GIDA
THE JOURNAL OF FOOD
E-ISSN 1309-6273, ISSN 1300-3070

Research/Araştırma
GIDA (2020) 45(5)989-996
doi: 10.15237/gida.GD20079

DETERMINATION EXTRACTION CONDITIONS FOR MAXIMUM PHENOLIC COMPOUNDS IN THE KIWIFRUIT USING RESPONSE SURFACE DESIGN

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Received/Geliş: 16.06.2020; *Accepted* / Kabul: 17.09.2020; *Published online* / Online baskı: 30.09.2020

Kasnak, C., Palamutoğlu, R. (2020). Determination extraction conditions for maximum phenolic compounds in the kiwifruit using response surface design. GIDA (2020) 45(5)989-996 doi: 10.15237/gida.GD20079.

Kasnak, C., Palamutoğlu, R. (2020). Yanıt yüzey tasarımı kullanılarak kivi meyvesindeki maksimum fenolik bileşikler için ekstraksiyon koşullarının belirlenmesi. GIDA (2020) 45(5)989-996 doi: 10.15237/gida.GD20079.

ABSTRACT
The study aims to determine the extraction parameters for maximal phenolic compounds and antioxidant activity from kiwifruit by using response surface methodology (RSM). Box-Behnken experimental design of RSM was applied to determine the effect of extraction parameters. Three independent variables were selected at three levels: methanol concentration (A: 60-75-90 %), ultrasound time (B: 5-10-15 min.), and mass of sample (C: 1-2-3 g). Conditions for maximum phenolic yield were found as A = 90%, B= 7.71 min, and C = 3 g, under this condition, DPPH of 88.96 %, ABTS of 739.07 mg/100g FW and TPC of 299.14 mg/100g fresh weight has been obtained.
Keywords: Kiwifruit, response surface methodology, total phenolic matter, antioxidant activity

YANIT YÜZEY TASARIMI KULLANILARAK KİVİ MEYVESİNDEKİ MAKSİMUM FENOLİK BİLEŞİKLER İÇİN EKSTRAKSİYON KOŞULLARININ BELİRLENMESİ

ÖZ
Bu çalışmanın ana odak noktası yanıt yüzey metodolojisi (RSM) kullanarak kivi meyvesinden olabildiğince fazla fenolik bileşik ve antioksidan aktivite için ekstraksiyon parametrelerini belirlemektir. Ekstraksiyon parametrelerinin etkisini belirlemek için RSM'de Box-Behnken deneysel tasarımı kullanıldı. Metanol konsantrasyonu (A: %60-75-90), ultrason süresi (B: 5-10-15 dakika) ve numune kütlesi (C: 1-2-3 g) olmak üzere üç seviyede üç bağımsız değişken seçildi. Bu şartlar altında maksimum fitokimyasal verim için koşullar A = % 90 metanol konsantrasyonu, B = 7.71 dakika ve C = 3 g olarak bulundu. Bu koşullar altındaki ekstraksiyon sonucu kivide DPPH % 88.96, ABTS 739.07 mg/100 g ve toplam fenolik içerik 299.14 mg/100 g tespit edildi.
Anahtar kelimeler: Kivi, yanıt yüzey metodolojisi, toplam fenolik madde, antioksidan aktivite

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INTRODUCTION

Kiwifruit, which belongs to the genus Actinidia, is a comestible fruit with kernels entombed in flesh. The fruits are of Chinese origin and produce over 4 million tons per year in the world (Li and Zhu, 2019; Vivek et al., 2016). Due to its high nutritional and medicinal value, it is considered one of the most prominent horticultural plants in the world. Kiwifruit is rich in bioactive compounds such as ascorbic acid, polyphenols, and flavonoids, which have important health benefits. Therefore, it has a high antioxidative capacity (Amodio et al., 2007). It is accepted that the consumption of foods with high antioxidant capacity has a positive effect on health by reducing oxidative stress (Kasnak and Palamutoğlu, 2015). Antioxidant activity can be determined using certain radicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) or can be estimated because it correlates with total phenolic and ascorbic acid contents (Moharram and Youssef, 2014).

Phenolics are important compounds found as secondary metabolites in all plants (Khoddami et al., 2013; Naczk and Shahidi, 2004). The extraction of phenolics from source materials is the first place in their analysis (Naczk and Shahidi, 2004). Improper extraction time, solvent purity, and plant material solvent rate can lead to insufficient extraction of polyphenols. Therefore, appropriate extraction parameters should be selected for the proper determination quantification of phenolics (Jakobek et al., 2015). RSM is a good option for choosing the right extraction parameters. For this reason, many studies have been conducted to determine the optimum extraction conditions using RSM (Şahin and Şamlı, 2013; Mushtaq et al., 2014; Belwal et al., 2016; Sharmila et al., 2016; Pandey et al., 2018; Qadir et al., 2019).

The extraction of phenolic compounds in fruits and plant material is influenced by its chemical structure, the extraction method used, sample particle size, sample amount, solvent type and concentration, storage time and conditions, as well as the presence of inhibitors (Robbins, 2003). Water, ethanol, methanol, ethyl acetate, acetone, and to lesser extent propanol, isopropyl alcohol, and combinations thereof are often used for the extraction of phenolics (Antolovich et al., 2000). In the pre-trials of this study, methanol was chosen as the solvent which gave good results. Recently, ultrasound-assisted extraction has been used successfully for the extraction of phenolic compounds (Arshadi et al., 2016). Here, cavitations bubbles on the surface of the cell walls are provided to break down the cell wall by mechanical and thermal effects and better penetration of the solvent into phenolics (Martínez-Patiño et al., 2019).

To determine total phenolic content and antioxidant activity accurately in fruits such as kiwifruit, the extraction conditions applied should be optimum. In this study, it was aimed to determine the most suitable extraction conditions to obtain maximum phenolic content with the use of RSM.

MATERIALS AND METHODS

Sample Preparation

Kiwifruits were purchased from markets in Afyonkarahisar, Turkey. The kiwifruits were selected for the same magnitude. The kiwifruits were brought to the laboratory without waiting. The fruits were washed, peeled, and grated. All of the chemicals used in the analysis were purchased from Sigma-Aldrich.

Extraction

Three different amounts (1g, 2g, and 3g) of the grated kiwifruits sample were homogenized at 11000 rpm in 20 mL of 60-75-90% methanol for 3 minutes. The homogenate was sonicated for 5-10-15 min with an ultrasonic bath (Daihan WUC.D03H, Korea) and after that, it was centrifuged at 4000 rpm for 15 minutes. The supernatant was filtered and kept at -18 °C until analysis. The filtrate was used in total phenolic content (TPC), DPPH, and ABTS analyzes and all results were determined on fresh weight.

Determination of DPPH radical scavenging activity

The antioxidant activity of the samples was determined by using the DPPH radical scavenging method(Brand-Williams et al., 1995).

4.10 M DPPH solution was prepared with 100% methanol. 400 µL of DPPH solution and 1600 µL of the sample solution was mixed and incubated for 30 minutes in the dark. All samples were read at the spectrophotometer (Optizen pop, Korea) at 517 nm. The antioxidant activity was evaluated using the formula. DPPH results are given as percentages.

$$Antioxidantactivity\ (\%) = \frac{(A_{Control} - A_{Sample})}{A_{Control}} x100 \quad (1)$$

Determination of ABTS radical scavenging activity

ABTS radical cation decolorization analysis was carried out by making some modifications according to by (Re et al., 1999). 1.8 mM ABTS was mixed with 0.63 mM potassium persulfate solution (1:1) and mix waited in the dark for twenty-four hours. The solution was mixed with methanol till the absorbance was 0.700 + 0.030 at 734 nm was obtained. 1980 µL of the prepared solution was mixed with 20 µL of the sample and read on the spectrophotometer. Results are given as the Trolox equivalent.

Total Phenolic Content (TPC)

0.5 mL of kiwifruit extract, 7 mL of pure water, and 0.5 mL of Folin-Chiocalteu reagent were mixed and incubated for three minutes. Then 2 mL of 20% Na_2CO_3 was added and mixed again. The mixture was allowed to stand at 25 °C water bath for 1 hour. It was read at 765 nm on the spectrophotometer. The concentration was determined with the gallic acid curve (Kaur and Kapoor, 2002). Results are given as gallic acid equivalent.

Experimental design and Statistical analysis

The optimum situations for the extraction process were appraised by Box-Behnken experimental design (BBD) of RSM using the Design Expert 11 trial program. The independent variables were methanol (60, 75, and 90%, A), ultrasound time (5, 10, and 15 min., B), and mass of kiwifruits (1, 2, and 3 g, C). The dependent variables were DPPH, TPC, and ABTS. The design used was BBD, based on these fifteen experiments were designed with 4 axial points, 4 cube points, and 7 center points in a cube. A three-level, three factors Box-Behnken experimental design is seen in Table 1. Two replicate runs were performed for each tuber.

Table 1 Actualvaluesusedforthe BBD and DPPH, ABTS and TPC of kiwifruitextract.

Std	PtType	Blocks	Run	Methanol %	Ultrasound Time	Mass of Sample	DPPH (%)	TPC (mg GAE/100g fw)	ABTS (mg TE/ 100g fw)
11	2	1	1	75	5	3	87.76	265.00	682.14
12	2	1	2	75	15	3	86.32	320.00	787.94
4	2	1	3	90	15	2	87.84	220.00	549.65
8	2	1	4	90	10	3	88.00	280.00	680.67
2	2	1	5	90	5	2	88.48	225.00	582.80
5	2	1	6	60	10	1	88.26	120.00	274.64
10	2	1	7	75	15	1	88.96	110.00	332.87
13	0	1	8	75	10	2	86.70	250.00	570.57
9	2	1	9	75	5	1	88.18	120.00	289.08
15	0	1	10	75	10	2	87.19	230.00	551.97
1	2	1	11	60	5	2	84.40	210.00	473.66
6	2	1	12	90	10	1	88.88	125.00	316.72
7	2	1	13	60	10	3	84.11	320.00	619.87
14	0	1	14	75	10	2	87.19	230.00	551.97
3	2	1	15	60	15	2	85.30	225.00	480.65

RESULTS AND DISCUSSIONS

Analysis of variance (ANOVA) and estimated regression of each response

Variance analysis is used for model validation. Average squares, degree of freedom, the sum of squares, F-value, and P-value are the parameters that control the effectiveness of the model. The variance of the mean data is evaluated by determining the F-value. Also, P-values validate the model statistically. According to the variance analysis, the parameters are more sensitive at higher F-values. Besides, the model for P values less than 0.05 is statistically approved (Majdi et al., 2019). The good fit model R^2 value should be more than 0.8 (Rana et al., 2018).

Response Surface Methodology

The trials were made according to BBD demonstrated in table 1, which proposes the impact of process factors in the increment of ABTS, DPPH, and TPC of the phytochemicals extracted from kiwifruit. The DPPH value of 88.96%, TPC of 3.2 mg/g, and ABTS of 7.88 mg/g were found to be maximal responses carried from correspondent runs 10, 12, and 12 respectively. Based on the outcomes made from the empirical runs, ANOVA statistics (Tables 2–4) were created. The interaction impacts of the singular process variables were confirmed using three-dimensional response surface plots. The generated equation (Eqs. 2) in coded level, taking account for the DPPH of independent variables, is given below:

$$DPPH = 87.03 + 1.39A - 1.01C + 0.8169AC + 0.7916C^2 \quad (2)$$

Table 2 ANOVA statisticsforthe DPPH of kiwifruit

Source	Sum of Squares	df	MeanSquare	F-value	p-value	
Model	31.67	9	3.52	13.45	0.0053	significant
A-Methanol	15.47	1	15.47	59.14	0.0006	significant
B-Ultrasound Time	0.0194	1	0.0194	0.0743	0.7961	
C-Mass of Sample	8.17	1	8.17	31.24	0.0025	significant
AB	0.5953	1	0.5953	2.28	0.1918	
AC	2.67	1	2.67	10.2	0.0241	significant
BC	1.24	1	1.24	4.72	0.0818	
A^2	0.9478	1	0.9478	3.62	0.1153	
B^2	0.0008	1	0.0008	0.0032	0.9568	
C^2	2.31	1	2.31	8.85	0.031	significant
Residual	1.31	5	0.2616			
Lack of Fit	1.15	3	0.382	4.72	0.1797	notsignificant
PureError	0.1618	2	0.0809			
Total	32.98	14				
R^2=0.9603 Adj R^2=0.8890						

Table 2 shows that the linear effect of methanol and sample mass, the methanol% mass effect, and the square effect of sample mass and sample mass are important for DPPH. Lack of Conformity the F-value 4.72 means that the Lack of Conformity is not critical to the pure error. The DPPH antioxidant activity had an R^2 value of 0.9603. This means that 96.03% of the changes in the DPPH antioxidant activity model have been explained. Good regression value demonstrated the credibility of the model.

Table 3 ANOVA statisticsforthe TPC of kiwifruit

Source	Sum of Squares	df	MeanSquare	F-value	p-value	
Model	63468.75	3	21156.25	53.32	< 0.0001	significant
A-Methanol	78.13	1	78.13	0.1969	0.6658	
B-Ultrasound Time	378.13	1	378.13	0.953	0.3499	
C-Mass of Sample	63012.5	1	63012.5	158.81	< 0.0001	significant
Residual	4364.58	11	396.78			
Lack of Fit	4097.92	9	455.32	3.41	0.247	notsignificant
PureError	266.67	2	133.33			
Total	67833.33	14				
R^2=0.9357 Adj R^2=0.9181						

Table 3 states that TPC develops with a significant linear effect of the sample mass. The F value of 53.32 indicates that the model is important. However, a single variable model is not suitable because the only mass of sample is important. The model could not be established because only one variable was significant. The study of the determination of phenolic compounds in apple by (Jakobek et al., 2015), the highest total phenolic content was observed by using 60% and 80% concentration of methanol (599 and 604 mg kg^{-1} FW. respectively).

Table 4 ANOVA statisticsforthe ABTS of kiwifruit

Source	Sum of Squares	df	MeanSquare	F-value	p-value	
Model	3.15E+05	3	1.05E+05	53.14	< 0.0001	significant
A-Methanol	9871.11	1	9871.11	5	0.0471	significant
B-Ultrasound Time	1904.52	1	1904.52	0.9642	0.3472	
C-Mass of Sample	3.03E+05	1	3.03E+05	153.47	< 0.0001	significant
Residual	21728.47	11	1975.32			
Lack of Fit	21497.97	9	2388.66	20.73	0.0469	significant
PureError	230.5	2	115.25			
Total	3.37E+05	14				
R^2=0.9355 Adj R^2=0.9179						

Table 4 explained that ABTS was developed by the significant linear effect of% methanol and sample mass. 53.14 Model F value indicates that the model is important. However, the model is not reliable because the lack of fit is significant ($p<0.05$). Our ratio of 20.024 indicates a sufficient signal. DPPH % was frankly studied through the pictorial presentment in the form of the 3D graphic (Figure 1).

Figure 1a shows the DPPH % antioxidant activity of the combination of ultrasound time and methanol by response surface plot for the optimized geometry. This figure defined that to get the highest DPPH % value (88.3317%) at the mass of sample of 2 g. the values of control factors were found as ultrasound time=5 minutes and methanol=90%. In the optimization of bioactive compound extraction by microwave parameters from *Kappaphycus alvarezii* using RSM by (Baskararaj et al., 2019), the best solvent was

reported to be methanol and the optimum methanol concentration was 80%. Figure 1b shows a cubic arrangement of the mean responses in the function of factors methanol, ultrasound time, and mass of sample for DPPH %. This figure portrayed that the highest DPPH antioxidant activity (89.4852%) was found with methanol 90%, ultrasound time 5 min., and mass of sample 3 g.

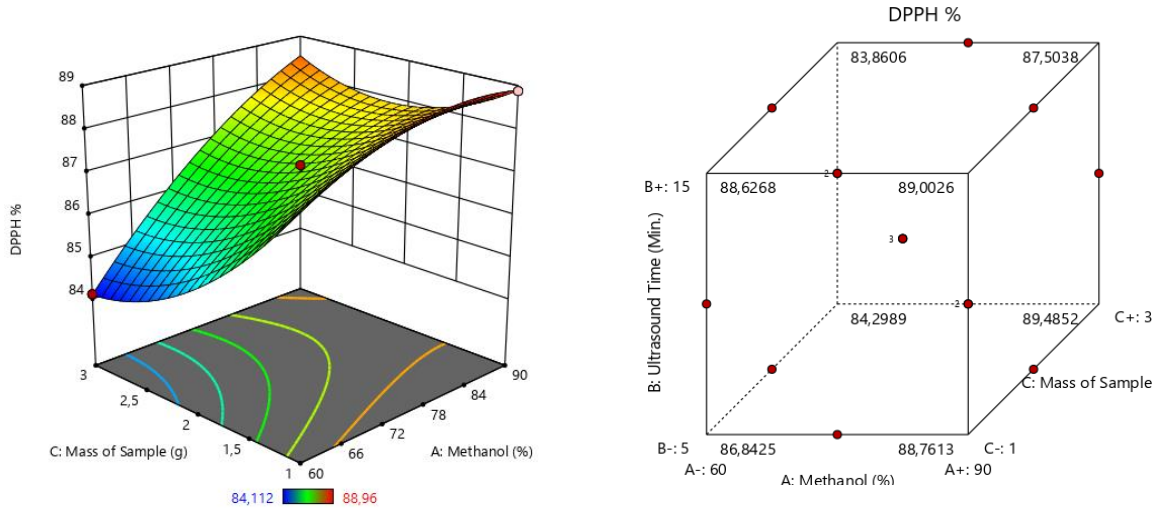

Figure 1 (a) Response surface for the effect of mass of sample (g) and methanol (%) on the DPPH % antioxidant activity at the ultrasound time at 10 min. (b) Cubic arrangement of the mean responses in function of factors methanol, ultrasound time and mass of sample for DPPH %.

The parameters applied in the study of RSM assisted extraction of bioactive contents in *Nephelium lappaceum L.* fruit peel by (Prakash Maran et al., 2017) are 50° C extraction temperature, 20 W ultrasound power, 20 minutes extraction time and 1: 18.6 g / mL solid-liquid rate. Results under that conditions, total anthocyanin (10.26 ±0.39 (mg/ 100 g)), phenolics (552.64 ± 1.57 (mg GAE/100 g)), and flavonoid (104± 1.13 (mg RE/100 g)) contents were found. In the research conducted by (Sharmila et al., 2019), response surface analysis estimated the optimal level of sonication power, flower mass, extraction time and methanol concentration at 30W, 2 g, 15 min., and 100% respectively for the maximum response of pigment absorbance (3.46), TPC (246.6 mg/g, and antioxidant activity (55.7%). In their study on the ultrasound-assisted extraction of trademarked teas by (Afroz Bakht et al., 2018), frequencies (26 kHz. 40 kHz), temperature (30. 40 and 50° C) and power (30%. 40 and 50%) were applied over a fixed period of 30 minutes. They reported that in both the ultrasonic frequencies, 40° C temperature, and 40% power combination exhibited the highest cumulative yield. In contrast to our study here, the ultrasonic bath assisted extraction process has worked.

CONCLUSIONS

In our study, the maximum yield conditions for the extraction of phenolic contents in the kiwifruit were determined using the RSM using BBD. Only the model could be installed for DPPH. The DPPH model was statistically highly significant ($P<0.01$). The model established for ABTS was not reliable because a lack of fit turned out to be important. The model could not be established in TPC because the model where a single variable is significant is not suitable. The order of importance of the three applications for DPPH was determined as follows: Methanol concentration > Mass of Sample > Ultrasound Time. The maximum phenolic efficiency for this process was found to be mass of sample= 3 g, methanol concentration: 90 %, and ultrasound

time: 7.71 min. Values were determined with the help of a response optimizer to maximize all of the DPPH, ABTS, and TPC responses.

CONFLICT OF INTEREST

The authors express no conflict of interest associated with this work.

AUTHOR CONTRIBUTIONS

CK designed the research. CK and RP conducted experiments of the research. CK and RP made statistical analyzes. CK wrote the article. All authors contributed the article and approved the submitted version.

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