series HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a DAD was employed for HPLC-DAD. After filtration, beverages were injected and separated using a Poroshell 120 EC-C18 column (4.6 $\times$ 150 mm, particle size 2.7  $\mu\text{m}$ ; Agilent Technologies). Injection volume was 10  $\mu\text{L}$ . Mobile phase contained 0.1% (v/v) trifluoroacetic acid as a solvent A and acetonitrile as a solvent B, and was applied as follows: 0 min, 5% B; 10 min, 23% B; 15 min, 50% B; 20 min, 50% B;

23 min, 100% B; 25 min, 100% B; 27 min, 5% B, 30 min, 5% B. Flow rate was 0.4 mL/min. Detection was performed at 280–320 nm. In the case of curcuminoids, a range of 425–470 nm was used. For MS analysis, a 6120 Agilent Quadrupole LC/MS equipped with an electrospray ionization interface was used in a negative ionization mode. Nitrogen at a flow rate of 1.3 L/min was used as drying gas. Nebulizer pressure was 40 psi, gas drying temperature was 350°C, and capillary voltage was 3500 V. OpenLab CDS ChemStation Edition software (Agilent Technologies) was used for data analysis. The compounds were first detected using a single MS scan in the 100–1100 *m/z* range, followed by a targeted search based on the peaks showing major signals in the HPLC-DAD chromatograms. Tentative identification of the compounds was based on retention time ( $t_R$ ) of HPLC-DAD separation and MS signal after comparison with the  $t_R$  of the standard and/or the literature data. The quantitative results were reported as peak areas (arbitrary units).

### Statistical analysis

Data are reported as mean  $\pm$  standard deviation ( $n=3$ ). One-way analysis of variance (ANOVA) and Tukey's test were used to examine the differences between samples ( $p<0.05$ ). All statistical analyses were done using the Statistica 8.0 software (Statsoft, Tulsa, OK, USA).

## RESULTS AND DISCUSSION

### Effect of ultrasound treatment on total soluble phenolic content

The TSP of ultrasound-treated (T1-T9) and untreated beverages are shown in Table 1. The highest TSP was noted for T6 ( $X_{\text{ET}}$  20 min,  $X_{\text{PC}}$  0.8 s,  $X_{\text{SA}}$  100%), and the lowest TSP was determined for T1 ( $X_{\text{ET}}$  10 min,  $X_{\text{PC}}$  0.4 s,  $X_{\text{SA}}$  30%), which were 8.79 and 5.81 mg GAE/mL, respectively. The highest value for 100%  $X_{\text{SA}}$  was in line with the literature. Nadeem *et al.* [2018] found that a sonication amplitude over 70% resulted in the highest TSP when applied to a carrot/grape beverage. Other authors reported similar relationship for different products, like blueberry, lime and mango juice, treated at high sonication amplitudes [Zou & Hou, 2017]. Statistically significant effects ( $p<0.05$ ) and the regression coefficient of the experimental model ( $R^2=0.995$ , Table 2) suggest that the model adequately describes the functional relationship between the experimental factors and the response variable.

The determined TSP showed a high correlation with the predicted value ( $R^2$  adjusted=0.993, Table 2). The correlation between predicted and experimental values suggests that the use of RSM allowed to accurately and reliably determine the optimum UT conditions for this variable.

Figure 1A shows the effects of beverage UT on TSP, which increased significantly at longer exposure time and higher sonication amplitudes. Thus, the highest TSP values were obtained when combining extended exposure time and maximum sonication amplitudes. On the other hand,  $X_{\text{PC}}$  was not a significant factor in the UT effect on TSP. Martinez-Guerra & Gude [2016] described a similar behavior of PC during the optimization of biodiesel production by UAE. The use of PC and continuous sonication

TABLE 2. Lineal and quadratic model equation coefficients and statistical parameters of experimental design for optimizing ultrasound treatment of beverage from mango and carrot with added turmeric. Response variables: total soluble phenolic content (TSP), total carotenoid content (TC), Trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP).

	TSP	TC	TEAC	FRAP
Intercept	-13.220*	81.633**	85.591*	0.747**
$X_{ET}$	2.013**	-5.803*	10.6343*	-0.026**
$X_{ET}^2$	-0.045**	0.095*	-0.228*	0.0005**
$X_{SA}$	0.820**	-2.060*	4.706**	0.005*
$X_{SA}^2$	-0.006**	0.017*	-0.045**	-0.00002
$X_{ET}X_{SA}$	-0.089**	0.307**	-0.597**	0.00008
$X_{ET}^2X_{SA}^2$	0.001**	-0.003**	0.006**	NSI
$X_{ET}^2X_{SA}$	0.002**	-0.006*	0.014**	NSI
$X_{ET}^2X_{SA}^2$	NSI	0.00005**	-0.0001**	NSI
R <sup>2</sup>	0.995	0.975	0.965	0.972
R <sup>2</sup> adjusted	0.993	0.965	0.950	0.960

\* $p < 0.05$ ; \*\* $p < 0.001$ ; NSI, no significant interaction between factors; R<sup>2</sup>, regression coefficient;  $X_{ET}$ , exposure time;  $X_{PC}$ , pulse cycle;  $X_{SA}$ , sonication amplitude.

for similar reaction conditions were compared. About 87% of biodiesel yield was obtained but was limited by the increase in temperature and the extraction time.

It is well-known that UT enhances the release of phenolics by collapsing the cell *via* cavitation in its surroundings [Nadeem *et al.*, 2018]. Hence, higher amplitudes promote cavitation, which results in the maximum release of phenolic compounds [Maran & Priya, 2015]. In nature, phenolic compounds are bound to cellulose, hemicellulose, and pectin,

which are part of the cell wall or are soluble in a vacuole. Cell walls expand with the enlargement of pores in cell membranes generated during the initial stages of cavitation, facilitating the higher diffusivity of water into the cell. Prolonged exposure of the sample to cavitation will lead to the collapse of cell walls, which results in the maximum diffusion of cell contents into the solvent, resulting in a higher extraction rate as the ultrasound amplitude increases [Maran & Priya, 2015].

### Effect of ultrasound treatment on total carotenoid content

The TC of beverages treated in different conditions (T1-T9) ranged from 27.93 to 53.71  $\mu\text{g}$   $\beta$ -carotene E/mL (Table 1). The treatments with the highest TC were T5 ( $X_{ET}$  20 min,  $X_{PC}$  0.6 s,  $X_{SA}$  65%) and T8 ( $X_{ET}$  30 min,  $X_{PC}$  0.6 s,  $X_{SA}$  65%), while the lowest TC was found for T6 ( $X_{ET}$  20 min,  $X_{PC}$  0.8 s,  $X_{SA}$  100%) and T9 ( $X_{ET}$  30 min,  $X_{PC}$  0.8 s,  $X_{SA}$  100%). Lower values of  $X_{SA}$  (30% and 65%) resulted in a higher TC, e.g.  $X_{SA}$  of 30, 65, and 100% resulted in values of 42.87, 42.09, and 34.72  $\mu\text{g}$   $\beta$ -carotene E/mL, respectively. For TC, a high regression coefficient ( $R^2=0.975$ ) and significant interactions (lack of fit,  $p < 0.05$ ) with the predicted model were determined (Table 2). The model showed that the significant parameters ( $p < 0.05$ ) were  $X_{ET}$  and  $X_{SA}$ , quadratic terms, linear-quadratic, and quadratic interaction. The effect of treatment parameters on TC was different to that on TSP, since applying high  $X_{SA}$  decreased TC in the treated beverages (Figure 1). This agrees with results reported by Mercado-Mercado *et al.* [2018], where UT led to decreased contents of lutein,  $\beta$ -carotene, and  $\beta$ -cryptoxanthin in mango-based beverages. Likewise, ultrasound-treated guava beverages showed a decrease in lycopene as ET and SA increased [Campoli *et al.*, 2018]. The authors suggested that the decrease in TC 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]. High sonication amplitudes generate a greater number of cavitations

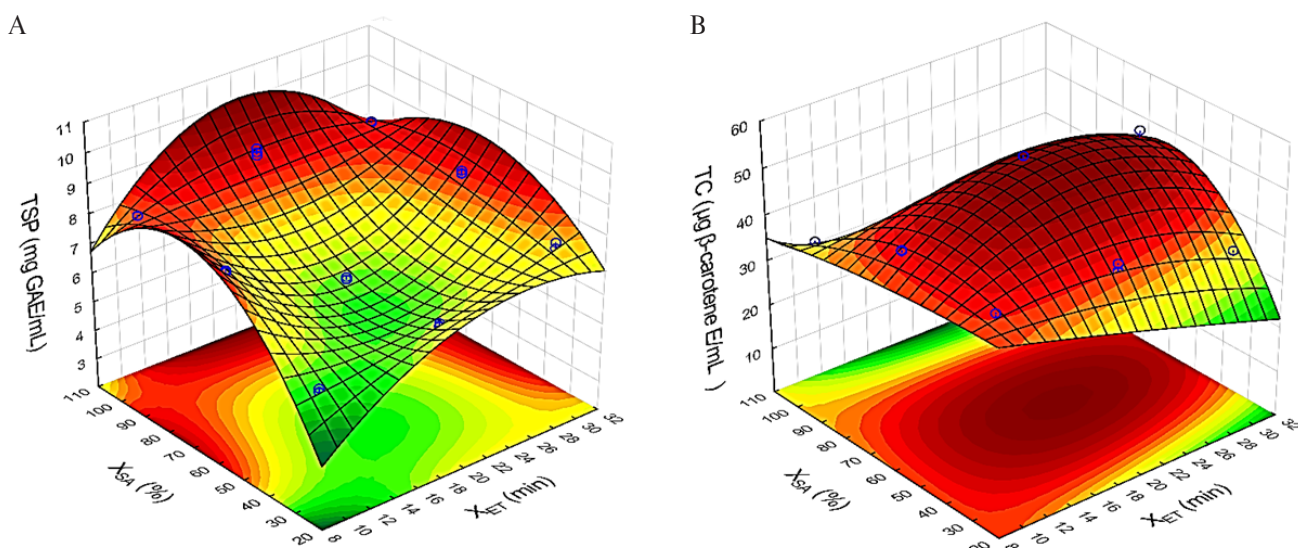


FIGURE 1. Response surface plots of optimization of ultrasound treatment of beverage from mango and carrot with added turmeric. Effect of exposure time ( $X_{ET}$ ) and sonication amplitude ( $X_{SA}$ ) on total soluble phenolic content (TSP) (A) and total carotenoid content (TC) (B).

Blue dots represent the experimental points. GAE, gallic acid equivalents.

TABLE 3. Predicted and experimental values of total soluble phenolic content (TSP), total carotenoid content (TC), Trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP) for optimized ultrasound treatment of beverage from mango and carrot with added turmeric.

Parameter	Predicted value	Experimental value
TSP (mg GAE/mL)*	8.81 <sup>b</sup>	11.48±0.33 <sup>a</sup>
TEAC (mmol TE/mL)*	195.31 <sup>a</sup>	199.38±5.10 <sup>a</sup>
FRAP (mmol TE/mL)*	0.87 <sup>b</sup>	1.5±0.06 <sup>a</sup>
TC (µg β-carotene E/mL)**	53.91 <sup>a</sup>	52.86±1.23 <sup>a</sup>

\*For optimal ultrasound treatment parameters: 21 min of ultrasonication time at 100% sonication amplitude and 0.7 s pulse cycle. \*\*For optimal ultrasound treatment parameters: 20 min of ultrasonication time at 65% sonication amplitude and 0.6s pulse cycle. Different letters in the same row indicate significant differences ( $p < 0.05$ ) between predicted and experimental values. GAE, gallic acid equivalent; TE, Trolox equivalent.

in the external environment, which then promote isomerization and oxidation reactions by the action of hydrogen peroxide; a highly reactive compound that is generated under UT conditions [Sun *et al.*, 2017]. In contrast, treatments of medium  $X_{SA}$  conditions (65%) with intermediate and prolonged extraction times (20 or 30 min) showed the highest TC values (Table 1). The conditions in this study that maximized TC agree with the results reported by Jabbar *et al.* [2014] regarding the behavior of TC, lycopene, and β-carotene in carrot juice under different ultrasound conditions. The authors reported that when amplitudes of 70% were applied at 20 kHz, an increased content of these compounds was observed. This behavior is attributed to the fact that the sonication amplitude used does not promote isomerization reactions or any others that alter their molecular structure; consequently, the carotenoid content increased by mechanical disruption of the cell wall.

### Effect of ultrasound treatment on antioxidant capacity

Figures 2A and 2B show the effects of UT on the AOC of beverage. For both, TEAC and FRAP, the maximum values were found when the operating conditions were applied at high  $X_{SA}$  (80–100%). This finding is consistent with the results shown in Table 1, which shows the highest TEAC for the beverage sonicated with an amplitude of 100% for 20 min (195.84 mmol TE/mL). On the other hand, the highest FRAP was obtained at  $X_{SA}$  of 100% and  $X_{ET}$  of 30 min (0.97 mmol TE/mL). In both cases, it is evident that exposure to 100% ultrasound amplitudes favors the increase of antioxidant capacity in the beverages subjected to ultrasound treatment. In the case of TEAC, the maximum values were obtained with exposure times close to 20 min, while two maxima are shown for FRAP, regardless of  $X_{ET}$  (Figure 2). This indicates that  $X_{SA}$  is the main parameter that governs AOC since both show proportional variation.

For TEAC, optimum operating conditions were similar to those reported by Yeoh & Ali *et al.* [2017], who applied UAE for 15 min at 70% sonication amplitude to slices of fresh pineapple, and found that these conditions yielded the highest values for this technique. Similarly, Ramírez-Moreno *et al.* [2018] reported that the optimum conditions to increase the AOC in blackberry juices were an exposure time of 15 min with a sonication amplitude of 80%, values that resemble those found in the present work. Regarding FRAP, it has been reported that substantial increases were found using amplitudes between 40 and 80% [Chaikham *et al.*, 2016]. For example, an experiment performed on blackberry by-products showed that both SA and ET had a positive effect on AOC, as measured by FRAP [Romero & Yépez, 2014]. This can be explained by the fact that an increase in SA leads to more damage to the cell wall, causing compounds with antioxidant capacity to be released from the different cell structures [Hossain *et al.*, 2012].

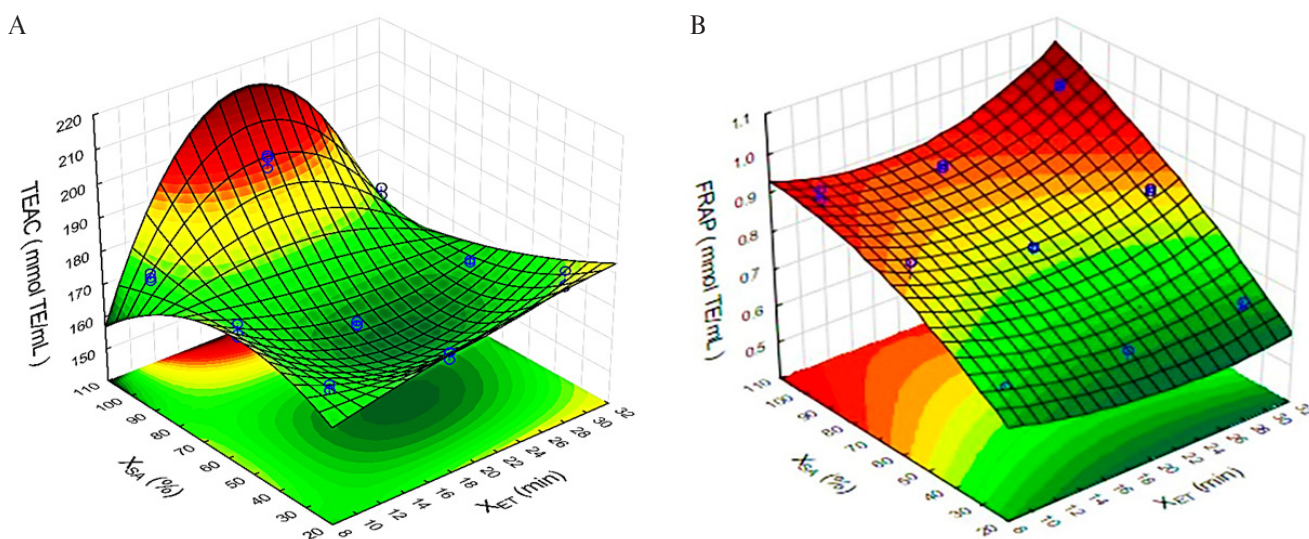


FIGURE 2. Response surface plots of optimization of ultrasound treated beverage from mango and carrot with added turmeric. Effect of exposure time ( $X_{ET}$ ) and sonication amplitude ( $X_{SA}$ ) on Trolox equivalent antioxidant capacity (TEAC) (A) and ferric reducing antioxidant power (FRAP) (B). Blue dots represent the experimental points. TE, Trolox equivalents.

TABLE 4. Chromatographic and mass spectrometric data, and quantification expressed as peak areas (arbitrary units) of phenolics in the control and the optimized ultrasound-treated (UT) beverages from mango and carrots with added turmeric.

Compound	Quantity		Retention time (min)	[M-H] <sup>-</sup> m/z
	Control beverage	UT beverage		
Phenolic acids				
<i>p</i> -Hydroxybenzoic acid	21,626 ± 1,399 <sup>a</sup>	6,041 ± 1,347 <sup>b</sup>	11.99	137
3,4-Dihydroxybenzoic acid	2,492 ± 506 <sup>b</sup>	7,221 ± 487 <sup>a</sup>	10.23	153
Gallic acid	14,956 ± 180 <sup>b</sup>	17,359 ± 157 <sup>a</sup>	5.79	169
Vanillic acid	16,617 ± 382 <sup>a</sup>	4,861 ± 839 <sup>b</sup>	14.10	167
Chlorogenic acid	4,514,332 ± 126,448 <sup>b</sup>	13,933,197 ± 259,247 <sup>a</sup>	10.00	353
Ferulic acid	24,415 ± 747 <sup>a</sup>	23,500 ± 138 <sup>a</sup>	17.70	193
<i>p</i> -Coumaric acid	20,835 ± 5,807 <sup>a</sup>	20,338 ± 5,519 <sup>a</sup>	17.85	163
Curcuminoids				
Curcumin	71,302 ± 11,628 <sup>a</sup>	65,446 ± 1,470 <sup>a</sup>	3.85	367
Demethoxycurcumin	232,359 ± 32,115 <sup>a</sup>	83,260 ± 4,260 <sup>b</sup>	4.23	337
Bisdemethoxycurcumin	112,036 ± 14,018 <sup>a</sup>	86,724 ± 6,911 <sup>b</sup>	4.19	307
Flavonoids				
(-)-Gallocatechin	116,369 ± 12,069 <sup>a</sup>	94,727 ± 13,064 <sup>b</sup>	4.58	305
(-)-Gallocatechin gallate	4,712 ± 315 <sup>b</sup>	7,403 ± 447 <sup>a</sup>	1.20	457
(+)-Catechin	531,674 ± 4,627 <sup>b</sup>	643,371 ± 5,494 <sup>a</sup>	9.62	289
Kaempferol	6,683 ± 729 <sup>b</sup>	22,135 ± 2,771 <sup>a</sup>	21.37	285
Quercetin	20,197 ± 4,384 <sup>b</sup>	84,501 ± 1,707 <sup>a</sup>	20.63	301
Xanthonoids				
Mangiferin	60,689 ± 15,781 <sup>b</sup>	80,078 ± 7,877 <sup>a</sup>	14.21	421

Values are expressed as mean ± standard deviation. Different letters in the same row denote significant differences ( $p < 0.05$ ).

### Optimization of UT conditions and model validation

Optimization of UT conditions was performed by superimposing the contour graphs obtained from each dependent variable using the response surface. The present study performed two optimizations, one based on TSP and AOC (TEAC and FRAP) and the other based on TC. The predictive model showed that the optimal conditions for TSP and AOC were obtained by submitting the beverage to 21 min of ultrasonication at an amplitude and pulse of 100% and 0.7 s, respectively. For TC, the optimum values were 20 min at 65% amplitude and pulse of 0.6 s. The optimum values are shown in Table 3. The most favorable UT conditions were applied to the beverage to validate the proposed predictive model, and to confirm if the experimental values obtained coincide with those predicted by the mathematical model.

In the case of TSP and FRAP, it was found that the experimental values were higher than those predicted by the mathematical model. This phenomenon could be explained by the presence of other antioxidants not considered in the present work such as ascorbic acid or thiols, which were capable to disrupt the results. Nevertheless, for TC and TEAC, the predicted results matched with the experimental results

obtained at optimal UT conditions, which were validated by the RSM model with a good correlation.

### Identification of bioactive compounds by HPLC-DAD-MS

A total of 16 compounds (seven phenolic acids, three curcuminoids, five flavonoids and a xanthonoid) were identified in the control, as well as in the optimized UT beverages (Table 4). For phenolic acids, ultrasound treatment enhanced the quantity of 3,4-dihydroxybenzoic acid, gallic acid, and chlorogenic acid in the beverage. No significant differences were noted for the *p*-coumaric and ferulic acids between treated and control beverages, while abundance of vanillic acid and *p*-hydroxybenzoic acid decreased in the treated sample. Turmeric was the source of three curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) detected in the control and UT beverages (Table 4). A lower quantity of curcumin, demethoxycurcumin, and bis-demethoxycurcumin was determined in the UT beverage, as compared to the control.

The five flavonoids in the UT beverage were found in the following order of quantity: (+)-catechin > (-)-gallocatechin > quercetin > kaempferol > (-)-gallocatechin gallate (Table 4). Furthermore, their contents increased with UT,

except for (–)-gallocatechin, which decreased. A higher mangiferin quantity in the UT beverage than in control was also noted. The phenolic profile of the beverages agrees with those reported elsewhere for mango pulp, carrot juice, and turmeric. Gallic acid, *p*-coumaric acid, ferulic acid, vanillic acid, quercetin, (–)-gallocatechin, kaempferol, and mangiferin have been reported in mango pulp [Barrón-García *et al.*, 2020; Lee *et al.*, 2021; Quirós-Sauceda *et al.*, 2017]. The presence of chlorogenic acid, (+)-catechin, *p*-coumaric acid, and ferulic acid was determined in carrots [Formica-Oliveira *et al.*, 2017; Jabbar *et al.*, 2015], while gallic acid, protocatechuic acid, (+)-catechin, chlorogenic acid, (–)-epicatechin, ferulic acid, curcumin, demethoxycurcumin, bisdemethoxycurcumin, and quercetin have been reported in turmeric [Ali *et al.*, 2014; Pal *et al.*, 2020].

It is known that ultrasound induces acoustic cavitation and the rupture of plant cells [Jing *et al.*, 2015], which facilitates solvent penetration and increases the solubility of the solutes. Conversely, it is also possible that ultrasound treatment may degrade some compounds or promote reactions between others, which may result in a decrease in their content. Also, ruptured cells allow the release of various compounds into the solvent, such as insoluble and cytosolic substances, thereby limiting the solubility of the compounds of interest [Zhao *et al.*, 2007].

## CONCLUSION

In the present study, an ultrasound treatment was applied to increase contents of soluble phenolic compounds and carotenoids in a beverage from mango and carrot with added turmeric, using response surface methodology to optimize working parameters. Exposure time and sonication amplitude were shown to affect the dependent variables, while pulse cycle did not show any effect. Optimization and model validation data showed that TEAC and TC were accurately predicted by the model but TSP and FRAP were not. The data suggest that an adequately optimized ultrasound treatment of a beverage can improve its TSP. Contents of most of the individual phenolics of the beverage also increased in response to the optimized UT. Total carotenoid content changed by UT in a more variable manner; it was generally increased by the treatments, but with an evident decrease for more drastic conditions, such as longer exposure time and higher sonication amplitude.

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## CONFLICT OF INTEREST

Authors declare no conflicts of interest.

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## REFERENCES

- Aguilar-Hernández, G., García-Magaña, M.D.L., Vivar-Vera, M.D.L.Á., Sáyago-Ayerdi, S.G., Sánchez-Burgos, J.A., Morales-Castro, J., Anaya-Esparza, L.M., Montalvo González, E. (2019). Optimization of ultrasound-assisted extraction of phenolic compounds from *Annona muricata* by-products and pulp. *Molecules*, 24(5), art. no. 904. <https://doi.org/10.3390/molecules24050904>
- Aguilar-Hernández, G., Vivar-Vera, M.D.L.Á., García-Magaña, M.D.L., González-Silva, N., Pérez-Larios, A., Montalvo-González, E. (2020). Ultrasound-assisted extraction of total acetogenins from the soursop fruit by response surface methodology. *Molecules*, 25(5), art. no. 1139. <https://doi.org/10.3390/molecules25051139>
- Ahmad, K., Imran, M., Ahmad, T., Ahmad, M.H., Khan, M.K. (2020). Impact of ultrasound processing on physicochemical and bioactive attributes of grape based optimized fruit beverage. *Pakistan Journal of Agricultural Sciences*, 57(4), 1117–1124. <https://doi.org/10.21162/PAKJAS/20.8680>
- Ali, I., Haque, A., Saleem, K. (2014). Separation and identification of curcuminoids in turmeric powder by HPLC using phenyl column. *Analytical Methods*, 6(8), 2526–2536. <https://doi.org/10.1039/C3AY41987H>
- Alvarez-Parrilla, E., de la Rosa, L.A., Amarowicz, R., Shahidi, F. (2011). Antioxidant activity of fresh and processed Jalapeno and Serrano peppers. *Journal of Agricultural and Food Chemistry*, 59(1), 163–173. <https://doi.org/10.1021/jf103434u>
- Barrón-García, O.Y., Morales-Sánchez, E., Jiménez, A.R., Antunes-Ricardo, M., Luzardo-Ocampo, I., González-Jasso, E., Gaytán-Martínez, M. (2022). Phenolic compounds profile and antioxidant capacity of ‘Ataulfo’ mango pulp processed by ohmic heating at moderate electric field strength. *Food Research International*, 154, art. no. 111032. <https://doi.org/10.1016/j.foodres.2022.111032>
- Benzie, I.F., Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Analytical Biochemistry*, 239(1), 70–76. <https://doi.org/10.1006/abio.1996.0292>
- Bhardwaj, R.L., Pandey, S. (2011). Juice blends — a way of utilization of under-utilized fruits, vegetables, and spices a review. *Critical Reviews in Food Science and Nutrition*, 51(6), 563–570. <https://doi.org/10.1080/10408391003710654>



9. Campoli, S.S., Rojas, M.L., do Amaral, J.E.P.G., Canniatti-Brazaca, S.G., Augusto, P.E.D. (2018). Ultrasound processing of guava juice: Effect on structure, physical properties and lycopene *in vitro* accessibility. *Food Chemistry*, 268, 594–601. <https://doi.org/10.1016/j.foodchem.2018.06.127>
10. Chaikham, P., Kemsawasd, V., Apichartsrangkoon, A. (2016). Effects of conventional and ultrasound treatments on physico-chemical properties and antioxidant capacity of floral honeys from Northern Thailand. *Food Bioscience*, 15, 19–26. <https://doi.org/10.1016/j.fbio.2016.04.002>
11. Codex Alimentarius (2005). “Norma general del codex para zumos (jugos) y néctares de frutas.” Codex Stan, 247, 21.
12. Davis, A.R., Collins, J., Fish, W.W., Tadmor, Y., Webber III, C.L., Perkins-Veazie, P. (2007). Rapid method for total carotenoid detection in canary yellow-fleshed watermelon. *Journal of Food Science*, 72(5), S319–S323. <https://doi.org/10.1111/j.1750-3841.2007.00381.x>
13. Deogade, S.C., Ghate, S. (2015). Curcumin: therapeutic applications in systemic and oral health. *International Journal of Biological and Pharmaceutical Research*, 6(4), 281–90.
14. Formica-Oliveira, A.C., Martínez-Hernández, G.B., Díaz-López, V., Artés, F., Artés-Hernández, F. (2017). Effects of UV-B and UV-C combination on phenolic compounds biosynthesis in fresh-cut carrots. *Postharvest Biology and Technology*, 127, 99–104. <https://doi.org/10.1016/j.postharvbio.2016.12.010>
15. Fujiki, H., Sueoka, E., Rawangkan, A., Suganuma, M. (2017). Human cancer stem cells are a target for cancer prevention using (–)-epigallocatechin gallate. *Journal of Cancer Research and Clinical Oncology*, 143(12), 2401–2412. <https://doi.org/10.1007/s00432-017-2515-2>
16. Hasheminya, S.M., Dehghannya, J. (2022). Non-thermal processing of black carrot juice using ultrasound: Intensification of bioactive compounds and microbiological quality. *International Journal of Food Science & Technology*. <https://doi.org/10.1111/ijfs.15901>
17. Hossain, M.B., Brunton, N.P., Patras, A., Tiwari, B., O'Donnell, C.P., Martin-Diana, A.B., Barry-Ryan, C. (2012). Optimization of ultrasound assisted extraction of antioxidant compounds from marjoram (*Origanum majorana* L.) using response surface methodology. *Ultrasonics Sonochemistry*, 19(3), 582–590. <https://doi.org/10.1016/j.ultsonch.2011.11.001>
18. Jabbar, S., Abid, M., Hu, B., Muhammad Hashim, M., Saeeuddin, M., Lei, S., Wu, T., Zeng, X. (2014). Influence of sonication and high hydrostatic pressure on the quality of carrot juice. *Journal of Food Science & Technology*, 49(11), 2449–2457. <https://doi.org/10.1111/ijfs.12567>
19. Jabbar, S., Abid, M., Wu, T., Hashim, M.M., Saeeuddin, M., Hu, B., Lei, S., Zeng, X. (2015). Ultrasound-assisted extraction of bioactive compounds and antioxidants from carrot pomace: A response surface approach. *Journal of Food Processing and Preservation*, 39(6), 1878–1888. <https://doi.org/10.1111/jfpp.12425>
20. Jing, C.L., Dong, X.F., Tong, J.M. (2015). Optimization of ultrasonic-assisted extraction of flavonoid compounds and antioxidants from alfalfa using response surface method. *Molecules*, 20(9), 15550–15571. <https://doi.org/10.3390/molecules200915550>
21. Lee, F.Y., Vo, G.T., Barrow, C.J., Dunshea, F.R., Suleria, H.A. (2021). Mango rejects and mango waste: Characterization and quantification of phenolic compounds and their antioxidant potential. *Journal of Food Processing and Preservation*, 45(7), art. no. e15618. <https://doi.org/10.1111/jfpp.15618>
22. Manna, P., Gyasuddin, M., Manna, P. (2017). Development and quality evaluation of vegetable juice blend with coconut milk. *International Journal of Scientific & Engineering Research*, 8(7), 1871–1874.
23. Mercado-Mercado, G., López Teros, V., Montalvo-González, E., González-Aguilar, G.A., Álvarez-Parrilla, E., Sáyago-Ayerdi, S.G. (2018). Effect of ultrasound-assisted extraction on the release and *in vitro* bioaccessibility of carotenoids in mango (*Mangifera indica* L.) “Ataulfo”-based beverages. *Nova Scientia*, 10(20), 100–132. <https://doi.org/10.21640/ns.v10i20.1277>
24. Martínez-Guerra, E., Gude, V.G. (2016). Determining optimum pulse mode for ultrasound enhanced biodiesel production. *Journal of Industrial and Engineering Chemistry*, 35, 14–19. <https://doi.org/10.1016/j.jiec.2016.01.004>
25. Maran, J.P., Priya, B. (2015). Ultrasound-assisted extraction of pectin from sisal waste. *Carbohydrate Polymers*, 115, 732–738. <https://doi.org/10.1016/j.carbpol.2014.07.058>
26. Nadeem, M., Ubaid, N., Qureshi, T.M., Munir, M., Mehmood, A. (2018). Effect of ultrasound and chemical treatment on total phenol, flavonoids and antioxidant properties on carrot-grape juice blend during storage. *Ultrasonics Sonochemistry*, 45, 1–6. <https://doi.org/10.1016/j.ultsonch.2018.02.034>
27. Nolasco-González, Y., Chacón-López, M.A., Ortiz-Basurto, R.I., Aguilera-Aguirre, S., González-Aguilar, G.A., Rodríguez-Aguayo, C., Navarro-Cortez, M.C., García-Galindo, H.S., García-Magana de Lourdes, M., Meza-Espinoza, L., Montalvo-González, E. (2022). *Annona muricata* leaves as a source of bioactive compounds: Extraction and quantification using ultrasound. *Horticulturae*, 8(7), art. no. 560. <https://doi.org/10.3390/horticulturae8070560>
28. Ordóñez-Santos, L.E., Martínez-Girón, J., Arias-Jaramillo, M.E. (2017). Effect of ultrasound treatment on visual color, vitamin C, total phenols, and carotenoids content in Cape gooseberry juice. *Food Chemistry*, 233, 96–100. <https://doi.org/10.1016/j.foodchem.2017.04.114>
29. Pacheco-Ordaz, R., Antunes-Ricardo, M., Gutiérrez-Urbe, J.A., González-Aguilar, G.A. (2018). Intestinal permeability and cellular antioxidant activity of phenolic compounds from mango (*Mangifera indica* cv. Ataulfo) peels. *International Journal of Molecular Sciences*, 19(2), art. no. 514. <https://doi.org/10.3390/ijms19020514>
30. Pal, K., Chowdhury, S., Dutta, S.K., Chakraborty, S., Chakraborty, M., Pandit, G.K., Dutta, S., Paul, P.K., Choudhury, A., Majumder, B., Sahana, N., Mandal, S. (2020). Analysis of rhizome colour content, bioactive compound profiling and *ex situ* conservation of turmeric genotypes (*Curcuma longa* L.) from sub-Himalayan terai region of India. *Industrial Crops and Products*, 150, art. no. 112401. <https://doi.org/10.1016/j.indcrop.2020.112401>
31. Palafox-Carlos, H., Yahia, E.M., González-Aguilar, G.A. (2012). Identification and quantification of major phenolic compounds from mango (*Mangifera indica*, cv. Ataulfo) fruit by HPLC-DAD-MS/MS-ESI and their individual contribution to the antioxidant activity during ripening. *Food Chemistry*, 135(1), 105–111. <https://doi.org/10.1016/j.foodchem.2012.04.103>

32. Quirós-Sauceda, A.E., Chen, C.Y.O., Blumberg, J.B., Astiazaran-García, H., Wall-Medrano, A., González-Aguilar, G.A. (2017). Processing 'ataulfo' mango into juice preserves the bioavailability and antioxidant capacity of its phenolic compounds. *Nutrients*, 9(10), art. no. 1082.  
<https://doi.org/10.3390/nu9101082>
33. Ramírez-Moreno, E., Zafra-Rojas, Q.Y., Arias-Rico, J., Ariza-Ortega, J.A., Alanís-García, E., Cruz-Cansino, N. (2018). Effect of ultrasound on microbiological load and antioxidant properties of blackberry juice. *Journal of Food Processing and Preservation*, 42(2), art. no. e13489.  
<https://doi.org/10.1111/jfpp.13489>
34. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9–10), 1231–1237.  
[https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3)
35. Romero, J.C., Yépez, V.B. (2014). Ultrasound as pretreatment to convective drying of Andean blackberry (*Rubus glaucus* Benth). *Ultrasonics Sonochemistry*, 22, 205–210.  
<https://doi.org/10.1016/j.ultsonch.2014.06.011>
36. Rojas, M.L., Leite, T.S., Cristianini, M., Alvim, I.D., Augusto, P.E. (2016). Peach juice processed by the ultrasound technology: Changes in its microstructure improve its physical properties and stability. *Food Research International*, 82, 22–33.  
<https://doi.org/10.1016/j.foodres.2016.01.011>
37. Saeeduddin, M., Abid, M., Jabbar, S., Hu, B., Hashim, M.M., Khan, M.A., Xie, M., Wu, T., Zeng, X. (2016). Physicochemical parameters, bioactive compounds and microbial quality of sonicated pear juice. *International Journal of Food Science & Technology*, 51(7), 1552–1559.  
<https://doi.org/10.1111/ijfs.13124>
38. Savasari, M., Emadi, M., Bahmanyar, M.A., Biparva, P. (2015). Optimization of Cd (II) removal from aqueous solution by ascorbic acid-stabilized zero valent iron nanoparticles using response surface methodology. *Journal of Industrial and Engineering Chemistry*, 21, 1403–1409.  
<https://doi.org/10.1016/j.jiec.2014.06.014>
39. Šeregelj, V., Vulić, J., Četković, G., Čanadanovć-Brunet, J., Šaponjac, V.T., Stajčić, S. (2020). Natural bioactive compounds in carrot waste for food applications and health benefits. *Studies in Natural Products Chemistry*, 67, 307–344.  
<https://doi.org/10.1016/B978-0-12-819483-6.00009-6>
40. Sun, J., Li, X., Lin, X., Mei, Z., Li, Y., Ding, L., Bai, W. (2017). Sonodegradation of cyanidin-3-glucosylrutinoside: degradation kinetic analysis and its impact on antioxidant capacity *in vitro*. *Journal of the Science of Food and Agriculture*, 97(5), 1475–1481.  
<https://doi.org/10.1002/jsfa.7887>
41. Wei, S.D., Chen, H., Yan, T., Lin, Y.M., Zhou, H.C. (2014). Identification of antioxidant components and fatty acid profiles of the leaves and fruits from *Averrhoa carambola*. *LWT – Food Science and Technology*, 55(1), 278–285.  
<https://doi.org/10.1016/j.lwt.2013.08.013>
42. Wu, T., Lv, H., Wang, F., Wang, Y. (2016). Characterization of polyphenols from *Lycium ruthenicum* fruit by UPLC-Q-TOF/MSE and their antioxidant activity in Caco-2 cells. *Journal of Agricultural and Food Chemistry*, 64(11), 2280–2288.  
<https://doi.org/10.1021/acs.jafc.6b00035>
43. Yeoh, W.K., Ali, A. (2017). Ultrasound treatment on phenolic metabolism and antioxidant capacity of fresh-cut pineapple during cold storage. *Food Chemistry*, 216, 247–253.  
<https://doi.org/10.1016/j.foodchem.2016.07.074>
44. Yıkıms, S. (2020). Sensory, physicochemical, microbiological and bioactive properties of red watermelon juice and yellow watermelon juice after ultrasound treatment. *Journal of Food Measurement and Characterization*, 14(3), 1417–1426.  
<https://doi.org/10.1007/s11694-020-00391-7>
45. Yirsaw, B.D., Megharaj, M., Chen, Z., Naidu, R. (2016). Reduction of hexavalent chromium by green synthesized nano zero valent iron and process optimization using response surface methodology. *Environmental Technology & Innovation*, 5, 136–147.  
<https://doi.org/10.1016/j.eti.2016.01.005>
46. Zhao, C.L., Chen, Z.J., Bai, X.S., Ding, C., Long, T.J., Wei, F.G., Miao, K.R. (2014). Structure-activity relationships of anthocyanidin glycosylation. *Molecular Diversity*, 18(3), 687–700.  
<https://doi.org/10.1007/s11030-014-9520-z>
47. Zhao, S., Kwok, K.C., Liang, H. (2007). Investigation on ultrasound assisted extraction of saikosaponins from *Radix Bupleuri*. *Separation and Purification Technology*, 55(3), 307–312.  
<https://doi.org/10.1016/j.seppur.2006.12.002>
48. Zhou, B., Yi, H., Tan, J., Wu, Y., Liu, G., Qiu, Z. (2015). Anti-proliferative effects of polyphenols from pomegranate rind (*Punica granatum* L.) on EJ bladder cancer cells *via* regulation of p53/miR-34a Axis. *Phytotherapy Research*, 29(3), 415–422.  
<https://doi.org/10.1002/ptr.5267>
49. Zou, Y., Hou, X. (2017). Sonication enhances quality and antioxidant activity of blueberry juice. *Food Science and Technology*, 37(4), 599–603.  
<https://doi.org/10.1590/1678-457x.27816>