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ORIGINAL PAPER

Effect of thermosonication and thermal treatments on antidiabetic, antihypertensive, mineral elements and in vitro bioaccessibility of bioactive compounds in freshly squeezed pomegranate juice

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Abstract

Pomegranate fruit and its products have been recognized as 'miracle fruit' due to their nutritional content, organoleptic properties and health benefits. There is no detailed study about the optimization of thermosonication (TS) conditions for the popular drink, freshly squeezed pomegranate juice. Bioactive components in freshly squeezed pomegranate juice treated with thermosonication were increased using the response surface method (RSM) and artificial neural network (ANN). ANN had higher correlation than RSM and as a result of optimization, thermosonication treatment conditions of 49.50 °C, 10.5 min and 72.50% amplitude were determined.Thermosonication-treated pomegranate juice (TS-PJ) had higher antidiabetic and antihypertensive effects than thermal pasteurized pomegranate juice (P-PJ). TS did not affect physicochemical parameters ($p > 0.05$). Both treatments reduced the microbial load after treatment and TS-PJ was superior in terms of sensory attributes. As a result of thermosonication treatment, increases in Ca, Fe and Na elements were detected. For in vitro simulated gastrointestinal medium TS-PJ better preserved the bioavailability of bioactive compounds. The results obtained may be useful for the industrial production of thermosonication-treated pomegranate juice.

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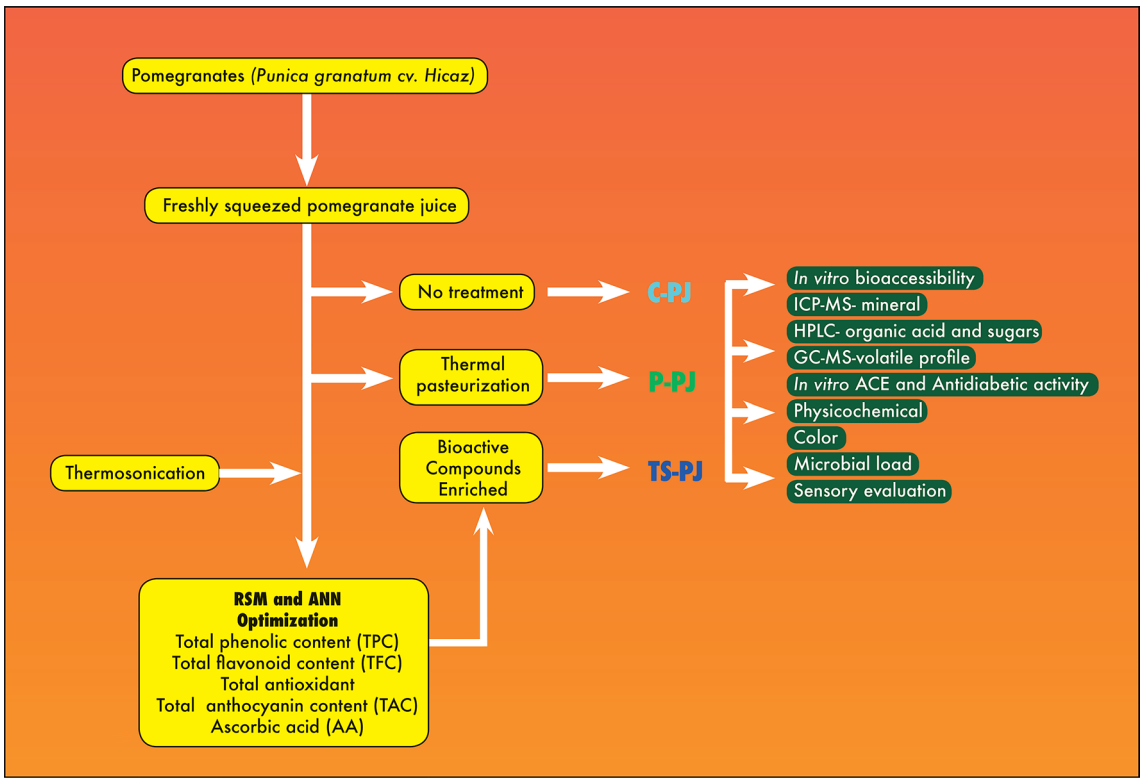
Graphical abstract



Keywords Thermosonication · Artificial neural network · Bioaccessibility · Antidiabetic · Antihypertensive · Pomegranate juice

Introduction

The pomegranate (*Punica granatum*) is an ancient perennial plant species of the *Punicaceae* family and is considered a 'miracle fruit' with seeds being consumed as food, juice and functional food. Significant modern pharmacological and clinical evidence highlighted the wide medicinal applications of pomegranate fruit parts and juice. Superior to other juices, pomegranate juice (PJ) is a fortified source of dietary polyphenols with potential antioxidant capacity. PJ's polyphenols include tannins, anthocyanins, and flavonoids. The presence of these beneficial phytochemicals is directly linked to positive health benefits (effects such as therapeutic effects, lipid and sugar metabolism and anti-inflammatory effects) [1].

The consumption of PJ has increased significantly since the scientific literature reported therapeutic benefits attributable to its antioxidant, antimicrobial, anti-carcinogenic and anti-inflammatory properties. Increasing consumer demands for fresh and high-quality food products increased interest in non-thermal technologies for the preservation of juices. Although thermal pasteurization is the most widely used preservation technology, it has adverse effects on the nutritional and sensory quality of fruit juices. Conversely, non-thermal technologies are viable alternatives for protection [2]. There has been an increase in non-thermal processing technologies in the food industry due to consumer demand for minimally processed, high-quality foods. Among the new food processing techniques, the combination of ultrasound application with controlled heat is called thermosonication (TS). In order to ensure the effectiveness of the TS treatment, it is necessary to consider variables such as the pH of the product, amplitude, temperature and duration of the treatment. Thermosonication was recently researched in food processing and was found to be simple, reliable, environmentally friendly and highly effective in achieving microbial decontamination and protection [3]. It was reported that the thermosonication process is effective for carrot (*Daucus carota*) juice [4], pineapple, grape and cranberry juices [5], barberry juice [6], anthocyanin-enriched tomato juice [7], hog plum (*Spondias mombin* L.) juice [8], orange juice whey drink [9], fruit (*Haematocarpus validus*) juice [10] and beetroot (*Beta vulgaris* L.) juice [11] with minimal change in nutritional and sensory properties.

Process modeling approaches such as response surface methodology (RSM) and artificial neural networks (ANN) are required to efficiently optimize process conditions. The thermosonication process and optimization of fruit and vegetable juices was extensively researched, but the application of TS treatment to enrich the bioactive components of pomegranate juice and the comparison of RSM and ANN optimization techniques for freshly squeezed pomegranate juice have not been investigated so far. At the same time, the aim was to compare the physicochemical, sensory, mineral, antidiabetic, antihypertensive, aroma profile and bioavailability features of freshly squeezed pomegranate juice, TS-treated freshly squeezed pomegranate juice and thermal pasteurized freshly squeezed pomegranate juice.

Material

Pomegranates (*Punica granatum* cv. Hicaz) were purchased from a local market in Tekirdag, Turkey, in August 2020. Robust pomegranates were chosen from among the fruit. Intact arils were manually separated from the whole pomegranates. The pomegranate juice was squeezed using a home juicer (SJ -3143, Sinbo, Istanbul, Turkey) and filtered through double layered sterilized muslin cloth to remove coarse particles and impurities from the pomegranate juice. Freshly squeezed pomegranate juice was transferred to 100 ml sterile bottles. Freshly squeezed pomegranate juice without thermosonication treatment was called the untreated sample (control pomegranate juice, C-PJ). Samples were stored at -18 ± 1 °C until analysis. Tests were performed three times.

Thermal pasteurization and thermosonication treatments

Thermal pasteurization treatment (P-PJ) was conducted in a water bath (Wisd-Model WUC-D06H, Daihan, Wonju, Korea). The sterile bottle was pasteurized at 75 ℃ for 15 s. After P-PJ treatment, the juice was cooled down in an ice bath and manually packed into sterile bottles. For thermosonication treatment, 100 mL of freshly squeezed pomegranate juice was processed using a 200 W ultrasonic processor (Hielscher Ultrasonics Model UP200St, Berlin, Germany) at a frequency of 26 kHz. TS parameters studied included temperature (40, 45, 50, 55 and 60 °C), processing duration (4, 6, 8, 10 and 12 min) and amplitudes (60%, 65%, 70%, 75% and 80%) in constant mode. An ice-water bath was used to prevent overheating during ultrasound processing. All the treatments were done in the dark during ultrasound to avoid any possible interference of light. After thermosonication treatment, the pomegranate juice samples were immediately cooled in an ice bath. Samples were stored at -18 ± 1 °C until analysis. Pomegranate juice samples were named TS-PJ (thermosonication-treated pomegranate juice) after optimization. Tests were performed three times.

Modelling procedure for response surface methodology and artificial neural networks

The response surface method (RSM) and artificial neural networks (ANN) were used to understand the effect of thermosonication treatments on the bioactive components in the freshly squeezed pomegranate juice. While temperature (X_1, 40–60 °C), time (X_2, 4–12 min) and amplitude (X_3, 60–80%) are independent factors, total phenolic content (mg GAE/100 mL), total flavonoid content (mg CE/100 mL), total anthocyanin content (mg C3G/100 mL), ascorbic acid (mg/mL), DPPH (% inhibition), and CUPRAC (% inhibition) were response variables. For RSM, Central Composite Design (CCD) was implemented using Minitab software (version 19, Minitab software, State College, PA, USA) to optimize thermosonication processing of freshly squeezed pomegranate juice. a five-level, three-factor, two replicates experimental design was created. There were 40 trial points for optimization. The following quadratic-polynomial formula was used to create the equation models:

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

Definition of this formula is as follows: the dependent variable (y); the intercept term (β_o); the first order (linear) equation coefficient (β_i); the quadratic equation coefficient (β_{ii}); the two-factor cross-interaction coefficient (β_{ij}); and X_i and X_j are independent variables.

ANN was implemented using the MATLAB Neural Network Toolbox (MATLAB Version R2020b-Mathworks Inc., Natick, MA, USA) to optimize thermosonication processing of freshly squeezed pomegranate juice. The model parameters from our previous study were used [12]. The learning rates were set as 0.441, 0.003, 0.054, 0.001, 0.093 and 0.008, respectively. ANNs were trained using 100 iterations. The best performing epochs of the responses were 237, 100, 102, 100, 101 and 150, respectively. The performance of the system was measured using the plot regression function. The neural network created includes 3 inputs, 10 hidden layers and 6 output layers (Fig. 1).

To clarify the performance of ANN models, determination coefficient (R^2), root mean square error (RMSE) and absolute average deviation (AAD) were compared between RSM and ANN models. The formulas are written as follows:

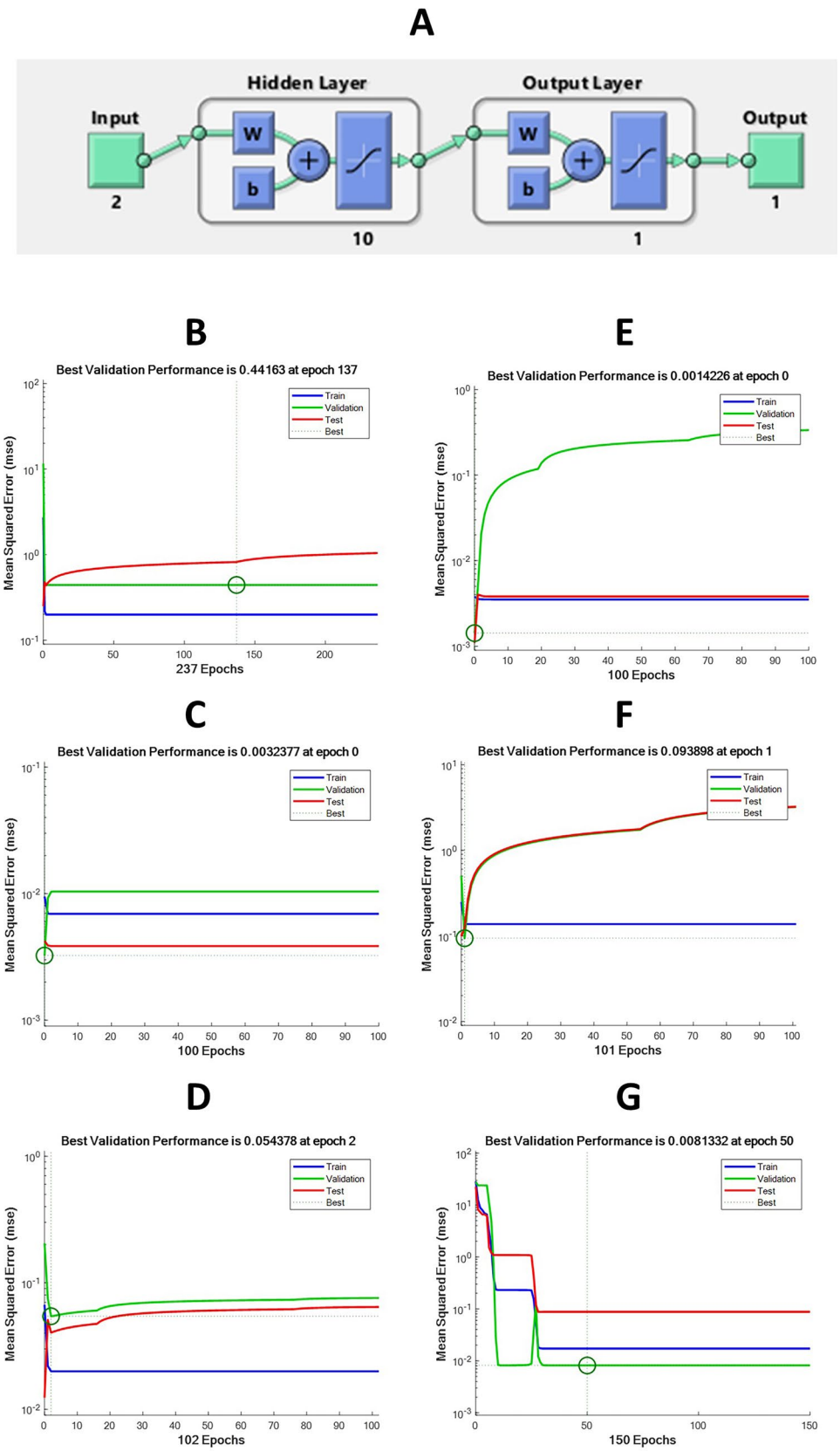


Fig. 1 Optimal architecture of the developed ANN model (**A**) and performance plot for the ANN model (**B**). **B** Total phenolic content; **C** Total flavonoid content; **D** Total anthocyanin content **E** Ascorbic acid F Radical scavenging activity; **G** Cupric reducing antioxidant capacity

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

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

$$ADD = \left(\frac{1}{n}\sum_{i=1}^{n} \left|\frac{Y_{Predicted} - Y_{Experimental}}{Y_{Experimental}}\right|\right) * 100 \quad (4)$$

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

Determination of bioactive compounds

TPC was determined using the Folin-Ciocalteu method as described by Singleton and Rossi (1965) with a spectrophotometer (Spectrum Instrument, SP-UV / VIS-300SRB, Australia) [13]. TPC was determined in triplicate for each treatment, sampling day, and replicate and results are expressed as mg of gallic acid equivalents per 100 mL. Total flavonoid concentrations were calculated colorimetrically by UV spectrophotometer (Spectrum Instrument, SP-UV / VIS-300SRB, Australia) according to the method applied by Zhishen et al. (1999) [14]. Antioxidant activity was assessed using two different methods: the scavenger 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and cupric ion reducing antioxidant capacity (CUPRAC) following the methodologies previously described by Grajeda-Iglesias et al. (2016) and Apak et al. (2006) [15, 16], respectively. Total monomeric anthocyanin content (TAC) was determined with the pH differential method described by Giusti and Wrolstad [16]. TAC was determined in triplicate for each treatment, sampling day, and replicate and results are expressed as mg of cyanidin 3-glucoside equivalents per 100 mL of juice. The ascorbic acid concentration was determined using Tillman's titrimetric method (2,6-Dichlorophenolindophenol sodium) [17].

Color and physicochemical analyses

Brix was measured at 20 °C using an optical refractometer (ATAGO brand RX-7000α model, Japan) and pH was measured with a pH meter (Hanna Instruments HI 2002 pH/ORP, Romania). The titration acidity was potentiometrically determined by titration of the samples with 0.1 N NaOH (Sigma-Aldrich, USA) solution to pH 8.1. From the sample, 5 ml was taken and 50 mL of distilled water was added and 10 mL of the sample was taken from the filtrate. The results are calculated as g citric acid/L.

L^*, a^* and b^* values of fruit juices were measured with a Hunter colorimeter (Color Measuring Device PCE-CSM 5, Germany). L^* is a measure of light and darkness between 0–100. 0 corresponds to black and 100 corresponds to white. In the color measurement system, positive (+) values of the* parameter indicate redness and negative (−) values indicate greenness. The positive (+) values of the b^* parameter indicate yellow and the negative (−) values represent blue. Chroma (C),hue angle (h), ΔE, and browning index (BI) were expressed according to the following Eqs. (1) and (2);

$$\text{Chroma, C} = (a^2 + b^2)^{1/2} \quad (5)$$

$$\text{h (hue angle)} = \tan^{-1}(b/a) \quad (6)$$

$$\Delta E = ((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)^{1/2} \quad (7)$$

$$BI = [100(x - 0.31)]0.17 \quad (8)$$

where

$$x = (a * +1.75L *)(5.645L * +a * -3.012b *) \quad (9)$$

All determinations were carried out three times per treatment.

Determination of microbial load

The treated formulations were submitted to serial dilutions in buffered peptone water 0.1% w/v. Potato dextrose agar (PDA, Merck, Germany) was used to count molds and yeasts (YMC), media was set to pH 3.5 during preparation using 100 g/L tartaric acid, incubating aerobically at 27 ℃ for 5 days. Plate count agar (PCA, Merck, Germany) was used to count total aerobic mesophilic bacteria (AMBC), with aerobic incubation at 37 ℃ for 48 h. The *Enterobacteriaceae* (EC) count was poured in VRBG (violet red bile agar, Merck, Germany) with an incubation of 24 h–37 ℃. After solidification of the agar, the plates were incubated at 37 °C for 48 h. Then the purple reddish colonies were counted. Each test was performed in three replicates and the results are expressed as $\log_{10}$ colony forming units per (CFU) milliliter ($\log_{10}$ CFU/mL) of sample.

In vitro-simulated gastrointestinal digestion analysis

An in vitro digestion model was used followed by dialysis according to the method of Minekus et al. [18]. 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 TPC, TFC, TAC, ascorbic acid and

antioxidant activity were performed after the gastric and intestinal phases and were determined in triplicate for each treatment and replicate.

ICP-MS-based mineral compound analysis

The elements in the samples (Ca, Cu, Fe, K, Mg, Na and Zn) were determined with an atomic absorption spectrometer (Perkin Elmer Analyst AA800) at wavelengths of 422.7, 324.8, 248.3, 766.5, 285.2, 589.0 and 213.9; C_2H_2 flow (L min-1) rates of 2.0, 1.7, 2.0, 2.4, 2.1, 1.9 and 1.8, and oxidant air flow (L min^{-1}) rates of 18.0, 14.0, 17.0, 13.0, 17.0, 17.0 and 19.3, respectively. A known volume of sample (0.3–0.5 g), 6.5 mL 65% nitric acid solution and 1.5 ml 30% hydrogen peroxide (H2O2) were injected into the Teflon container and then the mix was burned in a microwave oven at temperatures varying between 180 °C during 30 min (Anton Paar Multiwave GO). The acid digested samples were diluted with ultra-pure water in 50-mL volumetric flasks, and the mineral contents of samples were determined as mg/kg according to calibration curves which were prepared with Ca, Cu, Fe, K, Mg, Na and Zn standards (Merck). Phosphorus (P) in samples was determined with UV–Visible spectrophotometer (Shimadzu, UV-1601) at a wavelength of 400 nm. A known volume of sample (0.3–0.5 g), 6.5 mL 65% nitric acid solution and 1.5 ml 30% hydrogen peroxide (H_2O_2) were injected into the Teflon container and then the mix was burned in a microwave oven at temperatures varying between 180 °C during 30 min (Anton Paar Multiwave GO). The acid digested samples were diluted with ultra-pure water in 50-mL volumetric flasks. 5 mL sample solution was mixed with 10 mL of molybdovanadate (Merck) solution which was prepared by dissolving 1.25 g of ammonium monovanadate and 25 g of ammonium hepta molybdate tetrahydrate in 1 L of ultra-pure water including 70 mL 65% nitric acid solution. Phosphorus content of samples was determined as mg/kg according to calibration curves which were prepared with P standard (Merck).

Determination of organic acid contents

Organic acid contents were evaluated by using the AOAC official method (1995) [19]. Samples were filtered through a 0.45-µ nylon membrane to ensure removal of any particulate impurities that might be present and injected into a Shimadzu LC-20A series HPLC model SPD-20A ultraviolet and visible detector (UV–VIS) system. The mobile phase consisted of 0.2 M KH_2PO_4 (pH 2.4) with a flow rate of 0.8 ml min^{-1}. InertSustain C18 column (5 µm, 4.6 × 250 mm) was used, 10 µL sample was injected, and peaks were identified by comparing retention times with those of commercially available external standards. Citric, malic, acetic, lactic, tartaric, oxalic, fumaric and formic acids were used as external standards, and different concentrations of each standard solution were used to draw a linear regression calibration curve. Results are expressed as g of each organic acid equivalent per liter of sample.

Analysis of antihypertensive and antidiabetic

The ACE inhibitory activity assay was carried out with some modifications [20]. The method measures the absorbance of hippuric acid from ACE activity from hippuryl-L-histidylL-leucine (HHL). The antidiabetic activity of juice (α-glucosidase and α-amylase) was investigated according to the modified method [21]. Acarbose was used as positive control in antidiabetic analyses. Absorbance measurements were performed by UV–VIS spectrophotometer (SP-UV / VIS-300SRB, Spectrum Instruments, Melbourne, Australia).

Determination of contents of sugars

Sugar contents were determined by using the method of Michael et al. [22]. Samples were filtered through 0.45-µ nylon membrane to ensure removal of any particulate impurities that might be present. A Shimadzu LC-20A series HPLC model RID-20A refractive index detector (RID) system was used. The mobile phase consisted of acetonitrile (83:17, v/v) with a flow rate of 2 ml min^{-1}. Agilent Zorbax NH_2 column (5 µm, 4.6 × 250 mm) was used, 10 µL sample was injected, and peaks were identified by comparing retention times with those of commercially available external standards. Fructose, glucose and sucrose were used as external standards, and different concentrations of each standard solution were used to draw a linear regression calibration curve. Results are expressed as g of fructose and glucose equivalent per liter of sample.

Analysis of volatile compounds

Analysis of volatiles were performed by a solid-phase microextraction (SPME) method described by Yıkmış et al. (2021), using a GC–MS system (Shimadzu Corp.) [12]. The identifications were achieved by comparing the mass spectra of unknown compounds with those in Wiley 8 and NIST 05 mass spectral laboratory.

Sensory evaluation

The sensory evaluation was based on the method of Yıkmış (2019) with slight modification using 30 trained panelists (16 males and 14 females, from 20 to 25 years old) from Istanbul Gelisim University who had received sensory assessment training. The panelists participated in the sensory assessment by evaluating 5 different features of color, aroma, texture, taste and overall acceptability [23]. The

C-PJ, P-PJ and TS-PJ were presented to panelists who were asked to describe the differences between samples by using a 9-point hedonic scale, where 1 means very much disliked and 9 very much liked.

Statistical analysis

Data analysis was performed using SPSS 22.0 software (SPSS Inc., Chicago, USA), JMP (12.2.0 SAS Institute, Inc., Cary, NC, USA), and SigmaPlot 12.0 Software (Systat Software, Inc., USA). The experimental results are expressed as the means ± standard deviations (SD) of three replicates for each treatment. Multigroup comparisons of the means were carried out by one-way analysis of variance (ANOVA) test with post hoc contrasts using least significant difference (LSD) test and Duncan test. The statistical significance for all tests was set at $p < 0.05$.

Results and discussion

Optimization of bioactive components

Experimental and predicted results of the bioactive component values of freshly squeezed pomegranate juice samples at different levels of amplitude, different temperature and time periods are given in Table 1. The experimental data obtained were subjected to the second-order polynomial regression model. As the result of the RSM optimization, the second-order polynomial regression model for TPC, TFC, TAC, ascorbic acid, DPPH and CUPRAC responses are given in Eqs. 10–15

Table 2 shows the analysis of variance (ANOVA) for TPC, TFC, TAC, ascorbic acid, DPPH and CUPRAC. The linear effect of X_1 and X_2 factors on TPC and TFC values as a result of RSM was statistically significant ($p > 0.001$). However, the linear effect of the X_1 factor for TAC, ascorbic acid and DPPH was not statistically significant ($p > 0.05$). The linear effect of X_1 and X_2 factors on DPPH values was not statistically significant ($p > 0.05$). In the 2-way interaction, only TAC, ascorbic acid and CUPRAC X_2 and X_3 factors were not found to be significant ($p > 0.05$). The effects of X_1, X_2 and X_3 factors on the bioactive components were found to be statistically significant in cross-interactions ($p < 0.001$). The interactions of the variables were graphically represented by three-dimensional (3D) response surfaces for RSM and ANN, as shown in Fig. 2. As a result of the RSM model, the R^2 values showed high correlations for TPC, TFC, TAC, ascorbic acid, DPPH and CUPRAC at 98.55, 98.25, 97.37, 98.72, 98.61 and 96.98, respectively (Table 2).

As a result of the optimization, X_1, X_2 and X_3 values were determined as 49.50 °C, 10.5 min and 72.50% amplitude, respectively. As a result of thermosonication treatment applied to pomegranate juice samples, the TPC optimization value was determined as 335.21 mg GAE/100 mL, an increase of 2.9% compared to the C-PJ sample. The TFC value, on the other hand, was found to be 47.89 mg CE/100 mL as a result of the optimization, an increase of 6.8% compared to the C-PJ sample. As a result of the optimization, TAC 16.31 mg C3G/100 mL was detected, an increase of 5.7% compared to the C-PJ sample. As a result of the optimization, DPPH activity was determined as 63.99%, an increase of 9.8% compared to the TVJ sample.

$$\mathbf{TPC(mgGAE/100mL)} = 1094.5 + 1.923X_1 - 53.18X_2 - 17.133X_3 - 0.05168X_1^2 + 0.6990X_2^2 + 0.08220X_3^2 + 0.1294X_1X_2 + 0.02902X_1X_3 + 0.5147X_2X_3 \quad (10)$$

$$\mathbf{TFC(mgCE/100mL)} = 160.53 + 0.219X_1 - 7.823X_2 - 2.497X_3 - 0.007091X_1^2 + 0.10042X_2^2 + 0.011831X_3^2 + 0.01919X_1X_2 + 0.00442X_1X_3 + 0.07604X_2X_3 \quad (11)$$

$$\mathbf{TAC(mg}\boldsymbol{C}3\boldsymbol{G}\mathbf{/100mL)} = -190.7 + 2.235X_1 + 2.369X_2 + 4.089X_3 - 0.02682X_1^2 - 0.12117X_2^2 - 0.03233X_3^2 - 0.011338X_1X_2 + 0.00778X_1X_3 + 0.00413X_2X_3 \quad (12)$$

$$\mathbf{Ascorbicacid(}\boldsymbol{mg}\mathbf{/mL)} = -132.42 + 1.5520X_1 + 1.696X_2 + 2.7832X_3 - 0.018149X_1^2 - 0.08973X_2^2 - 0.021706X_3^2 - 0.00787X_1X_2 + 0.00457X_1X_3 + 0.00243X_2X_3 \quad (13)$$

$$\mathbf{DPPH(\%inhibition)} = -872.2 + 10.172X_1 + 11.09X_2 + 18.380X_3 - 0.11898X_1^2 - 0.5935X_2^2 - 0.14347X_3^2 - 0.0527X_1X_2 + 0.03018X_1X_3 + 0.0184X_2X_3 \quad (14)$$

$$\mathbf{CUPRAC(\%inhibition)} = -168.8 + 6.463X_1 - 2.636X_2 + 2.572X_3 - 0.04814X_1^21 + 0.0776X_2^2 - 0.01444X_3^2 - 0.05645X_1X_2 - 0.01992X_1X_3 + 0.06140X_2X_3 \quad (15)$$

Table 1 Experimental and predicted responses of RSM and ANN and results of C-PJ and P-PJ

Run no	Independent variables			Dependent variables																
	Tem-puture (X_1)	Time (X_2)	Ampli-tude (X_3)	TPC (mg GAE/100 mL)			TFC (mg CE/100 mL)			TAC (mg C3G/100 mL)			Ascorbic acid (mg/mL)			DPPH (% inhibition)			CUPRAC (% inhibition)		
				Experi-mental data	RSM pre-dicted	ANN pre-dicted	Experi-mental data	RSM pre-dicted	ANN pre-dicted	Experi-mental data	RSM pre-dicted	ANN pre-dicted	Experi-mental data	RSM pre-dicted	ANN pre-dicted	Experi-mental data	RSM pre-dicted	ANN pre-dicted	Experi-mental data	RSM pre-dicted	ANN pre-dicted
1	50	8	70	325.45	325.78	325.95	46.45	46.506	46.56	17.45	17.201	17.12	10.26	10.305	10.35	67.75	68.137	68.29	73.65	73.246	73.32
2	60	8	70	318.45	318.83	318.45	45.49	45.525	45.49	14.48	14.425	14.48	8.51	8.4307	8.51	56.40	55.889	56.40	66.50	66.462	66.50
3	50	8	70	325.45	325.78	325.95	46.45	46.506	46.56	17.45	17.201	17.12	10.26	10.305	10.35	67.75	68.137	68.29	73.65	73.246	73.32
4	50	8	70	324.86	325.78	325.95	46.41	46.506	46.56	17.45	17.201	17.12	10.26	10.305	10.35	67.75	68.137	68.29	73.15	73.246	73.32
5	50	8	80	332.95	333.43	333.08	47.56	47.575	47.57	13.75	13.816	13.76	8.09	8.0576	8.12	53.38	53.294	53.40	72.75	72.258	72.75
6	45	6	65	336.85	336.67	336.85	48.25	48.149	48.25	15.06	15.362	15.06	8.86	9.0042	8.87	58.47	59.542	58.48	72.70	72.139	72.70
7	50	8	70	324.86	325.78	325.95	46.41	46.506	46.56	16.88	17.201	17.12	10.45	10.305	10.35	68.97	68.137	68.29	73.15	73.246	73.32
8	50	8	70	325.45	325.78	325.95	46.80	46.506	46.56	16.88	17.201	17.12	10.45	10.305	10.35	68.97	68.137	68.29	73.65	73.246	73.32
9	55	6	65	330.8	330.85	330.80	47.22	47.273	47.33	15.04	15.146	15.04	8.84	8.8735	8.84	58.38	58.737	58.35	72.20	72.294	72.20
10	50	8	70	324.86	325.78	325.95	46.60	46.506	46.56	16.88	17.201	17.12	10.26	10.305	10.35	67.72	68.137	68.29	73.15	73.246	73.32
11	40	8	70	322.48	322.39	322.02	46.15	46.068	45.94	14.66	14.613	14.66	8.62	8.5499	8.62	56.91	56.589	56.83	70.45	70.402	70.45
12	50	8	70	325.45	325.78	325.95	46.45	46.506	46.56	17.07	17.201	17.12	10.45	10.305	10.35	68.97	68.137	68.29	73.65	73.246	73.32
13	45	10	75	334.55	334.08	335.09	47.79	47.695	47.79	15.21	15.29	15.21	8.95	9.0038	8.96	59.04	59.579	59.04	75.20	75.044	75.20
14	50	8	70	326.7	325.78	325.95	46.45	46.506	46.56	17.45	17.201	17.12	10.26	10.305	10.35	67.75	68.137	68.29	73.22	73.246	73.32
15	50	8	80	333.08	333.43	333.08	47.58	47.575	47.57	13.75	13.816	13.76	8.09	8.0576	8.12	53.38	53.294	53.40	72.75	72.258	72.75
16	45	10	75	335.62	334.08	335.09	47.95	47.695	47.79	15.26	15.29	15.21	8.97	9.0038	8.96	59.23	59.579	59.04	75.20	75.044	75.20
17	50	12	70	337.84	338.97	337.84	48.26	48.376	48.26	15.36	15.464	15.37	9.03	9.0172	9.02	59.62	59.629	59.62	74.81	74.812	74.81
18	50	8	70	326.3	325.78	325.95	46.61	46.506	46.56	17.08	17.201	17.12	10.45	10.305	10.35	68.97	68.137	68.29	73.15	73.246	73.32
19	55	6	75	322.03	321.44	321.68	46.00	45.859	45.95	15.08	15.301	15.08	8.87	8.9764	8.87	58.45	59.382	58.44	70.65	70.526	70.65
20	45	6	75	324.33	324.36	324.33	46.33	46.294	46.33	14.74	14.739	14.74	8.67	8.6501	8.67	57.23	57.169	57.22	71.82	72.363	71.82
21	55	6	65	330.8	330.85	330.80	47.44	47.273	47.33	15.04	15.146	15.04	8.84	8.8735	8.84	58.38	58.737	58.35	72.20	72.294	72.20
22	50	8	70	325.45	325.78	325.95	46.45	46.506	46.56	16.88	17.201	17.12	10.26	10.305	10.35	67.72	68.137	68.29	72.70	73.246	73.32
23	45	10	65	326.58	325.8	326.53	46.65	46.509	46.65	15.80	15.748	15.80	9.29	9.2607	9.29	61.34	61.216	61.30	72.31	72.364	72.29
24	40	8	70	321.55	322.39	322.02	45.94	46.068	45.94	14.62	14.613	14.66	8.60	8.5499	8.62	56.74	56.589	56.83	70.45	70.402	70.45
25	60	8	70	318.45	318.83	318.45	45.49	45.525	45.49	14.48	14.425	14.48	8.51	8.4307	8.51	56.40	55.889	56.40	66.50	66.462	66.50
26	55	10	65	326.02	325.15	326.15	46.57	46.4	46.59	14.82	14.997	14.82	8.72	8.8152	8.72	57.43	58.303	57.43	70.87	70.261	70.87
27	45	6	75	324.33	324.36	324.33	46.33	46.294	46.33	14.74	14.739	14.74	8.67	8.6501	8.67	57.23	57.169	57.22	71.82	72.363	71.82
28	50	4	70	335.84	334.95	335.33	47.98	47.848	47.98	15.27	15.061	15.27	8.85	8.7218	8.86	58.41	57.653	58.72	74.36	74.164	74.14
29	50	12	70	337.84	338.97	337.84	48.26	48.376	48.26	15.36	15.464	15.37	9.03	9.0172	9.02	59.62	59.629	59.62	74.81	74.812	74.81
30	50	4	70	334.82	334.95	335.33	47.83	47.848	47.98	15.22	15.061	15.27	8.85	8.7218	8.86	58.41	57.653	58.72	74.14	74.164	74.14
31	50	8	70	326.7	325.78	325.95	46.61	46.506	46.56	17.45	17.201	17.12	10.26	10.305	10.35	67.75	68.137	68.29	73.15	73.246	73.32
32	50	8	70	326.7	325.78	325.95	46.61	46.506	46.56	17.45	17.201	17.12	10.45	10.305	10.35	68.97	68.137	68.29	73.15	73.246	73.32
33	50	8	60	334.8	334.56	335.62	47.83	47.802	47.75	14.28	14.12	14.60	8.40	8.2116	8.33	55.44	54.286	54.96	70.94	71.346	70.94
34	50	8	60	333.65	334.56	335.62	47.66	47.802	47.75	14.24	14.12	14.60	8.25	8.2116	8.33	54.45	54.286	54.96	70.94	71.346	70.94
35	55	10	75	337.28	336.34	337.28	48.18	48.028	48.16	15.45	15.317	15.45	9.09	9.0153	9.07	59.95	59.684	59.98	70.45	70.949	70.45
36	55	10	65	326.27	325.15	326.15	46.61	46.4	46.59	14.83	14.997	14.82	8.72	8.8152	8.72	57.43	58.303	57.43	70.87	70.261	70.87
37	45	10	65	326.47	325.8	326.53	46.64	46.509	46.65	15.80	15.748	15.80	9.29	9.2607	9.29	61.34	61.216	61.30	72.29	72.364	72.29

Table 1 (continued)

Run no	Independent variables			Dependent variables													
	Temputure (X_1)	Time (X_2)	Amplitude (X_3)	TPC (mg GAE/100 mL)			TFC (mg CE/100 mL)			TAC (mg C3G/100 mL)			Ascorbic acid (mg/mL)			DPPH (% inhibition)			CUPRAC (% inhibition)		
				Experimental data	RSM predicted	ANN predicted	Experimental data	RSM predicted	ANN predicted	Experimental data	RSM predicted	ANN predicted	Experimental data	RSM predicted	ANN predicted	Experimental data	RSM predicted	ANN predicted	Experimental data	RSM predicted	ANN predicted
38	55	10	75	336.95	336.34	337.28	48.14	48.028	48.16	15.45	15.317	15.45	9.09	9.0153	9.07	59.95	59.684	59.98	70.45	70.949	70.45
39	45	6	65	336.85	336.67	336.85	48.25	48.149	48.25	15.06	15.362	15.06	8.86	9.0042	8.87	58.47	59.542	58.48	72.70	72.139	72.70
40	55	6	75	321.32	321.44	321.68	45.90	45.859	45.95	15.08	15.301	15.08	8.87	8.9764	8.87	58.45	59.382	58.44	70.65	70.526	70.65
Predictive capacity comparison of RSM and ANN models for five response variables				R2	0.985	0.99		0.98	0.989		0.973	0.978		0.987	0.995		0.986	0.995		0.969	0.993
				RMSE	0.69	0.57		0.12	0.09		0.19	0.17		0.09	0.05		0.6	0.36		0.33	0.16
				ADD (%)	0.17	0.12		0.21	0.13		0.98	0.59		0.8	0.4		0.81	0.36		0.36	0.11
TS-PJ	49.5	10.5	72.5	335.21			47.89			16.31			9.7			63.99			74.59		
C-PJ				325.44			44.62			14.86			11.28			60.36			70.48		
P-PJ				325.48			40.80			13.25			8.1			56.40			66.28		

TPC total phenolic content, *TFC* total flavonoid content, *TAC* total anthocyanin content, *DDPH* radical scavenging activity, *CUPRAC* cupric reducing antioxidant capacity, *GAE* gallic acid equivalent, *CE* catechin equivalent, *C3g* cyanidin 3-glucoside, R^2 coefficient of determination, *RMSE* root mean square error, *AAD* absolute average deviation, *RSM* response surface methodology, *ANN* artificial neural network, *C-PJ* untreated pomegranate juice, *TS-PJ* thermosonication-treated pomegranate juice, *P-PJ* thermal pasteurized pomegranate juice

CUPRAC activity was determined as 74.59% as a result of the optimization and it was determined that there was an increase of 5.5% compared to the TJV sample. At the end of the thermal pasteurization applied in the TJV sample, decreases were detected in all bioactive components, while increases occurred with thermosonication treatment (except ascorbic acid). As a result of the optimization, reductions in the amount of ascorbic acid (16% reduction) were detected, but it was preserved more than with thermal pasteurization. Increases in bioactive compounds were reported in similar reports about fruit and vegetable smoothies [24], black, red and white currant juices [25], prebiotic soursop whey beverage [26], cashew apple bagasse [27], beetroot (*Beta vulgaris* L.) juice [11] and orange juice whey drink [9]. The formation of hydroxyl radicals, increased mass transfer rates and the formation of micro-voids during the formation of cavitation with the effect of thermosonication may cause an increase in bioactive components [28, 29]. At the same time, the increase in phenolic substance content and flavonoid substance content with ultrasound treatments can explain the increase in total antioxidant amounts.

The statistical results for the parameters used to compare the RSM and ANN models are shown in Table 1. The R^2 values of the ANN and RSM models were found to provide sufficient experimental fit. R^2 was detected for RSM (0.985, 0.980, 0.983, 0.973, 0.986 and 0.969 for TPC, TFC, DPPH and CUPRAC respectively) and for ANN; R^2 (0.990, 0.989, 0.978, 0.995, 0.995 and 0.969 for TPC, TFC, DPPH and CUPRAC respectively). For the experimental data and the predicted data, ANN had higher fit, indicating it was an alternative or better approach than RSM. For RSME and ADD values, the ANN model gave better results for all bioactive values (Table 1). It was concluded that the ANN model was more reliable and had higher accuracy than the RSM model in terms of predictive ability and measured responses for bioactive compounds. Similar results to our study were reported for optimization of kidney bean antioxidants [30], cashew apple bagasse [27], cranberry pomace [31] and ultrasound-assisted extraction of phenolic compounds of garlic [32], with better results for ANN modeling.

Physicochemical properties and color

The pH, total soluble solids (°Brix) and TA results for the C-PJ, P-PJ and TS-PJ samples are shown in Table 3. The pH values of the samples were determined in the range of 3.30–3.31. There were no statistical differences in the pH, Brix and TA values of the pomegranate juice samples ($p > 0.05$). No significant differences were detected in pH, total soluble solids (Brix) and TA physicochemical values with ultrasound and thermosonication treatments applied to different fruit juices [4, 8, 33, 34].

Table 2 Corresponding p-values of linear, interaction and quadratic terms of regression coefficients obtained by RSM of responses for TPC, TFC, TAC, Ascorbic acid, DPPH and CUPRAC experiments

Source	DF	TPC (mg GAE/100 mL)		TFC (mg CE/100 mL)		TAC (mg C3G/100 mL)		Ascorbic acid (mg/mL)		DPPH (% inhibition)		CUPRAC (% inhibition)	
		F-Value	P-Value	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
Model	9	226.74	0.0000	187.24	0.0000	123.6	0.0000	257.26	0.0000	237.23	0.0000	106.88	0.0000
Linear	3	32.35	0.0000	26.09	0.0000	4.37	0.0110	8.16	0.0000	7.38	0.0010	76.36	0.0000
X_1	1	40.24	0.0000	37.05	0.0000	1.63	0.2120	2.54	0.1220	2.11	0.1570	211.96	0.0000
X_2	1	52.71	0.0000	35.5	0.0000	7.24	0.0120	17.44	0.0000	15.86	0.0000	5.63	0.0240
X_3	1	4.11	0.0520	5.71	0.0230	4.25	0.0480	4.52	0.0420	4.18	0.0500	11.47	0.0020
Square	3	403.06	0.0000	323.76	0.0000	359.62	0.0000	753.03	0.0000	694.35	0.0000	209.88	0.0000
X_1^2	1	134.28	0.0000	100.06	0.0000	501.77	0.0000	1033.28	0.0000	941.94	0.0000	497.95	0.0000
X_2^2	1	628.95	0.0000	513.75	0.0000	262.18	0.0000	646.59	0.0000	600.08	0.0000	33.09	0.0000
X_3^2	1	339.69	0.0000	278.57	0.0000	729.13	0.0000	1478.06	0.0000	1369.71	0.0000	44.79	0.0000
2-Way Interaction	3	244.8	0.0000	211.87	0.0000	6.8	0.0010	10.58	0.0000	9.95	0.0000	34.41	0.0000
X_1* X_2	1	42.88	0.0000	37.3	0.0000	6.36	0.0170	9.89	0.0040	9.41	0.0050	34.86	0.0000
X_1* X_3	1	13.48	0.0010	12.4	0.0010	13.43	0.0010	20.89	0.0000	19.28	0.0000	27.13	0.0000
X_2* X_3	1	678.05	0.0000	585.92	0.0000	0.61	0.4420	0.94	0.3390	1.15	0.2930	41.25	0.0000
Error	30												
Lack-of-fit	5	6.26	0.001	4.51	0.005	3.24	0.022	8.23	0	9.32	0	16.62	0
Pure error	25												
Total	39												
R^2		98.55%		98.25%		97.37%		98.72%		98.61%		96.98%	
Adj R^2		98.55%		97.73%		96.59%		98.34%		98.20%		96.07%	
Pred. R^2		97.29%		96.79%		95.89%		97.67%		97.44%		94.24%	

X_1: Tempature; X_2: Time; X_3: Amplitude; DF: Degree of freedom; TPC: Total phenolic content; TFC: Total flavonoid content; TAC:Total anthocyanin content; DDPH: Radical scavenging activity; CUPRAC: Cupric reducing antioxidant capacity; GAE: Gallic acid equivalent; CE: Catechin equivalent; C3g: cyanidin 3-glucoside

Color is an important quality parameter that directly affects the sensory quality and represents the freshness of pomegranate juice. The effects of different processing treatments on *L* *, *a* *, *b* *, C, h°, BI and ΔE of pomegranate juice samples are presented in Table 3. The L* value was higher in TS-PJ and P-PJ compared to C-PJ ($p < 0.05$). This means that processing gives a lighter appearance. In addition, TS treatment of pomegranate juice resulted in increased red hue ($p > 0.05$). A similar effect on *L** and *a** and values was also detected for thermosonication applied to tomato juice enriched with anthocyanin [7]. In fruit juices, the increased value in *L** can be explained by partial precipitation of unstable suspended particles due to oxidative darkening of the cloud value due to the homogenizing effect of ultrasound treatment [35]. With the treatment applied to blood fruit (*Haematocarpus validus*) juice, decreases were detected in the b value with increasing thermosonication parameters [10]; contrarily, an increase was detected with the treatment applied to pomegranate juice ($p < 0.05$). No significant changes were observed in BI (browning index) values of pomegranate juice ($p > 0.05$). To better define the effect of visual discoloration on pomegranate juice, the variation of color values (AE) was examined. TS treatment (3.20) showed slight color change compared to thermal pasteurization (3.70). Similar effects in ultrasound treatments were also detected in the blackberry juice study [6]. When the results were examined in general, it was determined that the color values of pomegranate juice caused changes in both treatments. Changes in the color values of pomegranate juice may be caused by cavitation, heat effects and physical and chemical effects caused by thermosonication.

Microbial load

Thermal pasteurization and TS processing were performed on pomegranate juice and their effects on inactivation of the natural microbial load were also evaluated. Microbial loads of C-PJ, P-PJ and TS-PJ samples are shown in Table 3. Enterobacteriaceae were not detected in any of the samples. Ultrasound and TS treatments were reported to be effective in minimizing the foodborne microbial load (aerobic mesophilic, yeast and mold) of barberry juice, orange, fruit (*Haematocarpus validus*), vegetable, hog plum (*Spondias mombin* L.), carrot and cherry, red grape and pomegranate juices [4, 6, 8, 10, 33, 34, 36]. In our study, YMC and AMCB were observed in the control pomegranate juice

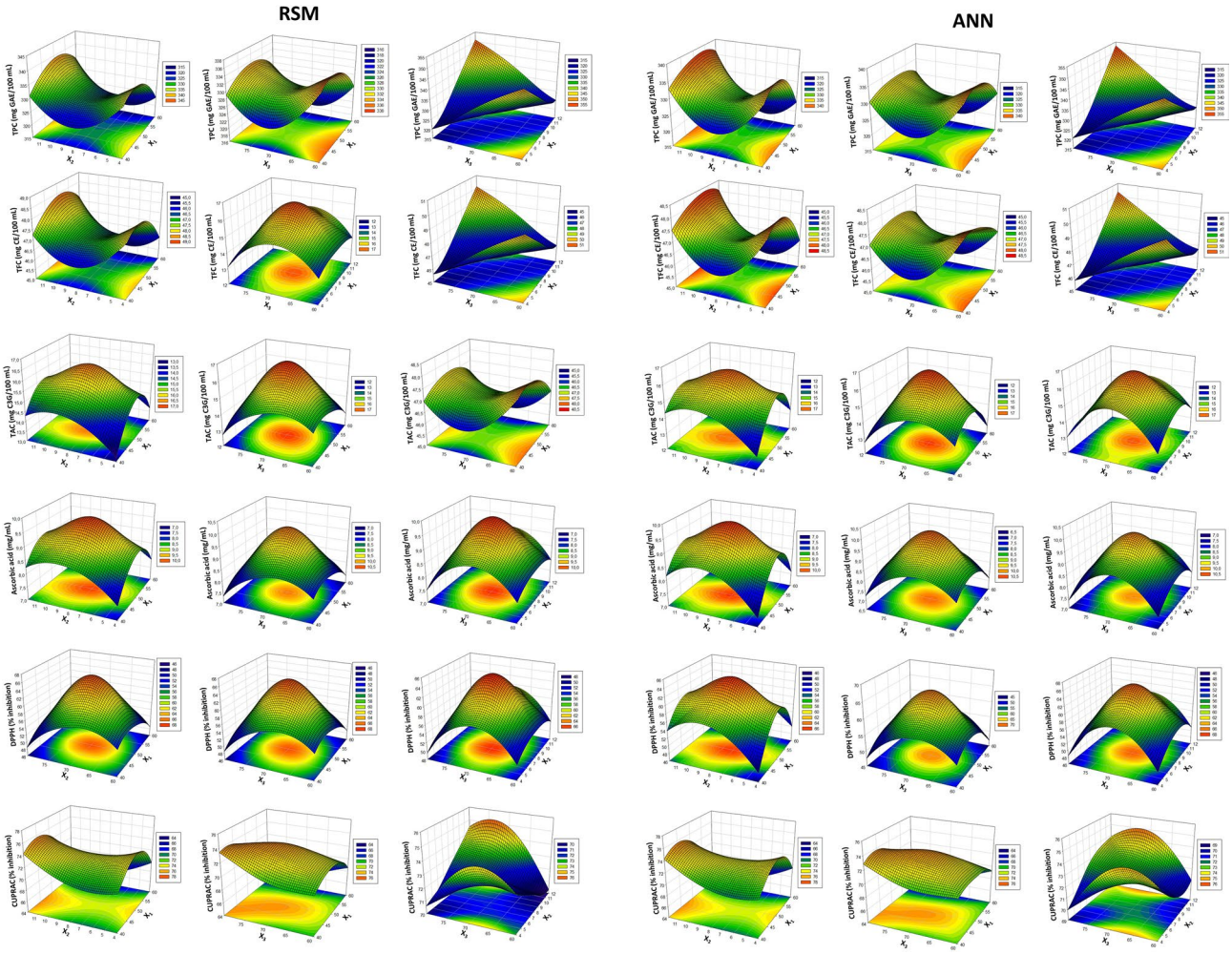


Fig. 2 Response surface plots (3D) for bioactive compound analysis as a function of significant interaction factors for RSM and ANN

sample. However, a net reduction in microbial load was found after thermal pasteurization and TS processes. The decrease in microbial load may be due to ultrasound, which changes the structure of the microbial cell membrane, and the increase in the heat sensitivity of microorganisms. [5]. Also, the reduction in microbial load during TS treatment can be attributed to physical and chemical mechanisms that occur during moderate heat exposure and cavitation.

Organic acid and sugar content

Organic acid and sugar contents of C-PJ, P-PJ and TS-PJ samples are shown in Table 3. The TS-PJ sample had higher rates of fructose (75.84 ± 0.93 g/L) and glucose (71.45 ± 0.30 g/L) compared to the other samples. Sugar components of the C-PJ sample were not significantly different from those of the TS-PJ sample ($p > 0.05$). Citric acid (12.97 ± 0.09 g/L) was detected in excess in the TS-PJ sample and was statistically different from P-PJ ($p < 0.05$). All samples did not show significant difference in terms of malic acid ($p > 0.05$). With ultrasound treatment applied to remove the bitterness in juice of the bitter variety of citrus fruit, reductions in organic acid and sugar components were detected at the end of the treatment, but there was no significant effect in our study [37]. Consistent with our study, no significant change was detected in glucose and fructose contents after ultrasound treatment applied to mandarin (*Citrus unshiu*) juice [38]. In addition, in a report Abid et al. (2014) detected significant increases in glucose and fructose values with 30-min ultrasound treatment applied to apple juice, but minimal increases were observed after thermosonication treatment applied to pomegranate juice [39].

ICP-MS mineral compound analysis results

The mineral composition of the C-PJ, P-PJ and TS-PJ samples is shown in Table 3. In the pomegranate juice samples, 7 different mineral elements (Ca, Mg, K, Zn, Fe, P

Table 3 Physicochemical, color, organic acid, sugar contents and minerals results of samples C-PJ, P-PJ and TS-PJ

Analyzes		Samples		
		C-PJ	P-PJ	TS-PJ
Physicochemical	pH	3.30 ± 0.00^{a}	3.31 ± 0.01^{a}	3.31 ± 0.01^{a}
	TSS (°Brix)	16.50 ± 0.00^{a}	16.17 ± 0.29^{a}	16.33 ± 0.29^{a}
	TA (%)	1.27 ± 0.00^{a}	1.27 ± 0.01^{a}	1.28 ± 0.01^{a}
Color	*L**	50.54 ± 0.17^{b}	53.93 ± 1.30^{a}	53.41 ± 0.30^{a}
	*a**	25.35 ± 0.29^{a}	25.21 ± 0.17^{a}	25.7 ± 0.22^{a}
	*b**	16.7 ± 0.03^{b}	18.14 ± 0.22^{a}	18.07 ± 0.04^{a}
	Chroma (C)	30.36 ± 0.26^{b}	31.06 ± 0.10^{a}	31.41 ± 0.16^{a}
	Hue angle (h°)	33.38 ± 0.26^{b}	35.74 ± 0.48^{a}	35.11 ± 0.27^{a}
	BI	74.78 ± 0.68^{a}	73.48 ± 1.15^{a}	74.63 ± 0.62^{a}
	ΔE	-	3.70 ± 1.16	3.20 ± 0.19
Microbial load	YMC (log CFU/mL)	5.45 ± 0.13	< 0.5	< 0.5
	AMBC (log CFU/mL)	2.85 ± 0.12	n.d	n.d
	EC (log CFU/mL)	n.d	n.d	n.d
Organic acid content	Malic acid (g/L)	0.37 ± 0.01^{a}	0.37 ± 0.1^{a}	0.37 ± 0.00^{a}
	Citric acid (g/L)	12.80 ± 0.07^{ab}	12.56 ± 0.12^{b}	12.97 ± 0.09^{a}
Sugar content	Glucose (g/L)	71.37 ± 0.16^{a}	67.72 ± 1.10^{b}	71.45 ± 0.30^{a}
	Fructose (g/L)	74.50 ± 0.13^{a}	74.51 ± 0.28^{a}	75.84 ± 0.93^{a}
Minerals	Ca (mg/L)	90.43 ± 0.04^{a}	82.74 ± 14.20^{a}	91.73 ± 0.01^{a}
	Mg (mg/L)	126.20 ± 0.14^{a}	125.5 ± 6.36^{a}	123.3 ± 3.54^{a}
	K (mg/L)	2205.5 ± 19.09^{a}	2226.5 ± 21.92^{a}	2093.5 ± 51.62^{a}
	Zn (mg/L)	185 ± 0.01^{a}	1.81 ± 0.00^{a}	1.59 ± 0.01^{b}
	Fe (mg/L)	0.61 ± 0.01^{b}	0.76 ± 0.08^{b}	0.96 ± 0.01^{a}
	P (mg/L)	268.55 ± 0.21^{b}	284.2 ± 0.00^{a}	263.9 ± 0.14^{c}
	Na (mg/L)	11.01 ± 0.53^{a}	10.92 ± 0.99^{a}	11.04 ± 1.00^{a}

Results are presented mean ± standard deviation (n = 3). Values with the different letters within line are significantly different ($p < 0.05$). C-PJ: Untreated pomegranate juice TS-PJ: Thermosonication-treated pomegranate juice; P-PJ: Thermal pasteurized pomegranate juice: TA: Titration acidity; YMC: Yeast and mold count; AMBC: Aerobic mesophilic bacteria count; EC: *Enterobacteriaceae* count; TSS: Total soluble solids; ΔE: Total color change; BI: Browning index

and Na) were detected. Significant increases in the amounts of mineral substances (Ca, Fe, K, Mg, P, Na and Zn) were detected after different ultrasound treatments applied to Brazilian nopal (*Opuntia ficus-indica*) beverage compared to control and thermal pasteurization samples [40]. As a result of TS treatment, significant increases were detected in Ca, Fe and Na elements compared to the control sample, while decreases were detected in Mg, K, Zn and P. Decreases in Mg and Zn elements and highest destruction in Ca element were detected in P-PJ samples and no statistical difference was determined for other samples ($p > 0.05$). The greatest increase in K element was detected in the P-PJ sample. Prebiotic soursop whey had less Mg and K after high-intensity ultrasound treatments than thermal pasteurization, which is in line with our study [26]. Decreases were detected in Zn after both treatments. TS caused an increase of 57.4% in Fe content compared to the C-PJ sample ($p < 0.05$). For P, the P-PJ sample was detected to contain 0.76 mg/L more than other samples ($p < 0.05$). TS treatment caused a minimal increase in Na ($p > 0.05$). Increases in Na and Ca and decreases in Mg were detected with ultrasound treatment applied to apple juice and grapefruit juice [39, 41], the same effects were detected with thermosonication treatment in our study. Ahmed et al. (2019) found increases in Fe and Ca minerals and decreases in Zn after different thermosonication treatments applied to wheat seedling juice [42]. In another study, increases in K and Mg were detected in pear juice treated with ultrasound [43], At the same time, Mazoor et al. (2020) stated that there were significant decreases in Fe, Ca and Zn, and a significant increase in K in the spinach juice sample that was treated with ultrasound [44];however, the opposite results were found for the same elements in our study.. Due to cavitation occurring during TS, the activity of substances in the cells increases and the migration of some minerals from the cells to the solution may cause an increase in mineral matter. This is the first study about the effect of TS on pomegranate juice minerals. Therefore, more research is required to better understand the sonochemical process and causes of some mineral matter reductions after thermosonication treatments.

ACE and Antidiabetic activity

Increasing the most commonly identified bioactive components in pomegranate juice by TS can explain the increase in ACE, α-glucosidase and α-amylase activities. The deterioration in cells caused by cavitation, the increase in bioactive compounds, and the fact that enzymes create an affinity with their active sites explains the increase in inhibition. The ACE inhibitory activity of C-PJ, P-PJ and TS-PJ samples is shown in Fig. 3A. ACE inhibitor activities of C-PJ, P-PJ and TS-PJ samples were determined as 35.86%, 34.32% and 39.82% inhibition, respectively. There was an 11% increase in ACE inhibition for the TS-PJ sample compared to the C-PJ sample ($p < 0.001$). As a result of thermal pasteurization treatment, there was a 4.3% decrease in the ACE inhibitory activity of pomegranate juice ($p < 0.001$). It was reported that ACE inhibitory activity increased with ultrasound and TS treatments applied to prebiotic soursop whey beverage, organic cherry laurel (*Prunus laurocerasus*) vinegar and tomato vinegar [9, 12, 26, 45].

It is important to control postprandial hyperglycemia with natural substances in diabetic treatment. Therefore, in our study, we tried to explain the antidiabetic effects of pomegranate juice and process conditions by reducing blood glucose absorption with α-glucosidase and α-amylase enzyme inhibitory activity. Bioactive compounds can show inhibitory properties for α-glucosidase and α-amylase enzymes in the hydrolysis of carbohydrates. Antidiabetic activity of C-PJ, P-PJ and TS-PJ samples is shown in Fig. 3(B-C). The TS-PJ sample showed the highest inhibition of α-glucosidase at 42.52%. An increase of 4.9% was detected compared to the C-PJ sample ($p < 0.05$). A 9.5% increase in α-amylase inhibition was detected for the TS-PJ sample compared to the C-PJ sample ($p < 0.001$). Regarding the antidiabetic activity of bitter gourd juice, pasteurization treatments (thermal and radiation) had no significant effect on α-amylase activity [46]. However, in our study, decreases were detected in α-glucosidase and α-amylase enzyme inhibitory activities as a result of thermal pasteurization. As a result of TS applied to pomegranate juice, in vitro antidiabetic effects were positively affected. The antidiabetic mechanism of TS in pomegranate juice can be explained by the increase in bioactive components as a result of cavitation, as reported in other studies [12, 45]. Therefore, in vitro ACE and antidiabetic (α-glucosidase and α-amylase) results showed that thermosonication-treated pomegranate juice could be a natural supplementary food.

Aroma profiles

Table 4 shows the volatile compounds identified in the C-PJ, P-PJ and TS-PJ samples. Principal component analysis (PCA) was used to evaluate the differences in volatile compounds between C-PJ, P-PJ and TS-PJ samples. The PCA plot in Fig. 4A shows the distribution of samples in 2 principal components. Eigenvector values in the score graph evaluating all pomegranate juice samples were obtained as 100% (PC1 = 82.5% and PC2 = 17.5%). PCA is suitable for distinguishing samples and grouping volatile compounds according to their spatial location. C-PJ is positively loaded on PC1 and PC2, P-PJ was negatively loaded on PC1 and positively loaded on PC2. However, TS-PJ was negatively loaded on PC1 and PC2 and was not grouped with volatile compounds. Hierarchical cluster analyses (HCA) were performed using the data obtained for C-PJ, P-PJ and TS-PJ. In HCA analysis, the Ward clustering method was used with distance calculations. The dendograms and constellation clusters of the C-PJ, P-PJ, and TS-PJ samples are shown in Fig. 4B-C. When the dendrogram is examined, the volatile aroma profiles of the most similar (green group) pomegranate juice samples were grouped first and the starting groups were combined according to their similarity. Finally, as the similarity decreased, all subgroups were combined into a single cluster. Classification is by distance in cluster analysis and constellations are separated by color. The green area (18), red area (9), blue area (3) and orange area (1) are divided into cluster groups. Pomegranate juice samples contained 30–31 volatile compounds, and the most identified groups were terpenes (7), alcohols (7), and aldehydes (6). The lowest amounts of volatile compounds were found in P-PJ (391.01 µg/kg), while the highest amounts of volatile compounds were found in C-PJ (599.13 µg/kg) and TS-PJ (445.81 µg/kg). TS was more affected by the total change, and the highest change was detected in limonene, hexanol and ethanol compounds compared to the C-PJ sample. Linalool, which is responsible for the floral, citrus and fruity aroma, suffered more damage in the P-PJ sample. In the reports of different effects on volatile compounds, as with the thermal pasteurization and ultrasound treatments applied to pomegranate juice, limonene and linalool compounds decreased after both treatments, as in our study [47]. Thermal pasteurization affected α-pinene compound more than TS ($p > 0.05$). At the end of TS applied to pomegranate juice, decreases in α-pinene, octanal and linalool compounds were similarly detected as effects of ultrasound to remove bitterness in citrus juice. [37]. Compounds considered Maillard reaction products, such as furfural, 5-hydroxymethylfurfural, and pyranone, were not detected, indicating that neither thermal pasteurization or TS treatments triggered the Maillard reaction. With 64.4% total volatile compound concentration in the TS-PJ sample, the least change was detected in alcohols that contributed most to the aroma of the pomegranate juice compared to the C-PJ sample. In the report by Tian et al. (2020), ultrasound treatment preserved the total alcohol volatile compounds of pomegranate juice more than thermal heat treatments, as in our study [47]. Similar

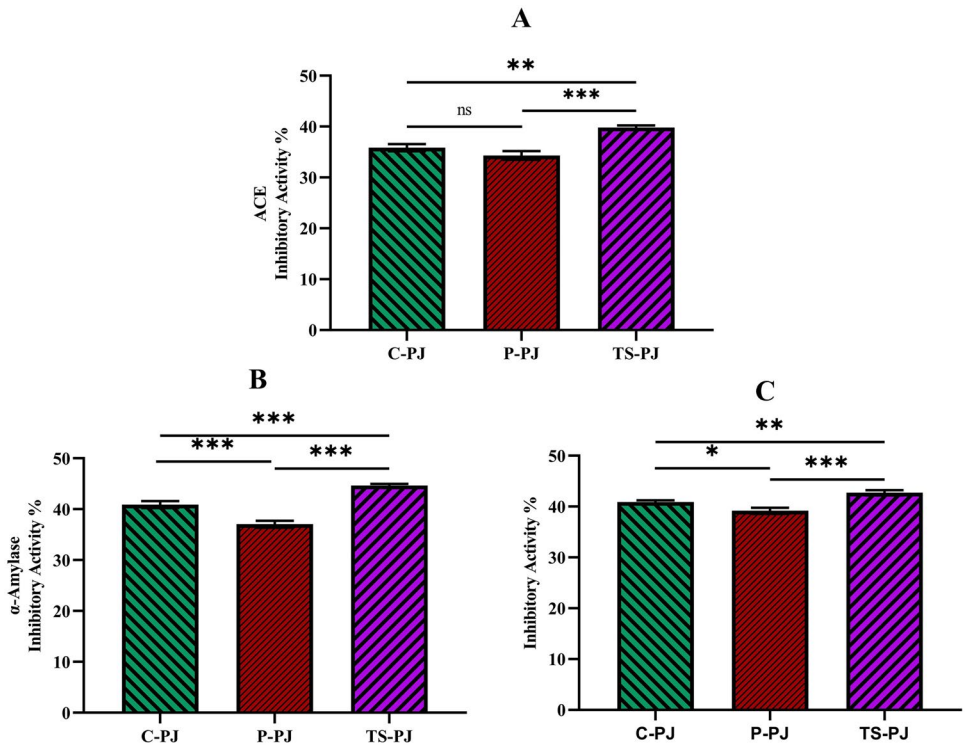


Fig. 3 **(A)** TPC, **(B)** TFC, **(C)** TAC, **(D)** Ascorbic acid, **(E)** DPPH, **(F)** CUPRAC and **(E)** Recovery (%) samples before and after the in vitro gastrointestinal digestion. Values represent mean values of three independent experiments ± S.D. Different lower case letters indicate significant differences between samples. Different capita letters indicate significant differences between digestive phases for the same treatment. The criterion for statistical significance was $p < 0.05$

results were found for TS treatment applied to grape juice, with reductions in volatile compounds including aldehydes and alcohols [48]. 6-Methyl-5-hepten-2-one, which significantly contributes to the fresh and green sensory properties of most fruits, was detected as 9.57 µg/kg and 12.79 µg/kg in P-PJ and TS-PJ samples, respectively ($p > 0.05$). The total for terpenes, alcohols, aldehydes, ketones and esters aroma compounds after TS treatment of pomegranate juice was higher than after thermal pasteurization. The same effect was reported in the ultrasound study by Cheng et al. (2020) using mandarin (*Citrus unshiu*) juice [38]. The positive effects of aroma compounds can be associated with possible synergistic effects of temperature and cavitation in TS technology [9]. At the same time, the effect of micro shock waves generated by cavitation during the TS process may cause reductions in some volatile aroma compounds.

Sensory analyses

The sensory appeal of the fruit juice for the consumer is important in terms of quality. The purpose was to assess the acceptability to the consumer by comparing C-PJ, P-PJ and TS-PJ samples. The sensory evaluations of the samples are shown in Fig. 4D. The overall acceptability of the TS-PJ sample was higher than the other samples. The aroma evaluations of the samples by the panelists were parallel to the results of the aroma profiles. Thermosonication treatment improved the sensory properties of freshly squeezed pomegranate juice more than thermal pasteurization. The sensory properties of carrot juice treated with TS were observed to be acceptable [4]. Similar to our findings, blood fruit (*Haematocarpus validus*) juice had the highest sensory scores for color, taste, texture and appearance as a result of thermosonication treatment [10]. Compared to thermal pasteurization, high acceptability was also observed for ultrasound-treated Brazilian nopal (*Opuntia ficus-indica*) beverage [40] and hog plum (*Spondias mombin* L.) juice [8]. Studies applying thermosonication to fresh grape juice [48] and orange juice whey drink [9] identified similar sensory properties (developed the evaluation criteria) to the control samples.

In vitro bioaccessibility

The effect of the in vitro gastrointestinal digestion model on the bioactive compounds (TPC, TFC, TAC, ascorbic acid, DPPH and CUPRAC) in the C-PJ, P-PJ and TS-PJ samples was evaluated. No extraction methods were applied to simulate the direct consumption and digestion conditions of the samples. The in vitro gastrointestinal digestion model results of the samples are shown in Fig. 5. When the results are evaluated in general, bioactive compounds were affected in all samples, even after passing through the gastrointestinal tract. Considering the effect of digestion process conditions on phenolic compounds, the best recovery was determined as 28.76% in the TS-PJ sample (Fig. 5G). The greatest loss of flavonoid substance at the end of intestinal digestion was detected in the P-PJ sample (Fig. 5B). When the in vitro gastrointestinal effects of phenolic substance and flavonoid

Table 4 Determination of volatile profiles of C-PJ, P-PJ, and TS-PJ

Volatile compounds	RI	Samples		
		C-PJ (µg/kg)	P-PJ (µg/kg)	TS-PJ (µg/kg)
Ethyl acetate	886	21.03 ± 0.72^{a}	12.22 ± 0.90^{b}	15.29 ± 1.17^{b}
3-methyl butanal	920	3.16 ± 0.59^{a}	1.83 ± 0.12^{a}	1.92 ± 0.15^{a}
Ethanol	929	69.12 ± 4.47^{a}	43.46 ± 1.87^{b}	52.79 ± 1.45^{b}
α-pinene	1022	57.97 ± 3.03^{a}	36.19 ± 1.38^{b}	41.89 ± 1.75^{b}
Hexanal	1081	12.16 ± 1.53^{a}	7.71 ± 0.74^{a}	10.1 ± 0.93^{a}
3-Methylbutyl acetate	1124	3.10 ± 0.36^{a}	2.35 ± 0.13^{a}	3.20 ± 0.23^{a}
β-Myrecene	1158	18.99 ± 2.93^{a}	15.23 ± 1.10^{a}	14.62 ± 1.05^{a}
α-Terpinene	1184	9.08 ± 0.47^{a}	4.50 ± 0.73^{b}	7.38 ± 0.90^{ab}
Limonene	1196	96.32 ± 6.26^{a}	70.05 ± 2.96^{b}	69.13 ± 6.51^{b}
3-methyl-1-butanol	1204	10.93 ± 2.78^{a}	5.09 ± 0.45^{a}	8.37 ± 1.07^{a}
Ethyl hexanoate	1236	1.02 ± 0.23^{a}	1.16 ± 0.38^{a}	0.91 ± 0.17^{a}
ρ-cymene	1274	37.19 ± 4.77^{a}	21.14 ± 2.70^{b}	24.91 ± 3.44^{ab}
Hexyl acetate	1276	6.84 ± 1.48^{a}	2.84 ± 0.53^{b}	2.95 ± 0.40^{ab}
Octanal	1288	29.12 ± 4.23^{a}	17.14 ± 2.23^{a}	18.58 ± 2.17^{a}
6-Methyl-5-hepten-2-one	1342	19.05 ± 1.65^{a}	9.67 ± 1.26^{b}	12.79 ± 0.88^{b}
Hexanol	1356	77.21 ± 5.63^{a}	54.13 ± 3.33^{b}	59.86 ± 5.10^{ab}
2-Nonanone	1388	5.15 ± 0.62^{a}	4.18 ± 0.48^{a}	3.73 ± 0.41^{a}
3-Hexenol	1384	3.91 ± 0.61^{a}	4.25 ± 0.58^{a}	3.57 ± 0.54^{a}
Nonanal	1396	16.54 ± 1.70^{a}	8.54 ± 1.30^{b}	12.83 ± 1.85^{ab}
Hexyl butanoate	1418	1.99 ± 0.41^{a}	0.84 ± 0.26^{a}	1.17 ± 0.16^{a}
Acetic acid	1458	7.32 ± 1.55^{a}	5.09 ± 0.93^{a}	6.11 ± 0.38^{a}
Decanal	1502	18.31 ± 1.49^{a}	14.54 ± 1.17^{a}	14.19 ± 1.16^{a}
Benzaldehyde	1542	8.48 ± 1.18^{a}	3.49 ± 0.66^{b}	6.17 ± 0.59^{ab}
Linalool	1548	23.07 ± 4.08^{a}	16.34 ± 1.58^{a}	19.85 ± 1.82^{a}
1-Octanol	1562	7.57 ± 0.77^{a}	7.27 ± 1.22^{a}	6.05 ± 0.32^{a}
β-Caryophyllene	1574	15.63 ± 2.24^{a}	10.74 ± 0.40^{a}	13.62 ± 1.61^{a}
1-Nonanol	1662	8.92 ± 1.46^{a}	6.34 ± 0.64^{a}	8.04 ± 0.13^{a}
3-Methyl butanoic acid	1682	1.01 ± 0.30^{a}	n.d	0.59 ± 0.18^{ab}
α-Terpineol	1690	5.99 ± 0.72^{a}	2.81 ± 0.51^{b}	2.69 ± 0.34^{b}
Hexanoic acid	1851	1.98 ± 0.23^{a}	1.02 ± 0.09^{b}	1.55 ± 0.23^{ab}
Octanoic acid	2064	0.97 ± 0.21^{a}	0.85 ± 0.08^{a}	0.96 ± 0.07^{a}
Total (µg/kg)				
Esters		33.98	19.41	23.52
Alcohols		200.73	136.88	158.53
Aldehydes		87.77	53.25	63.79
Ketones		24.2	13.85	16.52
Acids		11.28	6.96	9.21
Terpenes		241.17	160.66	174.24

RI Retention Index, *n.d.* not determined, *C-PJ* untreated pomegranate juice, *TS-PJ* thermosonication-treated pomegranate juice, *P-PJ* thermal pasteurized pomegranate juice. Results are presented mean ± standard deviation (n = 3). Values with the different letters within line are significantly different ($p < 0.05$)

substance losses were compared, Desseva and Mihaylova (2019) found similar results to pomegranate juice, where flavonoid substances were more affected [49]. When the effects of the TAC value of the samples are examined, they were affected more than the phenolic and flavonoid amounts (Fig. 5C). TAC loss of 89% was detected in the P-PJ sample. TS was found to preserve 1.77 mg C3G/100 mL more than thermal pasteurization. Polyphenols are highly sensitive to alkaline conditions and can be degraded by alkaline pH, leading to the observed losses in bioactive components [50]. High decreases were observed in the ascorbic acid values of the samples within the gastric digestive system and it was

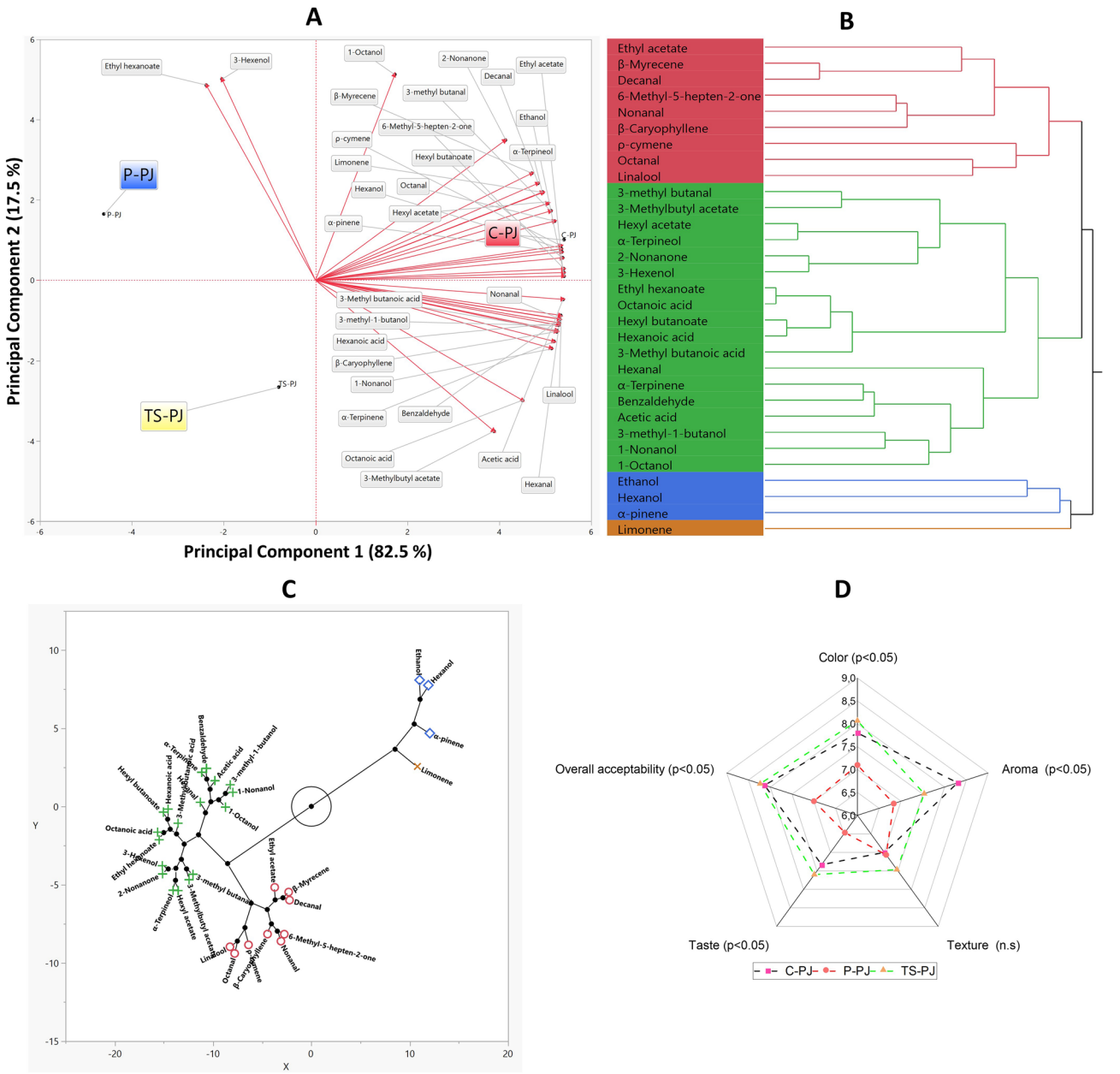


Fig. 4 **A** PCA bi-plot of volatile compounds in freshly squeezed pomegranate juice samples **B** Dendrogram for hierarchical cluster analysis of samples and identified organic volatile compounds. The samples were clustered according to red, green, blue and orange colors **C** Constellation after hierarchical cluster analysis of samples and identified volatile compounds **D** Results of sensory analysis chart for freshly squeezed pomegranate juice samples

not detected in intestinal digestion. It was thought that this decrease in ascorbic acid values may be due to the presence of oxygen during in vitro digestion. The results show that DPPH varied in the range of 26–35% from the initial activity of the pomegranate juice samples (Fig. 5E). DPPH antioxidant losses were found to be 85% in the P-PJ sample and 35.26% in the TS-PJ sample. Recovery of CUPRAC antioxidant values of C-PJ, P-PJ and TS-PJ samples were determined as 28.59%, 25.36% and 32.59%, respectively. In the study of tomato juice enriched with anthocyanins, TS samples were found to have higher bioaccessibility compared to thermally treated ones, which is in line with our study [7]. After non-thermal technology was applied to fermented vegetable juices, the bioavailability of total phenolics, anthocyanins and flavonoids decreased after in vitro digestion [36]. Barba et al. (2017) reported that thermal processing can increase or decrease the bioaccessibility of bioactive compounds depending on both the food matrix

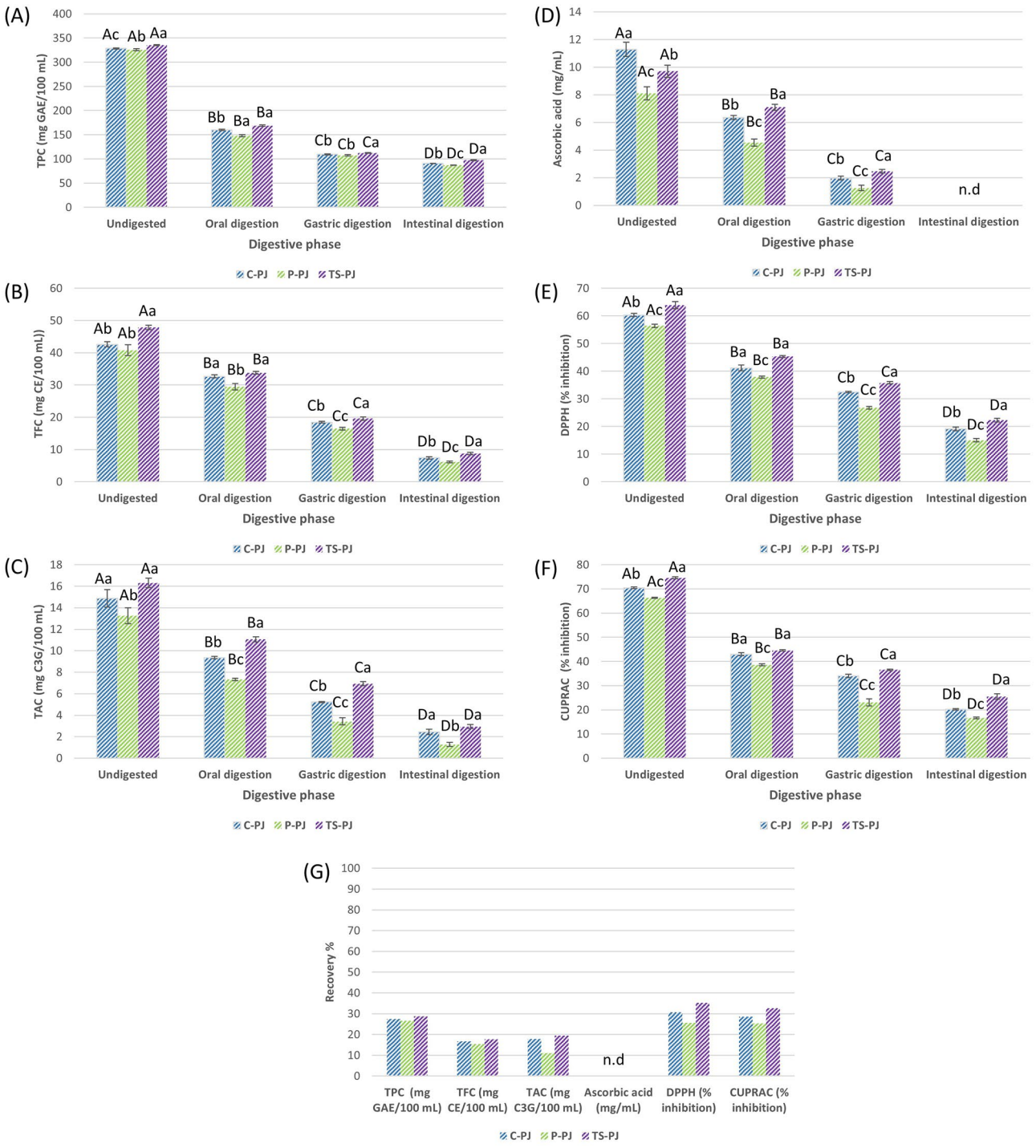


Fig. 5 **A** TPC, **B** TFC, **C** TAC, **D** Ascorbic acid, **E** DPPH, **F** CUPRAC and **E** recovery (%) of samples before and after in vitro gastrointestinal digestion. Values represent mean values of three independent experiments ± S.D. Different lower case letters indicate significant differences between samples. Different capital letters indicate significant differences between digestive phases for the same treatment. The criterion for statistical significance was $p < 0.05$

and the target compound [51]. However, in our study, thermal pasteurization reduced the bioavailability of bioactive compounds and thermosonication provided better results in recovery.

Conclusion

The aim of this study was to optimize the bioactive compounds with RSM and ANN modeling of different

thermosonication conditions and to investigate the effect on different parameters according to thermal pasteurization. ANN modeling provided superior results than RSM modelling. The quality of pomegranate juice treated with TS also improved compared to pasteurized pomegranate juice. TS and thermal pasteurization affected the volatile profile. Significant microbial inactivation was achieved and sensory parameters were also improved. Treatment with TS increased Ca, Fe and Na elements. TS increased the antidiabetic and antihypertensive effects of pomegranate juice. Treatment with TS preserved the in vitro bioavailability of the bioactive compounds (except ascorbic acid). Hence, thermosonication can be considered a better alternative to heat treatment of pomegranate juice. Further research studies are required to determine the properties of pomegranate juice treated with thermosonication during storage. This also constitutes preliminary research for in vivo studies.

Data Availability All data generated or analysed during this study are included in the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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