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Araştırma Makalesi/ Research Article

Yüzey Yanıt Metodolojisi Kullanılarak Sarı Karpuz Suyunun Bazı Biyoaktif, Duyusal ve Mikrobiyolojik Özelliklere Termosonikasyonun Etkisi

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ÖZ

Bu çalışmada, ılımlı ısı ile bir ultrasound işlemi olan termosonikasyonun sarı karpuz suyuna uygulanması ve proses koşullarının yüzey yanıt metodu kullanılarak optimizasyonu hedeflenmiştir. Bu amaçla, sarı karpuz suyu üretilmiş ve örneklere 26 kHz frekansta, farklı sıcaklıklarda (30, 35, 40, 45 ve 50 ° C), farklı zamanlarda (2, 4, 6, 8 ve 10 dakika) ve farklı genliklerde (%40, %45, %50, %55 ve %60) termosonikasyon işlemi gerçekleştirilmiştir. Toplam fenolik madde (TPC), toplam flavonoid madde (TFC), toplam antioksidan kapasite (1,1-difenil -2- pikrilhidrazil (DPPH), kuprik iyon indirgeme antioksidan kapasite (CUPRAC)) ve renk değerleri (*L* *, *a* * ve *b* *) proses koşullarının optimizasyonu için değerlendirilmiştir. Aynı zamanda, pastörize sarı karpuz suyu (PW) ile işlenmemiş sarı karpuz suyu (C) arasındaki farklar incelenmiştir. Optimizasyonun sonunda, sarı karpuz suyu örneklerinin mikrobiyal güvenliği ve duyusal özellikleri değerlendirildi. Çalışma sonucunda, sarı karpuz suyu için termosonikasyon uygulanmış maksimum optimizasyon değerleri 38,3 ° C, 5,6 dakika ve 50,5 amplitüde olmuştur. Optimizasyonun sonunda, CUPRAC (0,214 mg TEAC/mL), DPPH (0,123 mg TEAC/mL), toplam flavonoid madde (41,28 mg CE/L) ve toplam fenolik madde (104,30 mg GAE/L) olarak belirlendi. Termosonikasyonla işlem görmüş sarı karpuz suyunun mikrobiyal değerler açısından güvenli olduğu bulundu ve panelistler tarafından en çok tercih edildiği tespit edildi. Sonuç olarak, sarı karpuz suyu üretiminde termosonikasyon teknolojisi başarıyla kullanılmıştır.

Anahtar Kelimeler- Sarı Karpuz Suyu, Termosonikasyon, Toplam Fenolik Madde, Toplam Flavonoid Madde, Tepki Yüzeyi Metodolojisi

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Effect of Thermosonication on Some Bioactive, Sensory Analysis and Microbiological Properties of Yellow Watermelon Juice Using Response Surface Methodology

ABSTRACT

In this study, the application of thermosonication, which is a moderate ultrasound process, on yellow watermelon juice and targeted optimization of the process conditions was completed using the surface response method. For this purpose, yellow watermelon juice was produced and thermosonication at different temperatures (30, 35, 40, 45 and 50 °C), different times (2, 4, 6, 8 and 10 min) and different amplitudes (40%, 45%, 50%, 55% and 60%) at 26 kHz frequency was applied to the samples. Total phenolic content (TPC), total flavonoid content (TFC), total antioxidant capacity (1,1-diphenyl- 2-picrylhydrazyl (DPPH), cupric reducing antioxidant capacity (CUPRAC)), and color values (L^*, a^* and b^*) were evaluated for optimization of process conditions. At the same time, the differences between pasteurized yellow watermelon juice (PW) and a control (C) of untreated yellow watermelon juice were investigated. At the end of the optimization, microbial safety and sensory properties of the yellow watermelon juice samples were evaluated. As a result of the study, the maximum optimization values for the yellow watermelon juice, with thermosonication applied, were 38.3 °C, 5.6 minutes and 50.5 amplitude. At the end of optimization, CUPRAC (0.214 mg TEAC/mL), DPPH (0.123 mg TEAC/mL), total flavonoid content (41.28 mg CE/L), and total phenolic content (104.30 mg GAE/L) were determined. Thermosonication-treated yellow watermelon juice was found to be safe in terms of microbial values and was most preferred by panelists. As a result, thermosonication technology was successfully used for yellow watermelon juice production.

Keywords- Yellow Watermelon Juice, Thermosonication, Total Phenolic Content, Total Flavonoid Content, Response Surface Methodology

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I. INTRODUCTION

In epidemiological studies, it is thought that the consumption of fruits and vegetables reduces the risk of many diseases due to bioactive substances [1]. Watermelon (*Citrullus lanatus*) has intense red color, sweet taste and excellent nutritional and functional properties. Some phenolic compounds include antioxidants, lycopene, B vitamins, phosphorus, potassium, magnesium, calcium and iron, citrulline and arginine. [2-4]. Therefore, watermelon and its products have large commercial volume as a functional food. About 93% of the total weight of watermelon is water. For this reason, fruit juice is one of the most suitable products for processing watermelon. However, watermelon is a heat-sensitive fruit because its quality and taste are affected by heat or long-time exposure to the atmosphere. The typical disturbing odor seen after heating watermelon juice is an industrial problem. High temperature sterilization in fruit juice processing can affect the aroma of watermelon juice [5, 6]. However, thermal processes are effective in preventing microbial load. Heat treatment also causes biochemical changes, nutritional losses, undesirable reactions, changes in product quality and high energy consumption. Today, consumer demand for functional foods is increasing. These products are expected to have excellent sensory and nutritive properties produced by alternative technologies to thermal processing [7, 8].

Ultrasonication was defined as a potential technology to meet the 5 log reduction requirements of the US Food and Drug Administration (FDA) related to microorganisms in fruit juices [9]. Research showed that it is a good alternative to thermal methods and has minimal impact on the quality of fruit juices [10, 11]. In ultrasound treatment, the effects of enzymes and microorganism inactivation are explained mainly in two cases. In terms of physical events, acoustic cavitation is the result of micro-jets and shock waves. In terms of chemical events, the formation of free radicals from the sonolysis of water vapor is a result of the collapse resulting from cavitation [12, 13]. However, antimicrobial agents are used in combination with applications such as pressure and temperature to increase the efficiency of ultrasound therapy. One of these applications is the thermosonication (TS) process which is used with moderate temperatures. It is a useful technology to increase microbial and enzymatic inactivation rates and also to increase product shelf life and to preserve nutrient content [8]. Many researchers have found that jamun [14], apple [15], grapefruit [16], carrot [17], orange [18] and mosambi [19] have least loss of quality and nutritional value of fruit juices.

Response surface methodology (RSM) is a mathematical and statistical procedure that is commonly applied for optimization studies and especially in food processing. The main purpose is optimization with the response surface method; meaningful results can be obtained by combining the interactions of many independent variables with one or more target data [20-22].

Limited information about thermosonication was found in the literature about the effects on watermelon juice and bioactive compounds [2]. However, no study of the effect of thermosonication treatment on the color and bioactive components of yellow watermelon juice, its sensory properties, and microbial safety was found in the literature. The aim of this study is to optimize the total phenolic content, total flavonoid content, total antioxidant and color values of yellow watermelon juice, which is thought to be higher in terms of bioactive components, using the surface response method. At the same time, sensory properties and microbial safety of pasteurized yellow watermelon juice and untreated yellow watermelon juice were compared.

II. MATERIALS AND METHODS

A. Juice sample preparation

Fresh yellow watermelon was collected from a local producer (Tekirdag, Turkey) and kept at 4 °C until experiments were carried out. Shells, stalks, seeds and ripened parts were discarded. A crushing process blender (Waring Commercial Blender Model HGB2WTS3, USA) was used to remove the juice which was then filtered through a sterilized double layer muslin cloth. Freshly extracted fruit juice was mixed with a vortex and selected as control (C) and sterilized and filled into 100 ml airtight bottles. A bottle was pasteurized at 90 °C in a water bath (Wisd-Model WUC-D06H, Daihan, Wonju, Korea) for 10 seconds and cooled to 20 °C, and selected as pasteurized yellow watermelon juice (PW). Other samples were treated with ultrasound. In the study, the UP200St ultrasound device from Hielscher Ultrasonics (Berlin, Germany) was used. Samples were stored at -20 °C until analysis. Tests were performed three times.

B. Ultrasound treatments

Sonication treatments were performed directly after fresh juice was extracted. Yellow watermelon juice was treated at 26 kHz frequency, for different times (2, 4, 6, 8 and 10 minutes), different temperatures (30, 35, 40, 45 and 50 °C) and amplitudes (40%, 45%, 50%, 55% and 60%). The sonication was performed at 26 kHz frequency with a 200 W ultrasonic processor (Model UP200St, Hielscher Ultrasonics, Teltow, Germany). All the sonication treatments were carried out in the dark to avoid any possible interference of light. Juice samples (sonicated) were kept in sterilized and air tight media bottles, and were stored at -20 °C until further analysis.

C. Experimental design

Juice was analyzed by using Minitab Statistical Analysis Software (Minitab 18.1.1) to optimizsthe effect of ultrasound on quality parameters. The Response Surface Method (RSM) was used. Central Composite Design (CCD) was chosen as the experimental design and a five-level, three-factor experimental design was created. There are 20 test points for optimization (Table 1 and Table 2). Model competence, R^2 and corrected-R^2 coefficients, lack-of-fit tests and ANOVA results were evaluated. Arguments were determined as temperature (X_1), time (X_2) and amplitude (X_3). Dependent variables were determined as total phenolic content (TFC), total flavonoid content (TFC), antioxidants (1,1-diphenyl- 2-picrylhydrazyl (DPPH), cupric reducing antioxidant capacity (CUPRAC)) and color values (L^*, a^*, b^*). The second order-polynomial equation, shown in the equation below, was used to create the model.

Equation (1):

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

where quality Y is the dependent variable, βo is the intersection term, βi is first order (linear) equation coefficient, βii is quadratic coefficient, βij is two-factor cross-correlation coefficient, and Xi and Xj are independent variables.

Table 1. Independent variables and their levels in RSM

	Factor levels				
Independent variable	**Lowest** (-1.68)	**Low** (-1)	**Center** 0	**High** (+1)	**Highest** (+1.68)
Temperature (Factor 1, X_1) (° C)	30	35	40	45	50
Time (Factor 2, X_1) (min.)	2	4	6	8	10
Amplitude (Factor 3, X_3) (%)	40	45	50	55	60

D. DPPH free radical method

A 100 µL sample was placed into the tube or 2 ml of 0.1 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) (Sigma-Aldrich, USA) was added to the standard. Pure water sample of 100 µL was used as a control sample. Absorbance versus pure water at 517 nm was read by stirring with vortex and incubation at room temperature for 30 minutes [23, 24]. A spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Melbourne, Australia) was used for absorbance measurements. Results are expressed in mg Trolox equivalent (TEAC)/L**.**

E. CUPRAC (cupric ion reducing antioxidant capacity) assay

Samples of 100 µL were placed in the tube, then 1 ml 10 mM $CuCl_2.2H_2O$ (Sigma-Aldrich, USA) solution, 1 ml 7.5 mM Neocuproine (Sigma-Aldrich, USA) solution and 1 ml 1 M ammonium acetate (pH = 7)

was added. Finally, 1 ml of water was added to the final volume of 4.1 ml and measured after 30 minutes at 450 nm on a spectrophotometer (SP-UV / VIS-300SRB, Spectrum Instruments, Melbourne, Australia) [25]. Results are expressed in mg Trolox equivalent (TEAC)/L.

F. Determination of total phenolic content

The total phenolic content was measured spectrophotometrically with the the Folin-Ciocalteu method [26]. Juice sample of 0.1 ml, 0.90 ml of distilled water and 5 mL of 0.2 N Folin Ciocalteu (Merck, Germany) solution were mixed with 4 mL of 7.5% Na_2CO_3 (Merck, Germany) solution. This was incubated for 2 hours in the dark at room temperature. The absorbance changes were determined with a spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Melbourne, Australia) at 765 nm. Gallic acid (Sigma Aldrich, Germany) was used as a reference standard and the results are expressed as mg gallic acid equivalent per liter of juice (mg GAE/L).

G. Determination of total flavonoid content

The total flavonoid content was modified by the aluminum chloride colorimetric analysis method [27]. An aliquot (1.0 mL) of juice sample was placed in different test tubes containing 4 mL of distilled water, then 0.3 mL of 5% $NaNO_2$ (Sigma-Aldrich, USA) was added and allowed to stand for 5 min. Later, 0.3 mL of aluminum trichloride 10% $AlCl_3.6H_20$ (Sigma-Aldrich, USA) was added and incubated for 5 min, followed by the addition of 2 mL of 1 M NaOH (Merck, Germany), and the total volume was made up to 10 mL with distilled water. Samples were allowed to incubate in the dark for 30 minutes. The absorbance changes were determined with a spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Melbourne, Australia) at 510 nm. TFC is expressed as mg catechin equivalent (CE) per liter.

H. Color analysis

Color analysis of the samples was completed using the Color Measuring Device PCE-CSM 5 (Color Measuring Device PCE-CSM-5, Spectrum Instruments, Meschede, Germany) and a liquid container. Colors are expressed in terms of *L** (darkness-lightness), *a** (greenery-redness), and *b** (blue-yellowish) color parameters. Color *L**, *a**, and *b** color parameters are expressed [28].

İ. Microbiological analysis

Serial dilutions of yellow watermelon juice were prepared in peptone water solution for the microbial count. Colony forming units (CFU) were determined by standard spreading and pouring plate methodologies. PCA (Plate Count Agar- Merck, Germany) was used for total aerobic plate count. Samples were incubated at 30 °C for 48 h. For yeast and mold count, PDA (Potato Dextrose Agar- Merck, Germany) was used. Samples were incubated at 24 °C for 3-5 days. Total *Enterobacteriaceae* count was determined in VRBG (Violet Red Bile Glucose Agar- Merck, Germany) incubated at 37 °C for 24 h. The pink-red ring and red precipitation colonies were evaluated. Results are given as log colony forming units (CFU) per milliliter of yellow watermelon juice.

J. Sensory analysis

Thermosonication treated and pasteurized yellow watermelon juice was evaluated using a 5-point hedonic scale (1 =" extremely disliked =; 5 = extremely liked ") by a total of 10 panelists for approval, color, texture (viscosity), taste and aroma (5 female, 5 male). Prior to sensory evaluation, the fruit juice samples were cooled, randomly coded with three-digit numbers, and the order of presentation was completely randomized for each panelist. The evaluation was carried out in the Department of Dietetics, Nutrition Branch of Tekirdağ Namık Kemal University (Tekirdag, Turkey). The results of sensory evaluation were assessed using analysis of variance (ANOVA) and Tukey's pairwise comparison test ($p < 0.05$)

K. Statistical Analysis

RSM (Minitab 18.1, Minitab, Inc, State College, Pensilvanya, United States) was used for the optimization of yellow watermelon juice processing. Significant differences between mean values of yellow watermelon juice

samples were determined by analysis of variance (one-way ANOVA) using Tukey's HSD (Honestly Significant Difference) test at a significance level of $p < 0.05$. Statistical analysis was conducted using SPSS 22.0 software ((SPSS Inc., Chicago, IL, United States)). 3D graphs of the obtained models were created using SigmaPlot 12.0 Statistical Analysis Software (Systat Software, Inc., San Jose, CA, United States). All values were obtained in triplicate.

III. RESULTS AND DISCUSSION

A. Determination of Total Phenolic Content and Total Flavonoid Content

Due to numerous useful properties (such as strong antioxidant, cancer and disease prevention properties), research has intensified to find residues of fruit, vegetable, plant, agricultural and agricultural industry which can be used as sources of bioactive phenolic compounds [29, 30]. Experimental design results for TPC are shown in Table 2. The equilibrium of the polynomial model indicating the effect of temperature, time and amplitude factors on the total phenolic content of yellow watermelon juice samples is shown in Table 4. Table 3 shows the results of the analysis of variance of TPC (mg GAE / L) of the yellow watermelon juice samples with different levels of temperature, time and amplitude factors applied.

It was observed that the model used in the study ($R^2 = 0.9923$) adapts to the level (Table 3). The linear effects of the temperature applied to the yellow watermelon juice samples on the TPC values were found to be statistically significant ($P<0.001$) and the effect of time was not statistically significant ($p>0.05$). The linear effects of ampicillin on TPC values were found to be statistically significant ($P<0.05$). Cross-effects on TPC values were found to be statistically significant ($p<0.001$). In the 2-way interaction, the effects of the factors on the TPC values were found to be statistically significant ($P<0.001$).

Table 2 Measured responses used in experimental design for RSM.

Sample	Encoded Independent Variables			Dependent Variables						
				Response 1	Response 2	Response 3	Response 4	Response 5	Response 6	Response 7
	Temperature (X_1)	Time (X_2)	Amplitude (X_3)	Total Phenolic Content (mg GAE/L)	Total Flavonoid Content (mg CE/L)	DPPH (mg TEAC/mL)	CUPRAC (mg TEAC/mL)	*L**	*a**	*b**
PW				84.16	30.13	0.092	0.183	26.54	10.26	13.14
C				95.19	38.25	0.113	0.195	27.62	10.74	13.85
1	1.68 (50)	0 (6)	0 (50)	87.76	34.102	0.113	0.197	27.44	10.31	11.26
2	1 (45)	-1 (4)	1 (55)	88.27	33.150	0.118	0.202	28.62	11.54	12.10
3	-1 (35)	1 (8)	1 (55)	96.71	42.373	0.115	0.201	28.12	11.42	14.57
4	-1 (35)	-1 (4)	-1 (45)	109.65	43.160	0.120	0.214	27.34	11.57	12.75
5	1 (45)	1 (8)	-1 (45)	86.32	37.170	0.104	0.210	26.36	10.76	13.78
6	1 (45)	1 (8)	1 (55)	103.96	41.180	0.119	0.208	27.78	10.61	12.37
7	1 (45)	-1 (4)	-1 (45)	91.74	38.405	0.124	0.195	28.06	10.82	14.21
8	0 (40)	0 (6)	0 (50)	102.78	40.468	0.123	0.215	29.35	11.10	14.59
9	-1 (35)	1 (8)	-1 (45)	92.03	35.529	0.104	0.214	28.04	11.27	13.35
10	-1 (35)	-1 (4)	1 (55)	94.25	39.179	0.109	0.211	26.43	12.54	13.49
11	0 (40)	0 (6)	0 (50)	103.16	41.260	0.124	0.212	29.24	11.23	14.76
12	0 (40)	0 (6)	0 (50)	103.74	41.230	0.124	0.214	28.98	11.32	14.65
13	0 (40)	0 (6)	0 (50)	104.13	41.118	0.124	0.214	29.41	11.13	14.72
14	0 (40)	0 (6)	-1.68 (40)	97.11	39.120	0.113	0.209	27.14	10.84	13.93
15	0 (40)	-1.68 (2)	0 (50)	84.35	37.630	0.112	0.203	27.05	12.36	13.78

Table 2 Measured responses used in experimental design for RSM.

16	0 (40)	0 (6)	1.68 (60)	102.54	40.780	0.120	0.205	27.85	11.85	13.01
17	-1.68 (30)	0 (6)	0 (50)	97.47	38.620	0.107	0.211	26.91	11.58	12.01
18	0 (40)	0 (6)	0 (50)	104.24	41.029	0.123	0.213	29.16	11.24	14.65
19	0 (40)	0 (6)	0 (50)	104.65	40.842	0.123	0.213	29.14	11.17	14.68
20	0 (40)	1.68 (10)	0 (50)	84.94	38.140	0.099	0.207	27.06	11.04	14.48

C: untreated yellow watermelon juice; PW: Pasteurized yellow watermelon juice; GAE: Gallic acid equivalent; DDPH: radical scavenging activity; CUPRAC: Cupric Reducing Antioxidant Capacity; *L**: represents luminance value *a**: represents red and green; *b**: represents yellow and blue

The 3D variation of the TPC according to temperature, time and amplitude is shown in Figure 1 (A). When the model of TPC is examined, a linear increase and decrease in TPC values was observed as temperature, time and amplitude amount increased. In the fifteenth experiment, the lowest TPC value was found with 40 °C, 2 minutes and 45%; the highest TPC value was found in the fourth experiment treated with 35 °C and 45% for 4 minutes (Table 2). The application of the thermosonication process to the yellow watermelon juice has positive effects on TPC values. At the end of the optimization, TPC was found to be 104.30 mg GAE/L for 38.3 °C, 5.6 minutes and 50.5 amplitude thermosonication (Table 6). Yellow watermelon juice treated with thermosonication was found to cause an increase of 8.7% compared to sample C. When the C (95.19 mg GAE / L) and PW (84.16 mg GAE / L) samples are compared, it was found that TPC decreased by 11.03 mg GAE/L.

Flavonoids are compounds that show strong antioxidant properties against free radicals in the body and have protective effects against diseases that adversely affect human health [31]. Experimental design results for TPC are shown in Table 2. According to the experimental design, the equilibrium of the polynomial model indicating the effect of temperature, time and amplitude factors on TFC of yellow watermelon juice samples are shown in Table 4. Table 3 shows the results of variance analysis of TFC values (mg CE / L) for yellow watermelon juice samples with different levels of temperature, time and amplitude factors applied.

Table 3 shows the results of variance analysis of total flavonoid content values (mg CE/L) of yellow watermelon juice samples with different levels of temperature, time and amplitude factors applied. According to the optimization, the predicted performance of the model for TFC (R^2 = 0.9916) was found to be successful (Table 3). The linear effects of temperature applied to yellow watermelon juice samples on TFC values were statistically significant (P<0.001). The time and amplitude effects were statistically significant for yellow watermelon juice (p <0.05). In the thermosonication process, temperature and time factors were found to be statistically significant for TFC values (P <0.001).

Cross-interaction of amplitude was also found to be statistically significant (P<0.05). In the 2-way interaction, the linear effects of temperature and amplitude interaction on TFC values were found to be statistically significant (P<0.05). The temperature, time and amplitude effect of the thermosonication process on yellow watermelon juice is shown in Figure 1 (B). While increasing the temperature, time and amplitude, increased TFC values up to a certain point; afterward reduction and changes are observed. The lowest TFC value was obtained at 45 °C, 4 minutes, and 55% in the second experiment; the highest TFC was detected in the fifteenth experiment treated with 35 °C and 45% for 4 minutes (Table 2). When the thermosonication process was applied to yellow watermelon juice, it was determined that it increased the TFC values. At the end of the optimization, TFC of 41.28 mg CE / L was determined as a result of thermosonication at 38.3 °C, 5.6 minutes and 50.5 amplitude (Table 6). Yellow watermelon juice treated with thermosonication was found to have an increase in TFC values of 7.3% compared to sample C. When C (38.25 mg CE/L) samples and PW samples (30.13 mg CE/L) were compared, it was found that TFC decreased by 8.12 mg CE/L. Similar results were found for total phenolic content and total flavonoid content of Kasturi lime juice [32], purple cactus pear juice [33], mango juice [34], apple juice [35] and blueberry juice [36]. The addition of hydroxyl radicals to the aromatic ring of phenolic compounds and the breakdown of the cell walls with the effect of cavitation may lead to an increase in TPC [32]. Furthermore, the removal of trapped active oxygen in the juice by sonication can contribute to the development of phenolic compounds [33]. However, some experimental data (1, 2, 5, 7, 9, 10 and 20) showed reductions in the amount of TPC compared to the C sample. The reduction of TPC can be caused by the formation of OH- radicals during cavitation, by opening of rings of hydroxyl radicals and by the formation of calcone, and by the effect of anthocyanins on the breakdown of bioactive compounds [34, 35].

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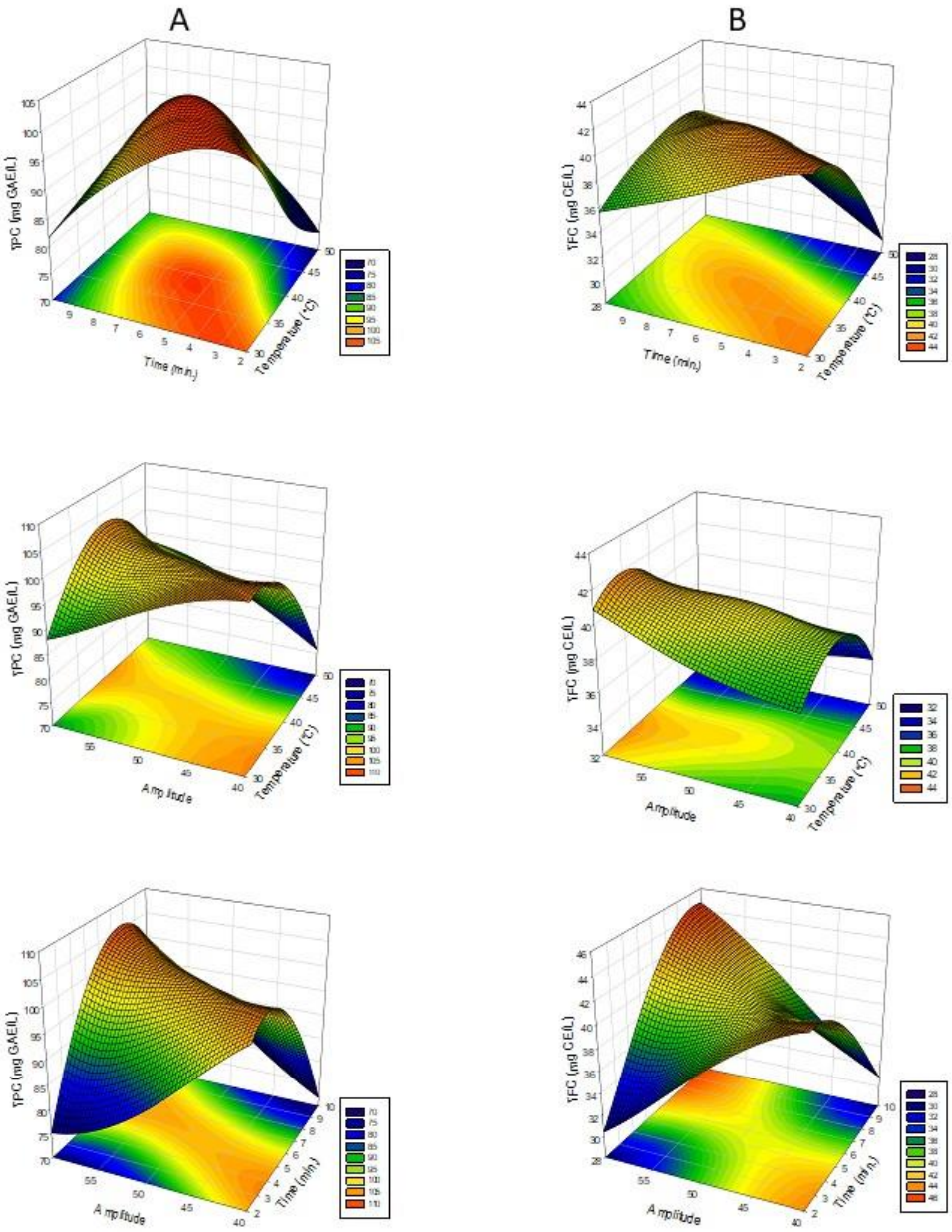

Figure 1. Response surface plots (3D) of TPC (A) and TFC (B) analysis as a function of significant interaction factors.

Table 3. Analysis of variance (ANOVA) of responses for total phenolic content, total flavonoid content, 1,1-diphenyl- 2-picrylhydrazyl (DPPH) and cupric reducing antioxidant capacity (CUPRAC) experiments

Source	DF	Total Phenolic Content (mg GAE/L)		Total Flavonoid Content (mg CE/L)		DPPH (mg TEAC/mL)		CUPRAC (mg TEAC/mL)	
		F-Value	P-Value	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
Model	9	142.46	0.000	130.99	0.000	352.08	0.0000	88.11	0.000
Linear	3	45.61	0.000	74.54	0.000	253.02	0.0000	83.04	0.000
X_1	1	121.61	0.000	204.14	0,000	132.28	0,0000	197.96	0.000
X_2	1	0.96	0.351	6.21	0,032	527.02	0,0000	26.25	0.000
X_3	1	14.27	0.004	13.27	0,005	99.75	0,0000	24.92	0.001
Square	3	244.14	0.000	120.19	0.000	584.36	0,0000	94.42	0.000
X_1*X_1	1	216.38	0.000	294.53	0.000	739.22	0,0000	176.52	0.000
X_2*X_2	1	637.96	0.000	132.85	0.000	1350.41	0,0000	138.6	0.000
X_3*X_3	1	26.7	0.000	15.12	0.003	212.26	0,0000	82.06	0.000
2-Way Interaction	3	137.62	0.000	198.23	0.000	218.85	0,0000	86.87	0.000
X_1*X_2	1	90.06	0.000	137.26	0.000	25.86	0,0000	146.55	0.000
X_1*X_3	1	86.33	0.000	18.36	0.002	24.12	0,0010	64.62	0.000
X_2*X_3	1	236.48	0.000	439.09	0.000	606.58	0,0000	49.45	0.000
Error	10								
Lack-of-Fit	5	2.62	0.157	1.6	0.310	1.67	0.294	0.44	0.809
Pure Error	5								
Total	19								
R^2		0.9923		0.9916		0.9969		0.9875	
Adj R^2		0.9853		0.9840		0.9940		0.9763	
Pred R^2		0.9502		0.9554		0.9820		0.9570	

DF: degree of freedom, *: multiplication. The term is significant at $p \leq 0.05$. The term is significant at $p \leq 0.01$. The term is significant at $p \leq 0.001$.

B. Determination of DPPH and CUPRAC

Antioxidants are compounds that inhibit the initiation or progression of oxidation reactions by preventing oxygen in the environment. At the same time, in the body they have antibacterial, anticarcinogen and cardiovascular disease-reducing effects [36]. Experimental design results for total antioxidants (DPPH and CUPRAC) are shown in Table 2. The equilibrium of the polynomial model indicating the effect of temperature, time and amplitude factors on the DPPH antioxidant capacity value according to the experimental design is shown in Table 4. Table 3 shows the results of variance analysis of DPPH (mg TEAC / mL) for yellow watermelon juice samples with different levels of temperature, time and amplitude factors applied during thermosonication. The model used in the study (R^2 = 0.9969) was found to be compatible with the level (Table 3). The linear effects of all the factors applied to yellow watermelon juice samples on DPPH values were statistically significant (P<0.001). Cross-interactions were found to be statistically significant for DPPH values (P<0.001). In the 2-way interaction, the effects of the factors on the DPPH values were found to be statistically significant (P<0.001).

Table 4. Predicted mathematical models for TPC, TFC, DPPH, CUPRAC, *L**, *a**, *b** values after ultrasound treatment

Dependent Variables	Polynomial
Total Phenolic Content (mg GAE/L)	$277.8 + 0.239\ X_1 - 24.26\ X_2 - 3.985\ X_3 - 0.11114\ X_1^2 - 1.1927\ X_2^2 - 0.03904\ X_3^2 + 0.3178\ X_1X_2 + 0.1245\ X_1X_3 + 0.5149\ X_2X_3$
Total Flavonoid Content (mg CE/L)	$7.4 + 3.655\ X_1 - 15.729\ X_2 + 0.428\ X_3 - 0.04641\ X_1^2 - 0.1948\ X_2^2 - 0.01052\ X_3^2 + 0.1404\ X_1X_2 - 0.02054\ X_1X_3 + 0.2511\ X_2X_3$
DPPH (mg TEAC/mL)	$-0{,}1042 + 0{,}009373\ X_1 - 0{,}01040\ X_2 + 0{,}002485\ X_3 - 0{,}000131\ X_1^2 - 0{,}001106\ X_2^2 - 0{,}000070\ X_3^2 - 0{,}000108\ X_1{*}X_2 + 0{,}000042\ X_1{*}X_3$
CUPRAC (mg TEAC/mL)	$0{,}1404 - 0{,}000441\ X_1 + 0{,}00277\ X_2 + 0{,}003622\ X_3 - 0{,}000098\ X_1^2 - 0{,}000542\ X_2^2 - 0{,}000067\ X_3^2 + 0{,}000395\ X_1{*}X_2 + 0{,}000105\ X_1{*}X_3$
L*	$-35{,}67 + 1{,}361\ X_1 + 2{,}959\ X_2 + 1{,}099\ X_3 - 0{,}02088\ X_1^2 - 0{,}13811\ X_2^2 - 0{,}01770\ X_3^2 - 0{,}06166\ X_1{*}X_2 + 0{,}01408\ X_1{*}X_3 + 0{,}02321\ X_2{*}X_3$
a*	$3{,}08 + 0{,}2324\ X_1 + 0{,}305\ X_2 + 0{,}1306\ X_3 - 0{,}002456\ X_1^2 + 0{,}03181\ X_2^2 + 0{,}001534\ X_3^2 + 0{,}00534\ X_1{*}X_2 - 0{,}00276\ X_1{*}X_3 - 0{,}02116\ X_2{*}X_3$
b*	$-120{,}51 + 3{,}945\ X_1 + 0{,}720\ X_2 + 2{,}227\ X_3 - 0{,}030967\ X_1^2 - 0{,}03761\ X_2^2 - 0{,}012617\ X_3^2 - 0{,}02286\ X_1{*}X_2 - 0{,}02741\ X_1{*}X_3 + 0{,}01476\ X_2{*}X_3$

X_1 = temperature (°C), X_2 = time (min.), and X_3 = amplitude. GAE: Gallic acid equivalent; DDPH: radical scavenging activity; CUPRAC: Cupric Reducing Antioxidant Capacity; *L**: represents luminance value *a**: represents red and green; *b**: represents yellow and blue

The lowest DPPH value was found with 40 °C, 10 minutes and 50% treatment in the twentieth application; the highest value was found to be 0.124 mg TEAC/mL in applications 7, 11, 12 and 13 (Table 2). The application of the thermosonication process to yellow watermelon juice showed positive effects on DPPH values compared to C and PW samples. At the end of the optimization, DPPH was determined to be 0.123 mg TEAC/mL with 38.3 °C, 5.6 min and 50% amplitude treatment as a result of thermosonication (Table 6). As a result of the optimization, it was found that the yellow watermelon juice treated with thermosonication caused an increase in the amount of antioxidants by 8.1% compared to the C sample.

The equilibrium of the polynomial model indicating the effect of temperature, time and amplitude factors on the CUPRAC antioxidant capacity value is shown in Table 4. Table 3 shows the results of CUPRAC (mg TEAC/mL) variance analysis of yellow watermelon juice samples with different levels of temperature, time and amplitude factors applied.

The model used in the study (R^2 = 0.9875) was found to fit the level (Table 3). All factors applied to yellow watermelon juice samples were found to be statistically significant on CUPRAC values ($P<0.001$). Cross-interactions of CUPRAC were found to be statistically significant ($P<0.001$). The effects of factors on CUPRAC values were found to be statistically significant in 2-way interaction ($P<0.001$). The change in antioxidant values according to temperature, time and amplitude is shown in Figure 2 (B). When the models of CUPRAC values are examined, a linear increase and decrease in antioxidant amounts was observed as temperature, time and amplitude amount increased. The lowest CUPRAC value was obtained with 45 °C, 4 minutes, and 45% treatment. The highest value was 0.215 mg TEAC/mL in the eighth application (Table 2). The CUPRAC values after application of the thermosonication process to the yellow watermelon juice were compared to the C and PW samples. At the end of the optimization, 38.3 °C, 5.6 minutes and 50% amplitude treatment caused CUPRAC of 0.214 mg TEAC/mL (Table 6).

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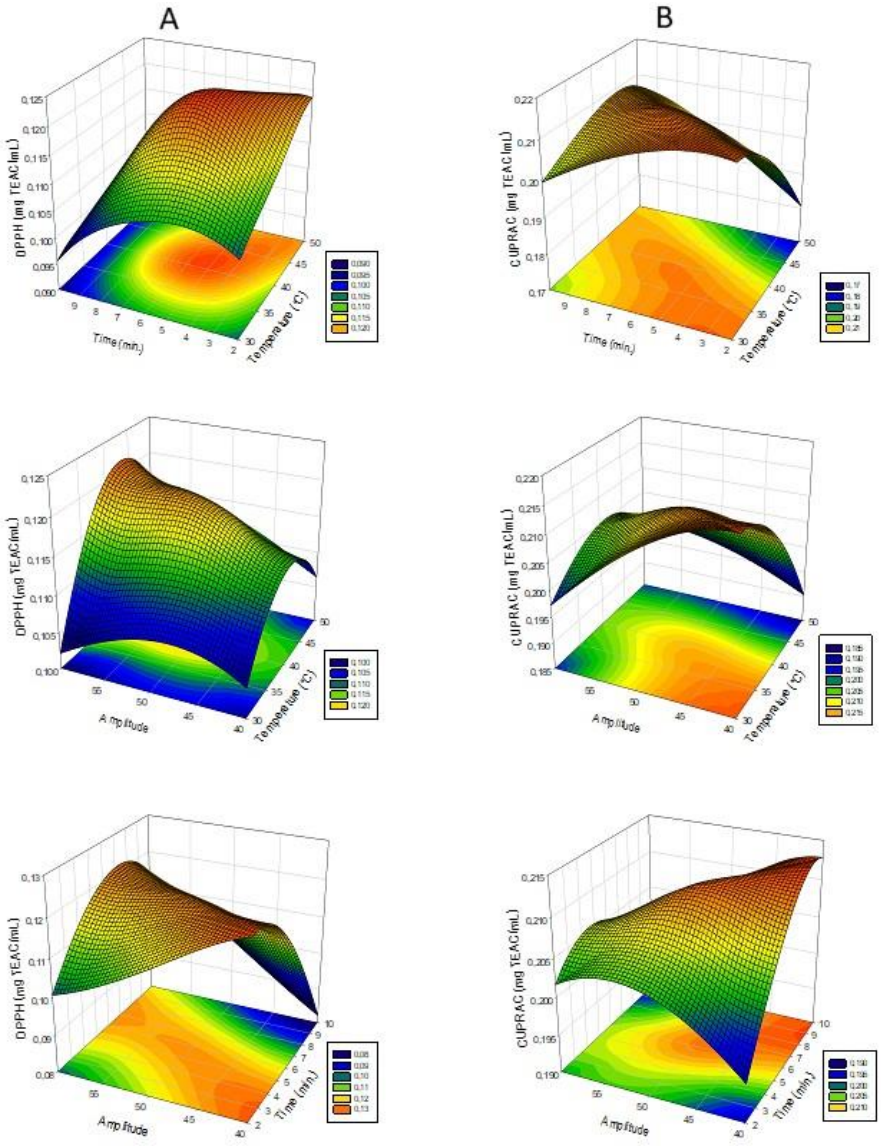

Figure 2. Response surface plots (3D) of DPPH (**A**) and CUPRAC (**B**) analysis as a function of significant interaction factors.

As a result of the optimization, yellow watermelon juice treated with thermosonication was found to cause an increase in the amount of antioxidants by 8.9% compared to the C sample. It was reported that ultrasound-treated purple cactus pear, apple juice, Kasturi lime, carrot-grape and grapefruit juices had increases in total antioxidant capacity in studies [32], [37-40]. The increase in clearance activity of the radicals was attributed to the increase in polyphenolic compounds and anthocyanins in blueberry juice during sonication [41]. The increase in the amount of phenolic compounds due to cavitation caused by the cutting force generated by sonication can be considered to have direct proportion to the total antioxidant capacity [32]. At the same time, by increasing the ultrasound time, Maillard reported that the cleaning of the reaction products also increased for oxygen radicals [42].

C. Determination of Color

Color is an important parameter affecting the quality of fruit juice during processing and storage. It also plays an important role in consumer satisfaction [32]. The equilibrium of the polynomial model indicating the

effect of temperature, time and amplitude factors on the *L**, *a** and *b** values as a result of surface analysis according to the experimental design is shown in Table 4.

Table 5. Analysis of variance (ANOVA) of responses in *L**, *a**, and *b** experiments.

Source	DF	*L**		*a**		*b**	
		F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
Model	9	95.81	0.000	95.89	0.000	184.24	0.000
Linear	3	9.94	0.002	232.42	0.000	47.64	0.000
X_1	1	10.9	0.008	309.66	0.000	47.78	0.000
X_2	1	0.04	0.839	250.69	0.000	40.85	0.000
X_3	1	18.87	0.001	136.92	0.000	54.3	0.000
Square	3	209.33	0.000	33.25	0.000	395.57	0.000
X_1*X_1	1	313.96	0.000	14.92	0.003	1138.58	0.000
X_2*X_2	1	351.56	0.000	64.07	0.000	42.98	0.000
X_3*X_3	1	225.5	0.000	5.82	0.037	189	0.000
2-Way Interaction	3	68.18	0.000	21.99	0.000	109.5	0.000
X_1*X_2	1	139.36	0.000	3.59	0.087	31.59	0.000
X_1*X_3	1	45.44	0.000	6	0,034	283.73	0,000
X_2*X_3	1	19.75	0.001	56.38	0,000	13.17	0,005
Error	10						
Lack-of-Fit	5	0.81	0.590	0.89	0.547	6.11	0.034
Pure Error	5						
Total	19						
R^2		0.9885		0.9885		0.9940	
Adj R^2		0.9782		0.9782		0.9886	
Pred R^2		0.9506		0.9463		0.9583	

DF: degree of freedom; *: multiplication. The term is significant at $p \leq 0.05$. The term is significant at $p \leq 0.01$. The term is significant at $p \leq 0.001$.

The model used in the study was found to be compatible with *L**, *a** and *b** color values (R^2 = 0.9885) (R^2 = 0.9885) (R^2 = 0.9940) (Table 5). All factors applied to yellow watermelon juice samples were found to be statistically significant (P<0.001). The time of thermosonication was not statistically significant (P>0.05). However, thermosonication temperature and amplitude were statistically significant for the *L** value (P<0.05). Cross-interaction of *L**and *b** color values was found to be statistically significant for the 3 factors applied (P<0.001). In the thermosonication process, the cross interaction of the amplitude with the color value a* was not statistically significant (P>0.05). The 3D graphs of the color values according to the temperature, time and amplitude are shown in Figure 3 (A, B, C). When the models of color values are examined, *a** linear increase and decrease in antioxidant amounts was observed as temperature, time and amplitude amount increased. In the 2-way interaction, the effects of temperature-time interaction factors on color values were not statistically significant (P<0.05).

In the ultrasound study applied to grape juice, *L** color values were reported to increase compared to the control sample [28]. Ultrasound applied to tomato juice was reported to cause higher *L**, *a** and *b** values than found in untreated samples [46]. Some treatments applied to the yellow watermelon juice showed parallels. At the same time, it was reported that the increase in *L** value may be due to the increase in the cloud value of the juice [47]. At the end of the optimization, with 38.3 °C, 5.6 minutes and 50.5 amplitude treatment, L*, a* and b* color values were 29.01, 11:43, and 14.55, respectively (Table 6). An increase in *L**, *a** and *b** values was detected compared to the control sample (Table 2).

Table 6. Maximum optimization values, according to the response surface method.

Variable	Setting			
Temperature (X1) (° C)	38.3			
Time (X1) (min.)	5.6			
Amplitude (X3) (%)	50.5			
Response	Fit	SE Fit	95% CI	95% PI
b*	14.5537	0.0454	(14.4526; 14.6549)	(14.2782; 14.8293)
a*	11.4262	0.0315	(11.3561; 11.4963)	(11.2353; 11.6171)
L*	29.0058	0.0583	(28.8758; 29.1357)	(28.6519; 29.3597)
CUPRAC (mg TEAC/mL)	0.214003	0.000365	(0.213191; 0.214816)	(0.211791; 0.216216)
DPPH (mg TEAC/mL)	0.122965	0.000238	(0.122434; 0.123496)	(0.121519; 0.124410)
Total Flavonoid Content (mg CE/L)	41.277	0.134	(40.979; 41.575)	(40.465; 42.089)
Total Phenolic Content (mg GAE/L)	104.295	0.374	(103.462; 105.128)	(102.026; 106.564)

GAE: Gallic acid equivalent; DDPH: radical scavenging activity; CUPRAC: Cupric Reducing Antioxidant Capacity; *L**: represents luminance value *a**: represents red and greenery; *b**: represents yellow and blue

D. Microbiological analysis

In this study, general microbiological analyses were found to be successful for yellow watermelon juice samples after optimization treatment (Table 7). Thermosonication is a new and applicable process used to replace conventional heat treatments. Ultrasonication was reported to be generally more effective when combined with mild heat. This combined process improves enzymatic and microbial inactivation by combined heat and cavitation. It does not produce an effect on the depolymerization of bacterial membranes and macromolecules without causing changes to fruit juice quality [8].

Table 7. Microbiological results for yellow watermelon juice with different processes applied

Sample	Microbiology		
	Total *Enterobacteriaceae* count (log CFU/ml)	**Total aerobic plate count (log CFU/ml)**	**Yeast and mold count (log CFU/ml)**
C	ND	2.14 ± 0.01^{a}	<1
PW	ND	ND	ND
UW	ND	ND	ND

ND: not detected, log CFU/mL, C: untreated yellow watermelon juice, PW: Pasteurized yellow watermelon juice, UW: thermosonication-treated yellow watermelon juice. All data are means ± SD, n = 3, Means within rows with differing subscripts are significantly different at least $p < 0.05$.

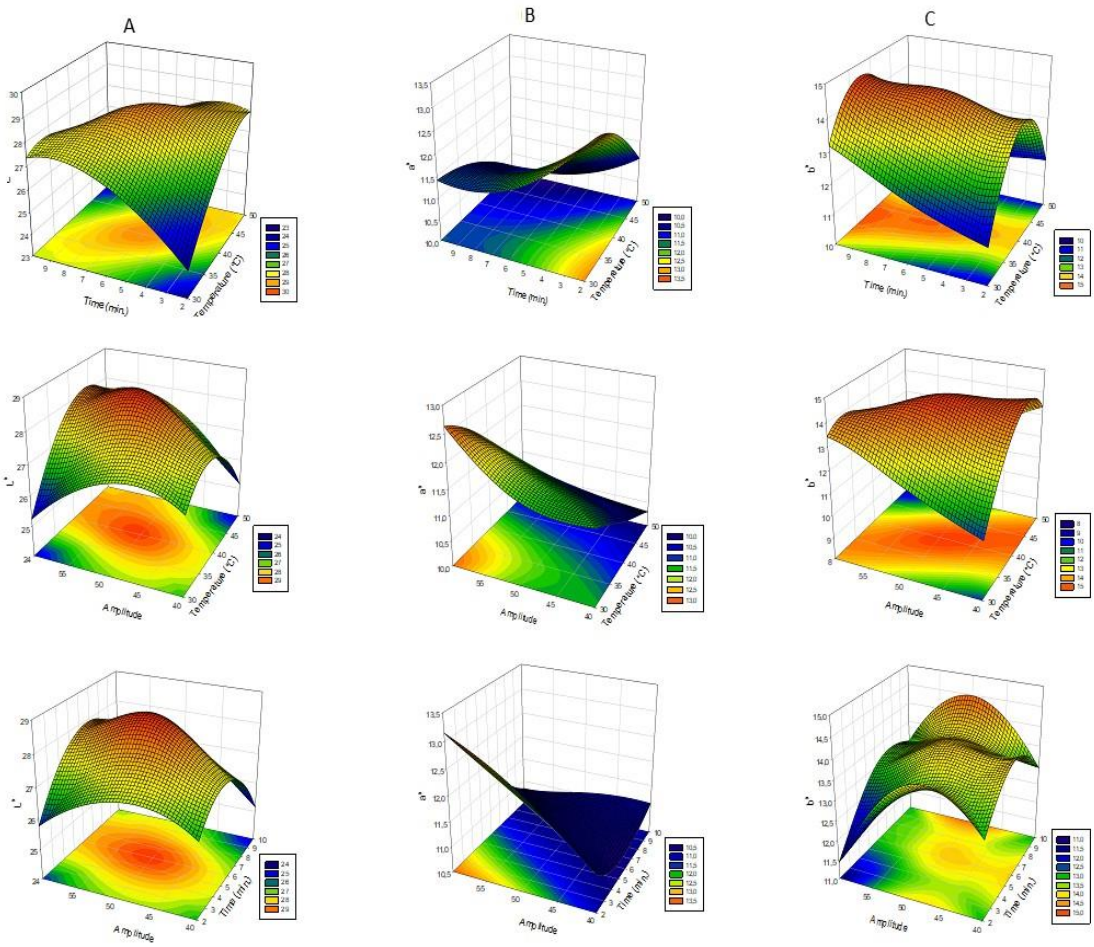

Figure 3. Response surface plots (3D) of L^* values (A), a^* values (B) and b^* values (C) as a function of significant interaction factors

E. Sensory analysis

Sensory analysis is a scientific discipline that is used to measure, analyze and interpret important properties of foods and materials through the senses of vision, smell, taste, touch and hearing [43]. As a result of surface response optimization for sensory analysis, yellow watermelon juice (40 °C, 6 minutes and 50 amplitude) and C and PW samples were compared. As shown in Table 7 and Figure 4, there was no significant change in thermosonication-treated yellow watermelon juice. The overall acceptability of the UW sample was found to be 4.20, and no statistically significant difference was found with the other samples ($P>0.05$). In the evaluation made by the panelists, the UW sample was found to be more successful than the C and PW samples. Researchers reported apple, carrot-grape, orange juice and cranberry juice with ultrasound operations applied generally had acceptable levels of sensory parameters compared to other samples [38], [44-46]. It was stated that the cavitation caused by the ultrasound application contributes to the improvement of the sensory parameters by causing oxygen removal from fruit juice [46]. This study was found to be in parallel with the literature and the sensory properties were not affected by the thermosonization process.

Table 7. Results of sensory analysis values for treated yellow watermelon juice

Sample	Sensory feature				
	Texture (viscosity)	Taste	Aroma	Color	Overall acceptability
C	4.10±0.57[a]	3.90±0.32[a]	4.10±0.57[ab]	4.20±0.42[a]	4.00±0.67[a]
PW	4.00±0.47[a]	3.80±0.63[a]	3.6±0.52[b]	4.00±0.47[a]	3.80±0.63[a]
UW	4.40±0.52[a]	4.20±0.42[a]	4.20±0.42[a]	4.50±0.53[a]	4.30±0.48[a]

All data are means ± SD, Means within rows with differing subscripts are significantly different at least $p < 0.05$. C: untreated yellow watermelon juice, PW: Pasteurized yellow watermelon juice, UW: thermosonication-treated yellow watermelon juice.

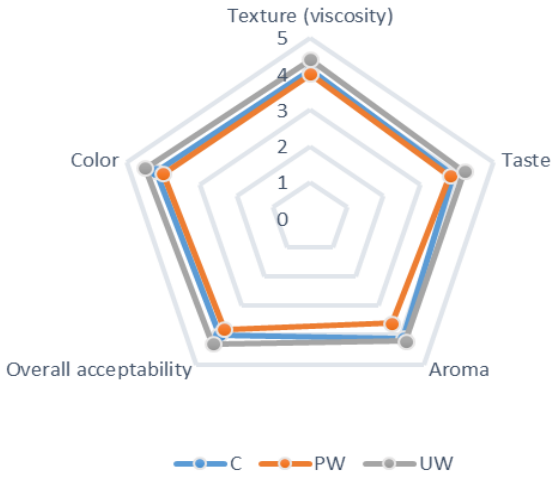

Figure 4. Results of the sensory analysis values chart for treated yellow watermelon juice

IV. CONCLUSIONS

In this study, the bioactive and color values of thermosonication applied to yellow watermelon juice and the effects were optimized with the surface response method. At the same time, the differences between the microbial safety and sensory properties of fresh pasteurized watermelon juice and fresh yellow watermelon juice were investigated. The results of the analysis showed that yellow watermelon juice treated with thermosonication had curative effects on bioactive properties compared to other samples. At the same time, it was microbially safe and it was most appreciated by the panelists in terms of sensory properties. According to these results, total phenolic substance, total flavonoid matter and total antioxidant substance (DPPH and CUPRAC) dependent variables were optimized. As a result of the thermosonication, the independent values for time and amplitude are 38.3 °C, 7.4 minutes and 50.5 amplitude, respectively. As a result, thermosonication technology was found to be successful for the production of yellow watermelon juice. Pilot scale studies are recommended for industrial production of the product.

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