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Optimization of Bioactive Components in Fresh Red Watermelon Juice of Ultrasound Assisted Extraction Conditions With Response Surface Methodology

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Abstract: In this study, optimization of bioactive components in fresh red watermelon juice which was applied ultrasound for different amplitude and time with response surface methodology (RSM) was performed. As a result of the optimization, lycopene, ascorbic acid, total phenolic content and DPPH were determined as 28.74 mg/100 mL, 4.34 mg/100 mL, 122.2 mg GAE/L and 54.26%, respectively. When compared to the fresh red watermelon juice samples applied ultrasound with control samples, it was found that lycopene, total phenolic content and DPPH values increase and ascorbic acid content decreased.

Keywords: Bioactive components, Fresh Red Watermelon Juice, Response Surface Methodology, Ultrasound

Taze Kırmızı Karpuz Suyundaki Biyoaktif Bileşiklerin Ultrases Destekli Ekstraksiyon Koşullarının Tepki Yüzeyi Metodolojisi ile Optimizasyonu

Öz: Bu çalışmada, farklı genlik ve sürelerde ultrases uygulanmış kırmızı karpuz suyundaki biyoaktif bileşiklerin tepki yüzeyi metodolojisi (TYM) kullanılarak optimizasyonu gerçekleştirilmiştir. Optimizasyon sonucunda ultrases uygulanmış örneklerin likopen, askorbik asit, toplam fenolik madde ve DPPH değerleri sırası ile 28.74 mg/100 mL, 4.34 mg/100 mL, 122.2 mg GAE/L ve %54.26 olarak tespit edilmiştir. Kontrol örneği ile kıyaslandığında ultrases uygulanmış kırmızı karpuz suyu örneklerinin likopen, toplam fenolik madde ve DPPH değerlerinde artış görülürken, askorbik asit içeriğinde azalma meydana gelmiştir.

Anahtar kelimeler: Biyoaktif bileşikler, taze kırmızı karpuz suyu, tepki yüzeyi metodu, Ultrases

1.Introduction

Watermelon [*Citrullus lanatus* (Thunb.)] is generally considered to be of Citrullus species which belongs to the *Cucurbitaceae* family of flowering plants (Seyed & Elnaz, 2006). Although the Middle East, Europe, the USA, Africa, Japan, and India are the most important watermelon producing areas, watermelon is cultivated in most parts of the world (Fehér, 1993). It is one of the most economically and a pleasant-tasting fruit (Olayinka & Etejere, 2018). Watermelon contains several bioactive compounds such as carotenoids, unsaturated fatty acids, xanthophylls, phenolic compounds, and citrulline (Zamuz et al., 2021). Bioactive compounds has properties; inhibition and induction of gene expression, enzymes, receptor activities (Santos et al., 2019). Carotenoids in waterlemon that give to the varios flesh colors; orange, red, yellow (canary and salmon). Flesh color is show of the potential health benefits of watermelon (Bang et al., 2007; Bang et al., 2010).

Food processing objective is producing more usable, compact, appealing, value-added, and shelf-stable product. The most traditional food processing method is heating for retard growth of foodborne pathogens and reduce microbial growth. These process can cause changes at color, taste, and textural modifications in heat-sensitive food (Singla & Sit, 2021). One of the green and innovative techniques is ultrasound-assisted processing (Bindes et al., 2019). Ultrasonication, as a novel non-thermal treatment, is applied in the food technology especially in biotechnology (Alijan et al., 2021). Many researchers have found that non-thermal treatment (ultrasound technology) of mango peel and juice (Santhirasegaram et al., 2013; Mercado-Mercado et al., 2018), grapefruit juice (Aadil et al., 2015), tomato juice (Zhang et al., 2019), rosehip nectar (Atalar et al.,2020), amazon fruits juice (De Souza Carvalho et al., 2020) had minimal losses to nutritional value and quality.

RSM is an statistical technique, less time-consuming and laborious. It has successfully and constantly been showed that it can be used in optimizing ingredients (Lee et al., 2006).

When the literature was searched, no study was found on the optimization of bioactive compounds in fresh red watermelon juice using the RSM. The objective of this study was to investigate the effects of various ultrasound treatment of fresh red watermelon to its antioxidant capacity, ascorbic acid, lycopene, and total phenolic contents as a result of RSM optimization.

2. Material and method

2.1. Watermelon juice samples preparation

Fresh red watermelons were carefully collected from local producers (Tekirdağ, Turkey) and kept at 4°C. The seeds, and ripe parts of the watermelons were removed from the outer pods then the remaining edible parts were crushed with the help of a blender (Waring Commercial Blender Model HGB2WTS3, USA) to prepare its juice. Watermelon juice after filtered through a sterilized double-layer muslin cloth, it was sterilized and filled into 100 mL airtight bottles. Untreated watermelon juice was chosen as the control (MJ-C). Watermelon juice was pasteurized in a water bath (90 °C) for 30 seconds, then quickly cooled to 20 °C (MJ-P). The UP200St- ultrasound device of Hielscher Ultrasonics (UP200St, Germany) was used in the study to treated with ultrasound. The device was operated at 26 kHz, 200 w. Samples were stored at -20 °C until analysis.

2.2. Experimental design

RSM was used to determine the effect of ultrasound technology on the lycopene, total phenolic content, ascorbic acid, and DPPH of the fresh red watermelon juice, Central Composite Design was chosen and a two-factor, five-level experiment design was created (Table 1). For the study, 13 trial designs were created. The lack-of-fit tests, R2 and corrected -R2 coefficients, and ANOVA results were model adequacy values. Independent variables were determined as duration within the range of m (time) and n (amplitude).

The following equation models was created with quadratic-polynomial equation formula;

$$y = b_o + b_1\, x_1 + b_2\, x_2 + b_{12} x_1 x_2 + b_{11} x_1^2 + b_{22} x_2^2 \qquad (1)$$

Table 1. Dependent and independent variables of RSM analysis and results of bioactive compounds

Çizelge 1. *RSM analizinin bağımlı ve bağımsız değişkenleri ve biyoaktif bileşenleri üzerine etkisi*

Run no.	Independent variables		Dependent variables							
	Time (m)	Amplitude (m)	Lycopene (mg/100 mL)		AA (mg/100 mL)		TPC (mg GAE/L)		DPPH (% inhibition)	
			Experimental data	RSM predicted	Experimental data	RSM predicted	Experimental data	RSM predicted	Experimental data	RSM predicted
1	8 (+1)	50(-1)	27.80	27.63	3.83	3.76	122.60	123.87	54.90	54.88
2	8 (+1)	70 (+1)	25.32	25.09	2.55	2.55	108.24	109.90	53.40	53.35
3	6 (0)	60 (0)	26.38	26.45	3.16	3.16	125.87	125.23	55.32	55.32
4	2 (-1.41)	60 (0)	28.64	28.72	5.07	5.04	107.41	106.68	51.55	51.54
5	6 (0)	60 (0)	26.38	26.45	3.16	3.16	125.87	125.23	55.32	55.32
6	6 (0)	60 (0)	26.38	26.45	3.16	3.16	125.87	125.23	55.32	55.32
7	6 (0)	60 (0)	26.38	26.45	3.16	3.16	125.87	125.23	55.32	55.32
8	10 (+1.41)	60 (0)	25.73	25.84	3.53	3.56	109.81	108.97	53.05	53.07
9	4 (-1)	70 (+1)	28.72	28.50	4.09	4.15	119.88	121.47	53.76	53.74
10	6 (0)	80 (+1.41)	27.44	27.56	3.16	3.13	106.71	105.71	51.57	51.59
11	4 (-1)	50 (-1)	27.25	27.09	3.67	3.65	108.80	110.00	52.94	52.96
12	6 (0)	60 (0)	26.38	26.45	3.16	3.16	125.87	125.23	55.32	55.32
13	6 (0)	40 (-1.41)	28.62	28.69	3.80	3.84	108.79	108.21	52.36	52.35
MJ-C			25.16		5.42		112.55		50.86	
MJ-P			21.45		2.70		104.86		46.64	

RSM: Response surface methodology TPC: Total phenolic content; AA: ascorbic acid; DDPH: radical scavenging activity; GAE: gallic acid equivalent; MJ-C: watermelon juice; MJ-P: thermal pasteurized watermelon juice

The coefficients of the polynomial were indicated by b_{12} (interaction effects), b_{11} and b_{22} (quadratic effects), b_1 and b_2 (linear effects), and b_0 constant term).

2.3. Determination of lycopene content

For the determination of lycopene concentration method was used as reported previously by Oms-Oliu et al., (2009) with some changes. The lycopene concentration (mg/L) of fresh red watermelon sample was estimated using the eguation below:

Lycopene concentration=Abs503×MW×DF×1000ε×L (2)

Molecular weight (MW) of lycopene; 536.9 g/mol, dilution factor (DF), path length (L) in cm, and molar extinction coefficient (ε) for lycopene;172.000 L/mol/cm.

2.4. Determination of total phenolic contents

Total phenolic content assay as described by Singleton & Rossi, (1965) was used. The dilution was carried out by taking 1.5 ml from the fresh red watermelon juice sample. Folin-Ciocalteau reagent (0.2 N, 2.5 mL) was added. After 2 mL of 7.5% Na_2CO was added, the prepared solution was incubated for 3 minutes. The red watermelon juice samples were left for 30 minutes in the dark at 24 °C (normal room temperature). Spectrophotometer (SP-UV / VIS-300SRB, Spectrum Instruments, Australia) was used to measurements absorbance (760 nm). Standard curve for gallic acid was constructed, results were given as mg GAE/L.

2.5. Determination of ascorbic acid contents

The Vitamin C (AA) content of the red watermelon samples was determined by Association of Official Analytical Chemists (AOAC) (Association of Official Analytical Chemists. [AOAC], 1990). Briefly, watermelon juice sample (five mL) was diluted with distilled water and two ml of the diluted solution was taken and glacial acetic acid (20%, 25 ml) was added. Titration was done using 0.05 g/100 mL DCIP (6-dichlorophenol indophenol) solution. Results were given as milligram ascorbic acid equivalents mg/100 with using standard curve for ascorbic acid.

2.6. DPPH free-radical scavenging activity

For the determination of DPPH radical scavenging activity of the fresh red watermelon samples, method, as described by Grajeda-Iglesias et al. (2016) was used with some changes. Shortly, two ml of 0.1 mM DPPH (Sigma-Aldrich, USA) was added to 100 µL of red watermelon juice sample as blank control and standard, or to tubes containing distilled water. The prepared solutions were incubated during 30 minutes under normal room conditions. Measurements were performed on tubes with a wavelength of 517 nm in a spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Australia). The percentage of inhibition of the DPPH value was calculated with the following formula:

$$\text{DPPH (\% inhibition)} = (T_0 - T_1)/T_0 * 100 \quad (3)$$

The T_0= absorbance value of control, T_1= absorbance value of the examined sample

2.7. Statistical analysis

Results were expressed as mean ± standard deviation (SD). All analyses were carried out in triplicate for each samples. Analysis of variance (one-way-ANOVA) was used for determination of the differences between the data with a significance level of p<0.05. SPSS 22.0 package software (SPSS Inc., Chicago, USA) was used for statistical analysis calculations.

3. Results and Discussion

3.1. Optimization of bioactive compounds

An innovative, non-toxic, environmentally friendly non-thermal pasteurization method that has emerged as an alternative to traditional thermal technologies to minimize changes in the organoleptic and nutritional properties of food is; ultrasound method (Bhargava et al., 2021; Fan et al., 2021; Valiati et al., 2022). Watermelon juice has attracted great attention from consumers in recent years due to its richness in lycopene, phenolic compounds, and high antioxidant activity. Since bioactive substances are sensitive to high-temperature processing, they hurt their nutritional and sensory properties. Therefore, non-thermal treatments are needed to enrich or further preserve the bioactive compounds of watermelon juice. One of the aims of this study is to increase the bioactive components with ultrasound. Optimization results of lycopene, ascorbic acid, total phenolic substance, and DPPH content of watermelon juice are shown in Table 1. The result of the optimization, the quadratic modeling equation of the lycopene value of watermelon juice is given below.

$$Lycopene\ (\frac{mg}{100\ mL)=} 29{,}44 + 1{,}980m - 0{,}233n + 0{,}05151m^2 + 0{,}004175n^2 - 0{,}04931mn \quad (4)$$

R^2 values, analysis of variance (ANOVA), discordance, and regression coefficients are shown in Table 2. Watermelon juice is a fruit juice included lycopene, an important beverage used in food industry such as nutraceutical supplements and energy drinks (Vani et al., 2021). In our study, significant increases were detected in lycopene amounts as a result of ultrasound treatments. When we examined the effects of time and amplitude, an increase in proportion to the amount of lycopene was found in general (Figure 1A). R^2 level of RSM modeling showed high agreement with 98.60% (Table 2). Two-way and one-way effects of modeling were not found to be statistically significant (p<0.05). As a result of the optimization, lycopene was determined as 28.74 mg/100 mL (Table 3). Compared to the MJ-C sample, a 14.23% increase in the amount of lycopene was detected. Similar increases were found in the amount of lycopene in ultrasound treatment applied to red watermelon juice (Yıkmış, 2020). However, another study found reductions in ultrasound treatment applied to guava juice. Significant reductions in the amount of lycopene were detected in thermal pasteurization (Campoli et al., 2018). The reason for this decrease in lycopene is that it undergoes oxidation and isomerization with thermal heat (Shi & Le Maguer, 2000).

Table 2. ANOVA results of bioactive compounds (Lycopene, ascorbic acid, TPC, and DPPH)
Çizelge 2. *Biyoaktif bileşiklerin (Likopen, askorbik asit, TPC ve DPPH) ANOVA sonuçları*

Source	DF	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
		Lycopene (mg/100 mL)		AA (mg/100 mL)		TPC (mg GAE/L)		DPPH (% inhibition)	
Model	5	98.5000	0.0000	541.1900	0.0000	95.1900	0.0000	7201.7800	0.0000
Linear	2	116.0900	0.0000	597.4100	0.0000	2.3400	0.1670	1498.5000	0.0000
m	1	201.6700	0.0000	973.4100	0.0000	2.1800	0.1830	2414.3900	0.0000
n	1	30.5200	0.0010	221.4100	0.0000	2.4900	0.1580	582.6000	0.0000
Square	2	67.4500	0.0000	544.0200	0.0000	191.9300	0.0000	15585.6600	0.0000
m^2	1	31.3500	0.0010	1088.0300	0.0000	234.4000	0.0000	17859.1300	0.0000
n^2	1	128.7600	0.0000	86.1700	0.0000	258.2400	0.0000	22074.8200	0.0000
2-Way Interaction	1	125.4100	0.0000	423.1100	0.0000	87.4000	0.0000	1840.6100	0.0000
m*n	1	125.4100	0.0000	423.1100	0.0000	87.4000	0.0000	1840.6100	0.0000
Error	7								
Lack-of-Fit	3								
Pure Error	4								
Total	12								
R^2		98.60%		99.74%		98.55%		98.98%	
Adj R^2		97.60%		99.56%		97.52%		99.97%	
Pred. R^2		90.34%		97.39%		90.03%		99.82%	

m: time; n: amplitude; DF: degrees of freedom; TPC: Total phenolic content; AA: ascorbic acid; DDPH: radical scavenging activity; GAE: gallic acid equivalent;

Table 3. Maximum optimization values according to RSM.
Çizelge 3. *RSM'ye göre maksimum optimizasyon değerleri.*

Variable	Setting			
Time (m) (min.)	8.8			
Amplitude (n) (%)	46.9			
Response (MJ-UT)	**Fit**	**SE Fit**	**95% CI**	**95% PI**
Lycopene (mg/100 mL)	28.74	0.202	(28.267; 29.222)	(28.111; 29.378)
AA (mg/100 mL)	4.346	0.047	(4.2339; 4.4579)	(4.1972; 4.4945)
TPC (mg GAE/L)	122.2	1.56	(118.52; 125.90)	(117.32; 127.10)
DPPH (% inhibition)	54.26	0.157	(53.884; 54.628)	(53.753; 54.758)

TPC: Total phenolic content; AA: ascorbic acid; DDPH: radical scavenging activity; GAE: gallic acid equivalent; MJ-UT: ultrasound-treated watermelon juice.

As a result of RSM, the ascorbic acid R2 value showed a high correlation with 99.74% (Table 2). As a result of the optimization, the quadratic polynomial equation of the ascorbic acid value of fresh red watermelon juice is given below.

$$AA\left(\frac{mg}{100\ mL}\right) = 3{,}141 + 0{,}2347m + 0{,}0136n - 0{,}07119m^2 + 0{,}000801n^2 - 0{,}02125mn \quad (5)$$

No statistically significant differences were found in the two-way interaction of m and n values in ultrasound treatment (p<0.001). The amount of AA in the MJ-C sample was determined as 5.42 mg/100 mL. It was determined that AA amount is decreased after ultrasound treatment. These decreases are observed when the three-dimensional graph of the modeling is examined (Figure 1B). After the optimization, AA was determined as 4.35 mg/100 mL in this study. However, ultrasound treatment preserved the ascorbic acid content more than thermal pasteurization. Similar effects were observed in ultrasound treatments applied to kiwi juice (Wang et al., 2019) and red grape juice (Margean et al., 2020). The decrease in the amount of ascorbic acid may be due to the sensitivity of ascorbic acid to the amount of heat generated as a result of cavitation.

Polyphenols are one of the non-nutritive bioactive components responsible for the taste, aroma, and color of vegetables and fruits (Dall'Asta et al., 2022). Today, there has been increasing interest to polyphenol-rich ingredients into foods. The effect of food processing technologies on the stability and biological activity of polyphenols is an important factor (Debelo et al., 2020). The effects of ultrasound parameters on total phenolic substance and DPPH are shown in Table 1. RSM optimization, the quadratic polynomial equations of TPC and DPPH values of fresh red watermelon juice are given below.

$$TPC\left(mg\ \frac{GAE}{L}\right) = -190{,}8 + 32{,}42m + 7{,}326n - 1{,}0878m^2 - 0{,}04567n^2 - 0{,}3180mn \quad (6)$$

$$DPPH\ (inhibition) = 7{,}903 + 4{,}1925m + 1{,}16118n - 0{,}18854m^2\_0{,}008385n^2 - 0{,}028974mn \quad (7)$$

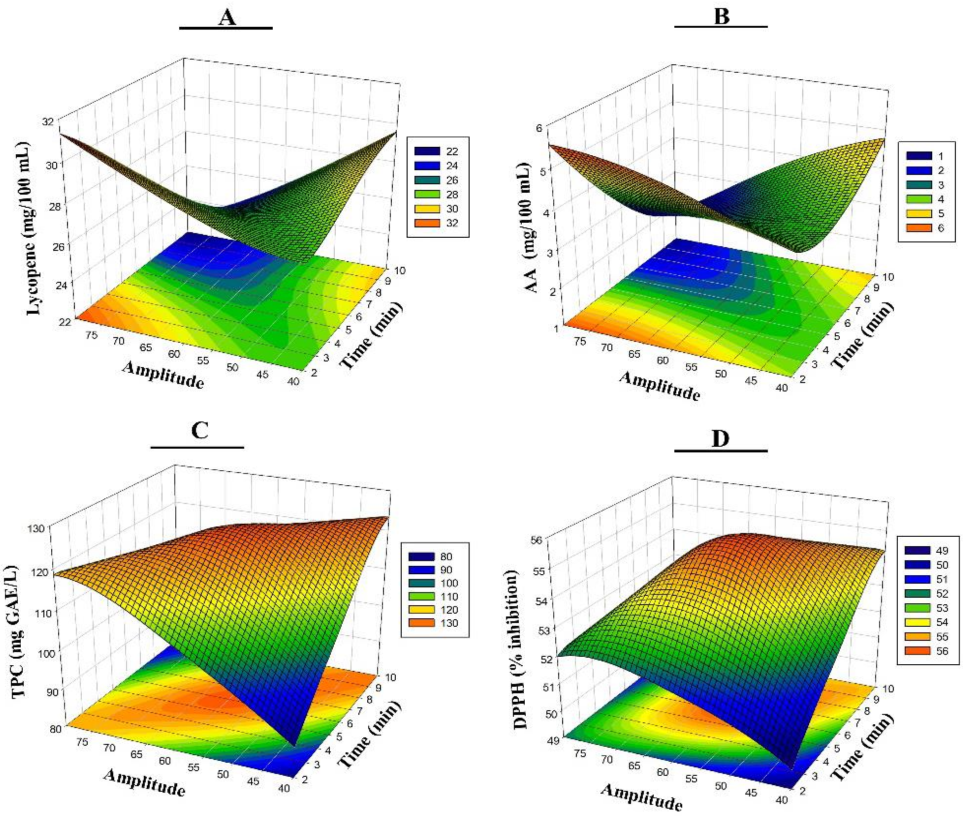

Figure 1. Response surface plots (3D) of lycopene (A), ascorbic acid (B), TPC (C), and DPPH (D), as functions of significant interaction factors.
Şekil 1. *Önemli etkileşim faktörlerinin fonksiyonları olarak likopen (A), askorbik asit (B), TPC (C) ve DPPH'nin (D) tepki yüzey grafikleri (3D).*

The results of the changes in DPPH antioxidant activity values during fermentation of watermelon juice samples applied with ultrasound at different times and amplitudes are expressed in Table 1. As a result of RSM, TPC and DPPH R2 values showed a high correlation with 98.55% and 98.98%, respectively (Table 2). Increases in the amounts of TPC and DPPH were detected with m and n. These increases are observed when the three-dimensional graph of the modeling is examined (Figure 1CD). The linear effects of the variables (m and n) on TPC were found to be statistically significant ($p>0.05$). However, linear effects on DPPH are insignificant ($p<0.001$). No significant effects on TPC and DPPH were detected in the two-way interaction of the variables ($p<0.001$). After the optimization, TPC and DPPH were determined as 122.20 mg GAE/L and 54.26%, respectively. Similar increases in TPC and antioxidant values with ultrasound were reported in functional beverage of Turkish (Doguer et al., 2021), chokanan mango juice (Santhirasegaram et al., 2013), strawberry juice (Wang, et al., 2019), lactic acid fermented mulberry juice (Kwaw et al., 2018), purple cactus pear (Zafra-Rojas et al., 2013), quince juice (Yıkmış et al., 2019) and gilaburu vinegar (Erdal et al., 2022) samples. Significant reductions were detected with thermal pasteurization. The effect originating from ultrasound is associated with the cavitation mechanism that occurs when bubbles form and burst during the propagation of sound waves in a liquid medium. When high-powered ultrasound is applied to food products or ingredients, these bubbles burst, producing free radicals across and intense shock waves the cell membrane (Aadil et al., 2013; Ordóñez-Santos et al., 2017; Wang et al., 2019; Yıkmış et al., 2021).

4. Conclusion

In this study, the optimization of bioactive components in fresh red watermelon juice using ultrasound with RSM was determined. Compared to the MJ-C sample, lycopene, total phenolic content, and DPPH values increased by 14.23%, 8.57%, and 6.69%, respectively. With the ultrasound treatment, a decrease of 19.81% in ascorbic acid content was detected. The results of this research have shown that ultrasound technology had a important effect on bioactive compounds in fresh red watermelon water.

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