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Extraction of Antioxidant Components by Microwave Assisted Homogenization from Artichoke Leaves

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Abstract: In this study, components having antioxidant properties were extracted by microwave assisted homogenization process from artichoke leaves which are considered as a food waste. Antioxidant capacity, total phenolic and total flavonoid content analyzes were applied to the obtained extracts. Extraction yields were determined by proportioning the antioxidant capacity of the extract obtained as a result of the microwave assisted homogenization processes to the those which were obtained by Soxhlet extraction. To achieve the highest extraction yield for the microwave assisted homogenization, the optimum conditions were determined using D-optimal design by 'desirability' function approach. According to the optimization results, the highest extraction yield was predicted as 95.91% with an application of 17.13 minutes of homogenization time, 180 W of microwave power and 11200 rpm of homogenization rate. According to the verification tests, there was no statistical difference between the experimental (96.08±0.83%) and the estimated data (95.91%) (P>0.05).

Key words: Artichoke leave, microwave assisted extraction, extraction yield, antioxidant capacity, optimization

Enginar Yapraklarından Mikrodalga Destekli Homojenizasyon ile Antioksidan Özelliğe Sahip Bileşenlerin Ekstraksiyonu

Özet: Bu çalışmada, mikrodalga destekli homojenizasyon işlemi ile atık olarak değerlendirilen enginar yapraklarından antioksidan özelliğe sahip bileşenler ekstrakte edilmiştir. Ekstraktlara antioksidan kapasite, toplam fenolik madde ve toplam flavonoid tayini analizleri uygulanmış ve ekstraksiyon verimi, uygulanan mikrodalga destekli homojenizasyon işlemi sonucunda elde edilen ekstraktın antioksidan kapasitesinin Soxhlet ekstraksiyon ile elde edilenlerin antioksidan kapasitesine oranlanması ile belirlenmiştir. Mikrodalga destekli homojenizasyonda en yüksek ekstraksiyon veriminin sağlanabilmesi için optimum koşullar D-optimal tasarım kullanılarak 'desirability' fonksiyonu yaklaşımı ile tespit edilmiştir. Elde edilen optimizasyon sonuçlarına göre, 17.13 dakika homojenizasyon süresi, 180 W mikrodalga gücü ve 11200 rpm homojenizasyon hızında en yüksek ekstraksiyon verimi %95.91 olarak tahminlenmiştir. Yapılan doğrulama testine göre deneysel veriler (%96.08±0.83) ile tahminlenen veriler (%95.91) arasında istatistiksel olarak farklılık bulunmamıştır (P>0.05).

Anahtar Kelimeler: Enginar yaprağı, mikrodalga destekli ekstraksiyon, ekstraksiyon verimi, antioksidan kapasite, optimizasyon

1. Introduction

The evaluation of food wastes is becoming increasingly important in terms of preventing environmental pollution and obtaining new products thereby create added value. Moreover, researching the presence of components having natural antioxidant properties in agricultural wastes, and releasing of these to the market after extraction processes are remarkable research subjects of today.

Artichoke (*Cynara scolymus* L.) is especially farmed in Mediterranean coasts of Europe and creates a significant proportion of waste after its processing (Biel et al. 2020). Artichoke is a vegetable with a height of 50- 150 cm from *Asteraceae* family and contains high-phenolic compound and inulin (Lattanzio et al. 2009, Salekzamani et al. 2019). The artichoke heart

part wrapped in the leaves is the industrial processed part of the plant and usually this part is consumed as food. The leaves, stems and root parts remaining as a result of the processing of the plant are considered as waste and these parts constitute 60-80% of the plant (Ergezer et al. 2018). It has been reported that artichoke has liver-protective, anticarcinogenic and cholesterol-reducing effects (Gebhardt and Fausel 1997). In addition, caffeic acid and apigenin, and luteolin flavonoids in the leaves show antioxidant activity (Lattanzio et al. 1994, Llorach et al. 2002). Since artichoke leaves are cheap and rich in important ingredients for health, extract of the leaves is thought to have a significant potential as a natural antioxidant source (Claus et al. 2015).

Microwave assisted extraction process has attracted attention as being one of the extraction techniques which reduces the extraction time and usage of the organic solvents when compared with the traditional methods (Routray and Orsat 2012, Ruiz-Aceituno et al. 2016). In microwave assisted extraction processes, microwave energy provides the heating of the solvent which present in the solution media and facilitates the penetration of the solvent into the sample tissue. Thus, the transition of the compound which desired to be extracted to the solvent occurs faster. Microwave assisted extraction processes might be substantial alternatives to the conventional solid-liquid extraction process for the extraction of phytochemicals. On the other hand, in microwave extraction processes, because of the variation of process parameters depending on the product, optimization of the conditions such as extraction time and applied microwave power is required (Li et al. 2012).

There are several studies in the literature where microwave assisted extraction is applied for different products. Bakić et al. (2019), in the study which they used microwave assisted extraction for the extraction of phenolic compounds from tomato peels, reported that they obtained a high amount of polyphenols (53.12 g kg^{-1}) in a short time such as 5 minutes. Pan et al. (2003) applied Soxhlet extraction, ultrasonic extraction and microwave assisted extraction processes for the extraction of polyphenol and caffeine from green tea leaves. When 4 minutes of extraction was applied, the highest extraction yield was obtained by microwave assisted extraction. Microwave assisted extraction process is a fairly new study subject for phenolic extraction from artichoke leaves (Mena-García et al. 2020). Even though there are several studies which determined the antioxidant capacity values of the artichoke leaf extracts (Gaafar and Salama 2013; Pandino et al. 2013; Zourro et al. 2014; Biel et al. 2020), to the best of our knowledge, there is no study in the literature which carried out for optimization of the process conditions.

In this study, extracts were obtained from the artichoke leaves by microwave assisted homogenization (MAH) and the optimization of the process parameters such as homogenization time (HT), microwave power (MP) and homogenization rate (HR) was carried out to achieve the maximum extraction yield using D-optimal design by 'desirability' function approach. The antioxidant capacity, total phenolic and total flavonoid content of the obtained extracts for all different conditions were determined.

2. Materials and Methods

2.1. Material

The leaves of the artichoke (*Cynara scolymus* L.) grown in Niksar/Tokat were used in the study. The leaves of the artichoke hearts (bracts) were dried using sun drying method and the moisture content was brought to <10%. Dried leaves were powdered using a blender (Sinbo SHB 3020, Turkey) and the samples under the sieve having a pore diameter of 630 µm were collected. The powder samples which are ready-to-use were stored at -18°C until analysis.

2.2. Determination of extraction conditions

MAH and Soxhlet extraction processes were applied for extraction of antioxidative components from the powdered artichoke

leaves. The ratio (w/v) of the sample and the solvent is determined as 3 g powder sample in 50 mL of solvent (distilled water or ethanol). Three different power levels (90, 180 and 360 W) were applied in a fixed time of 5 minutes in the MAH process using a household microwave oven (554 MD Arcelik, Turkey). Homogenization process was applied in three different rates (3600, 11200 and 20000 rpm) in the range of 5-25 minutes using a laboratory scale homogenizer (IKA Ultra-Turrax T-18 Basic, Germany). In addition, the temperature was kept constant at 25°C using a constructed ice bath assembly to prevent the samples from overheating. For MAH, distilled water used as the solvent and process variables were chosen as HT (min, X_1), MP (W, X_2) and HR (rpm, X_3) which will provide the maximum extraction yield and the optimum conditions determined using D-optimal design. The experimental design used in the study is shown in Table 1.

2.3. Determination of the extraction yield

The antioxidant capacity values of extracts obtained from extraction processes were compared to the antioxidant capacity value which was obtained by Soxhlet extraction, and extraction yields (%) were calculated for different conditions (Equation 1). Extraction yield was used as a response for the optimization.

$$\text{Extraction yield (\%)} = \frac{\text{Antioxidant capacity of the extracts obtained from MAH}}{\text{Antioxidant capacity of the extracts obtained from Soxhlet extraction}} \times 100 \qquad (1)$$

2.4. Soxhlet extraction

Traditional extraction (Soxhlet) method was used for the extraction of all phenolic compounds in powdered artichoke leaves. Three g of powder sample was weighed into the Soxhlet cartridge and extracted in a Soxhlet device with 200 mL ethanol for 24 hours. The extract containing ethanol was removed by a rotary evaporator (Buchi RE121 Rotavapor with Buchi 461 Water Bath, Germany) and then concentrated extract was recovered using 50 mL ethanol (same as the ratio used for the extraction processes, 3 g sample in 50 mL solvent) (See et al. 2016).

2.5. Analysis

In order to make the samples usable for the analysis after extraction, firstly the obtained suspensions were centrifuged at 9000 rpm for 5 minutes (Hettich EBA 21, Germany). After that, the supernatant phase was filtered using a coarse filter paper, and the filtrate was collected.

Determination of antioxidant capacity

DPPH solution (1.95 mL) at a concentration of 0.1 mM was added to 50 µL of extract. The samples were kept in dark for 30 minutes, then absorbance values were determined at 515 nm wavelength (PG Instruments T80, United Kingdom). The antioxidant capacities of the samples were expressed in mmol Trolox 100 g dry sample^{-1} (Brand-Williams et al. 1995). By application of Soxhlet extraction to the artichoke leaves, the antioxidant capacity value was calculated as 318.69 ± 2.89 mM trolox 100 g dry sample^{-1}.

Determination of total phenolic content

After mixing 250 µL of extract and 250 µL of 1:1 diluted Folin-Ciocalteau solution, 500 µL of Na_2CO_3 (210 g L^{-1}) solution and 4 mL of distilled water were added to the mixture. The samples which were kept at room temperature and in the dark for 25 minutes were subjected to 3800 rpm of centrifugation for 10 minutes. Total phenolic content was expressed in gallic acid equivalent (mg gallic acid 100 g dry sample^{-1}) after reading the absorbances of the supernatant phases at 725 nm wavelength (Claus et al. 2015). As a result of Soxhlet extraction, the total phenolic content of artichoke leaf powder was calculated as 1639.33 ± 18.86 mg gallic acid 100 g dry sample^{-1}.

Determination of total flavonoid content

The total flavonoid content of the samples was determined spectrophotometrically using aluminum chloride method. Four mL of pure water, 1 mL of extract and 0.3 mL of $NaNO_2$ (5%) were mixed, and the mixture was left incubation for 5 minutes. At the end of the incubation, 0.3 mL of $AlCl_3$ (10%) solution was added and the final mixture was left for 6 minutes of incubation. Then, 2 mL of NaOH (1 M) solution was added, and the total volume was completed to 10 mL using distilled water. The absorbance values of the samples were read at 510 nm and the total flavonoid content was calculated in terms of mg quercetin in 100 g dry sample (Gaafar and Salama 2013). Total flavonoid content of the artichoke leaf powder was calculated as 1522.27 ± 10.29 mg quercetin 100 g dry sample^{-1} by Soxhlet extraction.

2.6. Statistical Analysis

Statistical analyzes were carried out using SPSS 22.0 and Design Expert 7.0 package programs. The t-test was applied for determination of the difference between predicted and experimental values, and the Pearson coefficients were determined using SPSS 22.0 package program. The regression analysis and optimization study were performed using Design Expert 7.0 - Ease Inc., USA. Effects of the process variables on the extraction yield were investigated and the process was optimized according to the 'desirability' function approach to ensure the maximum extraction yield. According to the mathematical model, significant terms in the model for extraction yield were determined by variance analysis. For regression analysis of the extraction yield as a response, the model has been created as given in Equation (2).

$$\text{Extraction yield (\%)}=\beta_0+\sum_{i=1}^{k}\beta_i X_i+\sum_{i=1}^{k}\beta_i X_i^2+\sum_{i=1}^{k-1}\sum_{j=i+1}^{k}\beta_{ij}X_{ij} \qquad k=1, 2, 3 \qquad (2)$$

where, β represents the regression coefficient.

3. Results and Discussion

The extraction yields, total phenolic and total flavonoid contents obtained according to the D-Optimal design applied for the MAH process are given in Table 1. According to the results, it was determined that there was a positive correlation between the extraction yields and total phenolic contents with a correlation coefficient of 0.999. Also, a positive correlation having a correlation coefficient of 0.998 was observed between extraction yields and total flavonoid contents ($P<0.05$).

ANOVA table showing the effects of HT, MP and HR on the extraction yield was created according to the D-Optimal design applied for the MAH process and was given in Table 2. According to the data, the quadratic model obtained for the extraction yield is statistically significant ($P<0.01$) and the lack of fit is statistically insignificant at the 95% confidence level ($P>0.05$). Linear effects of HT, MP, HR, HT-MP interaction, HT-HR interaction, MP-HR interaction and the quadratic effect of HT have significant effects on the model response ($P>0.05$) (Table 2)

To understand what extent the obtained model for the extraction process by MAH meets the experimental data, R^2, adjusted R^2 (adj-R^2), adequate precision, predicted residual error sum of squares (PRESS) and coefficient of variation C.V. (%) were determined (Table 3). Results showed that, the obtained model was suitable to predict extraction yield values ($R^2>0.99$). Moreover, the values of R^2 and adj-R^2 are very close to each other (<0.3%) which reveals that the model does not contain statistically insignificant terms (Table 3) (Isleroglu et al. 2019).

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Table 1. Extraction yields, total phenolic and total flavonoid contents for extraction processes carried out by microwave assisted homogenization

Çizelge 1. *Mikrodalga destekli homojenizasyon ile ekstraksiyon işleminde ektraksiyon verimleri, toplam fenolik madde ve toplam flavonoid içerikleri*

HT (min) (X_1)	MP (W) (X_2)	HR (rpm) (X_3)	Extraction yield (%)	Total Phenolic Content (mg gallic acid/100 g dry sample)	Total Flavonoid Content (mg quercetin/100 g dry sample)
10	90	3600	31.95	536.87 (±5.84)	515.19 (±2.06)
25	90	3600	45.21	771.21 (±1.80)	732.03 (±4.12)
25	90	3600	46.68	777.56 (±0.90)	733.49 (±14.41)
5	90	11200	73.70	1227.18 (±11.68)	1152.62 (±6.17)
18.33	90	11200	87.15	1440.55 (±6.29)	1350.55 (±14.41)
5	90	20000	85.23	1378.32 (±3.59)	1302.52 (±16.47)
15	90	20000	84.08	1399.28 (±7.63)	1308.34 (±12.35)
25	90	20000	79.88	1335.14 (±1.80)	1266.14 (±18.52)
5	180	3600	34.69	576.88 (±8.08)	548.66 (±16.47)
20	180	3600	51.93	867.10 (±1.35)	819.35 (±32.93)
5	180	11200	86.97	1429.76 (±6.74)	1343.27 (±20.58)
15	180	11200	97.03	1594.87 (±2.25)	1494.62 (±8.23)
25	180	11200	91.91	1507.87 (±1.80)	1402.94 (±34.99)
5	180	20000	89.80	1486.91 (±1.35)	1392.75 (±16.47)
5	180	20000	89.21	1481.20 (±6.29)	1385.47 (±22.64)
25	180	20000	78.37	1314.81 (±1.80)	1239.94 (±22.65)
25	180	20000	76.63	1278.62 (±4.04)	1202.10 (±30.87)
5	360	3600	31.99	542.59 (±10.78)	521.01 (±7.34)
20	360	3600	44.34	743.90 (±6.74)	708.75 (±4.12)
20	360	3600	45.62	761.05 (±1.80)	717.48 (±16.47)
11.67	360	11200	77.09	1267.18 (±1.35)	1199.19 (±2.06)
11.67	360	11200	75.99	1264.01 (±5.39)	1184.64 (±30.87)
25	360	11200	70.18	1159.23 (±5.84)	1087.13 (±28.81)
5	360	20000	78.14	1294.49 (±3.59)	1203.56 (±12.35)
18.33	360	20000	72.29	1195.42 (±1.80)	1123.51 (±6.17)

HT: Homogenization time (min), **MP:** Microwave power (W), **HR**: Homogenization rate (rpm)
HT*: Homojenizasyon süresi (dak),* ***MP****: Mikrodalga gücü (W),* ***HR****: Homojenizasyon hızı (rpm)*

Table 2. ANOVA table representing the effects of linear, quadratic and interaction terms on the extraction yield

Çizelge 2*. Ekstraksiyon verimi üzerine lineer, kuadratik ve interaksiyon terimlerinin etkisini gösteren ANOVA tablosu*

Source	DF	Sum of Squares	F Value	*p* - Value
Model	14	10200.77	384.45	<0.0001
X_1	1	14.72	7.77	0.0192
X_2	2	560.12	147.77	<0.0001
X_3	2	8132.97	2145.64	<0.0001
X_1X_2	2	76.84	20.27	0.0003
X_1X_3	2	532.51	140.49	<0.0001
X_2X_3	4	247.00	32.58	<0.0001
X_1^2	1	106.13	56.00	<0.0001
Residual	10	18.95		
Lack of fit	5	14.77	3.53	0.0961
Pure error	5	4.18		
Total	24	10219.72		

X_1: Homogenization time (min), **X_2**: Microwave power (W), **X_3**: Homogenization rate (rpm), DF: Degree of freedom
X_1*: Homojenizasyon süresi (dak),* ***X_2****: Mikrodalga gücü (W),* ***X_3****: Homojenizasyon hızı (rpm), DF: Serbestlik derecesi*

Table 3. Statistical parameters for the extraction model

Çizelge 3. *Ekstraksiyon modeli için elde edilen istatistiksel parametreler*

Parameter	Extraction yield
R^2	0.9981
adj-R^2	0.9955
Adequate precision	59.806
PRESS	110.18
C.V. (%)	1.99

adj- R^2: Adjusted R^2, **PRESS**: Predicted residual error sum of squares, **C.V. (%)**: Coefficient of variation
adj-R^2*: Düzeltilmiş* R^2, ***PRESS****: Tahminlenmiş kalıntı hata kareler toplamı,* ***C.V. (%)****: Varyasyon katsayısı*

The second order polynomial model obtained for the extraction process with MAH and used for optimization given by Equation (3). To support the model, the interaction graphics showing the effects of process parameters on the extraction yield which ensures the success of the study and the relationship between predicted and experimental values are shown in Figure 1.

When Figure 1 was investigated, the lowest extraction yield values were seen at 3600 rpm HR (Figure 1a). On the other hand, the highest extraction yield values were obtained at 11200 rpm HR with an application of 180 W MP (Figure 1b). Moreover, for the HR of 11200 and 20000 rpm, the extraction yield values of 360 W MP were lower than those of 90 and 180 W. For 20000 rpm of HR, when HT was increased, extraction yields were decreased (Figure 1c). The relation between predicted and experimental values was shown in Figure 1d. According to the results, the linear equation of **y=1.0046x-1.1443** was obtained when the predicted extraction yield values (y-axis) and the experimental extraction yield values (x-axis) were plotted. The linear equation showed that predicted and experimental values of extraction yield are very close to each other proving that the model is appropriate.

$$\text{Extraction yield (\%)} = +71.19+1.91X_1-0.67X_{21}+6.69X_{22}-27.28X_{31}+14.76X_{32}+3.26X_1X_{21}-0.20X_1X_{22} +6.99X_1X_{31}+0.66X_1X_{32}-3.89X_{21}X_{31}-1.65X_{22}X_{31}+0.17X_{21}X_{32}+3.02X_{22}X_{32}-5.54X_1^2 \quad (3)$$

On the study of extraction by MAH, 12 solutions were calculated according to the numerical optimization which determined the optimum point. Among these solutions, the solution having the highest 'desirability' value (0.983) was chosen as the optimum point. The extraction yield was calculated as 95.91% at the optimum point which was having 17.13 minutes of HT, 180 W of MP and 11200 rpm of HR. After determination of the optimum point, validation tests were applied, and the experimental extraction yield was calculated as 96.08±0.83%. It was determined that there was no statistical difference between the predicted and experimental extraction yields (P>0.05).

The total phenolic contents of the obtained extracts by MAH are shown in Table 1. The total phenolic contents were ranged between 537 and 1595 mg gallic acid 100 g dry sample^{-1} and a positive correlation was observed between the total phenolic content and extraction yield. At the optimum point which was determined as 17.13 minutes of HT, 180 W of MP and 11200 rpm of HR the total phenolic content of the extract was determined as 1604.41±22.16 mg gallic acid 100 g dry sample^{-1}. The total phenolic content of the extract obtained by MAH at the optimum extraction yield in our study is higher than the extracts obtained from the outer leaves of the artichoke by classical extraction method (using 80% ethanol, extraction at 40°C for 4 hours, phenolic content of 4.39±0.81 mg gallic acid 100 g dry sample^{-1}) in the study of Ergezer and Serdaroğlu (2018). On the other hand, Gaafar and Salama (2013) were determined the total phenolic content of the artichoke leaves as 1836 mg gallic acid 100 g dry sample^{-1} using 80% ethanol as solvent and classical extraction method. According to the results of our study and the studies found in literature, total phenolic content of the artichoke leaves shows variations due to the factors such as genetic diversity and harvesting time (Ergezer and Serdaroğlu 2018). The total flavonoid contents of the obtained extracts by MAH are also shown in Table 1 and it was observed that there was a high correlation between total flavonoid content and extraction yield (correlation coefficient of 0.998), and total flavonoid content and total phenolic content (correlation coefficient of 0.999). At the optimum point the total flavonoid content of the optimum MAH extract was 1502.64±34.55 mg quercetin 100 g dry sample^{-1} which was in consistence with the results of Gaafar and Salama (2013).

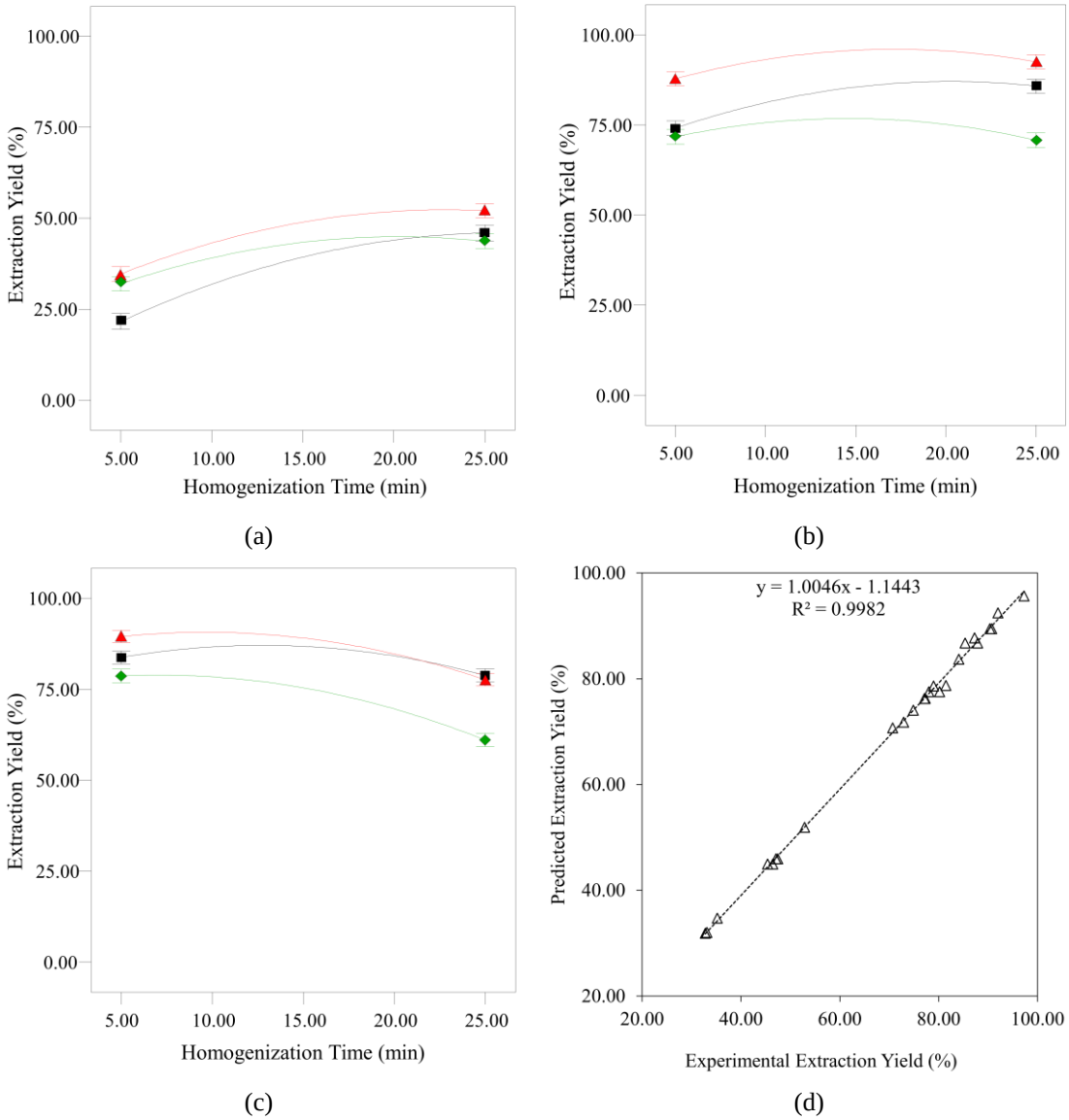

Figure 1. Effects of the process parameters on extraction yields at (a) 3600 rpm, (b) 11200 rpm and (c) 20000 rpm of homogenization rates (■: 90 W, ▲: 180 W and ◆: 360 W microwave power) and (d) relationship between experimental and predicted extraction yields

Şekil 1. *Proses parametrelerinin ekstraksiyon verimine etkisi (a) 3600 rpm, (b) 11200 rpm ve (c) 20000 rpm homojenizasyon hızı (■: 90 W, ▲: 180 W ve ◆: 360 W mikrodalga gücü) ve (d) deneysel ve tahminlenen ekstraksiyon verimleri arasındaki ilişki*

4. Conclusions

In this study, extracts having antioxidant properties were obtained using MAH process and the process parameters were optimized to obtain extracts having the highest antioxidant activity. According to the results, extracts having high antioxidant capacity were obtained at short times and at the room temperature by the application of MAH. Hence, this study revealed the evaluation of a waste product which can be used as a natural antioxidant source by novel extraction techniques. It is a drawback for the food industry to operate microwave assisted extraction systems because of their costs, but it is thought that the application of MAH process to the artichoke leaves can be advantageous in the long term because of the short extraction time and high yield of antioxidative compounds.

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