

Scientific paper

Optimization of Extraction Conditions of Bioactive Compounds by Ultrasonic-Assisted Extraction from Artichoke Wastes

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Abstract

In this study, bioactive compounds were extracted by ultrasonic-assisted extraction and classical extraction processes using distilled water as solvent from artichoke leaves which are considered as agricultural wastes. Antioxidant capacity, total phenolic and total flavonoid content values of the obtained bioactive extracts were determined, and extraction yields and times were evaluated to compare the extraction processes. Also, the optimum extraction conditions of ultrasonic-assisted extraction (extraction time and ultrasonic power) which provide the highest extraction yield were determined using D-optimal design by 'desirability' function approach. According to the results, bioactive extracts having high antioxidant capacity were obtained at shorter times and higher extraction yields were achieved by ultrasonic-assisted extraction process than classical extraction. The highest extraction yield was estimated as 98.46% with an application of 20.05 minutes of extraction time and 65.02% of ultrasonic amplitude for the ultrasonic-assisted extraction process.

Keywords: Artichoke, ultrasonic-assisted extraction, extraction yield, optimization, bioactive compounds

1. Introduction

One of the most important problems in the food industry is the management of waste produced during food processing. Especially in recent years, the increase in the world population and food consumption cause the formation of a large amount of waste products. The fruit and vegetable processing industry are currently concerned with the utilization of wastes (leaves, roots and water released after washing). Waste products obtained as a result of industrial processing of agricultural products may have rich natural antioxidant content. In general, this antioxidative effect is related with the chemical differentiations of phenolic compounds of these waste materials contain.¹ It is known that some plants have antimicrobial and antioxidant properties, and the production of extracts with antimicrobial and antioxidant properties from byproducts and wastes obtained during the production and processing of these plants are becoming increasingly important today. It is generally thought that the hydroxyl groups possessed by these extracts containing phenolic compounds are responsible for the antioxidant and antimicrobial properties.¹⁻² One of the products that has gained popularity in Turkey in recent years is artichoke (Cynara scolymus L.). Because of its rich content, artichoke and parts of the artichoke plant attract the attention of the food industry and health-oriented consumers.3 It is known that artichoke wastes constitute 60-80% of the total plant. In the food industry, artichoke wastes are used in the production of herbal food supplements and dietary fiber. In addition, it is thought that artichoke leaves can be used as a natural additive with antioxidant and antimicrobial effects due to their high phenolic content. In literature, the liver-protective properties, anticarcinogenic effects and cholesterol-lowering effects of artichokes were presented.⁵ It has also been reported that artichoke is a good source of antioxidants due to the significant amount of caffeic acid it contains. It is known that caffeic acid derivatives are the main phenolic substances found in the heart of artichokes. In addition, flavonoids such as apigenin and luteolin are found in artichoke and the leaves of artichokes as other phenolic compounds having antioxidant activity.6-7

Compounds with antioxidant properties have an important effect in delaying the oxidation of substrates. The strong effects of powerful but synthetic antioxidant substances such as BHT [2,6-bis (1,1-dimethylethyl) -4-methylphenol] used in the food industry and their negative effects on human health have been determined by some studies.⁸⁻⁹ The fact that consumers consider the components harmful to health and avoid the consumption of products having such synthetic additives accelerated the search of the food industry for natural and cheap additives suitable for use in foods. It is thought that extracts that can be an alternative to synthetic antioxidant substances can be obtained from a product such as artichoke which produces a high rate of waste and can be grown in terms of climate in Turkey. Being cheap and having high antioxidant activity, artichoke wastes may create an important potential in Turkey.¹⁰

The extraction process is based on the principle of obtaining the target components from the material with the highest efficiency and with the least damage to the target component. Conventional extraction methods used for the extraction of bioactive materials can be listed as classical extraction (directly treating the material with the solvent and mixing), decoction extraction, solvent extraction (liquid-liquid extraction) and steam distillation.¹¹ High pressure process, high hydrostatic pressure extraction and pulsed electric field processes can also be considered as conventional extraction methods. 12-14 These methods are frequently used for extraction of bioactive materials from plant materials and waste products. However, excessive solvent consumption and long extraction time are the main challenges of conventional extraction methods. 15 Solvents such as chloroform, chlorobenzene, acetone, ethanol, methanol and acetonitrile are generally used in these techniques. However, the toxic properties of the solvents and their residue in the target components made it necessary to develop environmentally friendly extraction techniques. In order to shorten the extraction time, increase the extraction yield and reduce the solvent usage novel extraction techniques are taking interest in the food industry. Moreover, it is vital to determine the suitable extraction method of the bioactive compounds from plants in terms of extraction yield.16

Most of the industrial applications have tended towards green technologies. Hence, the techniques for the extraction of polyphenols from food wastes should also be innovative and environmentally friendly. Microwave extraction, supercritical fluid extraction and ultrasonic-assisted extraction (UAE) are the most frequently used green extraction techniques recently. UAE has green impacts on the extraction process of bioactive compounds in terms of yield and short processing times when compared with classical extraction (CE) methods and has frequently been the subject of the literature due to its ease of use, portability and lower cost compared to other innovative techniques. ^{17–19} UAE has a lot of advantages when compared

with conventional extraction techniques such as higher extraction yield, short extraction time, lower extraction temperature and reduced usage of the solvent. Moreover, less number of structural and molecular changes of the material occur by the usage of UAE. 20,21 UAE is a developing extraction technology which can be suitable for scaling up. Patist et al.²² reported that ultrasonic applications in the food industry may be profitable when input and output costs were considered. Industrial scale UAE devices are being produced by companies such as REUS (France) and Hielscher (Germany).¹¹ Nevertheless, in literature, the studies involving the application of large-scale UAE devices are very rare. Because, while some process parameters can be the same when scale up is done such as solvent type and solvent material ratio and temperature, other process parameters like power and frequency of the ultrasonic device may differ due to the nonlinear nature of the process. However, in order to avoid this challenge, multi-mode devices which can ensure more intense cavitation have been designed by researchers^{11,23} and these studies can be very useful in the future for UAE process of the bioactive materials.

UAE is successfully applied for different kind of food products and industrial wastes in order to obtain bioactive materials. 11,21,24 In the UAE, the parameters affecting the process are mainly ultrasonic power, ultrasonic intensity or amplitude, duty cycle (the ratio of pulse duration and cycle time), solvent type, solvent to solid ratio, extraction time and extraction temperature. 1 In general, low power and high frequency ultrasound have been applied at UAE processes for food materials and wastes. Even though having substantial advantages over traditional extraction techniques, the success of UAE is mostly dependent to the optimization process. Optimization of the UAE process can ensure increased extraction rate and can prevent solvent wastage. 24

The study was aimed to show the effect of a green technology on the extraction of bioactive compounds from an agricultural waste and to make a comparison between the CE and UAE. Antioxidant capacity, total phenolic and total flavonoid contents of the obtained bioactive extracts from artichoke leaves at the different process conditions were determined. The process parameters which are extraction time (ET) and ultrasonic amplitude (UA) were investigated using D-optimal design by desirability function approach. Antioxidant capacity, total phenolic and total flavonoid contents of the obtained extracts at the different process conditions were determined. Also, CE was compared with UAE process in terms of extraction time and extraction yield.

2. Experimental

2. 1. Material

In the study, the leaves of the artichoke (*Cynara scolymus* L.) hearts were used which were grown in Tokat/ Turkey. The bracts were dried by sun drying method until

their moisture content were below 10%. After drying, dry leaves were powdered by a rotary blender (Sinbo SHB 3020, Turkey). Following to the sieving process using a sieve having 630 μ m pore diameters, the samples under the sieve were collected. Ready-to-use powdered samples were stored at –18 °C until analysis.

2. 2. Classical Extraction Processes

Powdered samples were mixed with distilled water using a magnetic stirrer for a period of 120-1440 minutes. The ratio (w v^{-1}) of the sample and the distilled water was applied as 3 g powder sample in 50 mL distilled water. Analyzes were carried out for the samples mixed for different durations (Table 1).

2. 3. Ultrasonic-Assisted Extraction Processes

For UAE process, distilled water was used as solvent and the ratio of powder sample to distilled water was 3 g 50 mL⁻¹ as it was done in CE process. UAE process was carried out using a laboratory scale sonicator (Q Sonica Q 500, 500 W, 20 kHz, ABD) having a 13 mm diameter probe. In order to prevent overheating of probe and samples, the a value was determined as 0.8. a value was calculated as $\alpha = t_{open}/(t_{open}+t_{closed})$. Here, t_{closed} indicates the time (s) that sonication is active, and indicates the time (s) that sonication is passive.²⁶ The optimum condition which ensured the highest extraction yield was determined using D-Optimal design. Independent process variables were selected as ET (min) (X_1) and UA (%) (X_2) and the limits of the process variables were applied in the range of 20-60 minutes and 30-80%, respectively. Moreover, the extraction temperature was kept constant at ~ 25 °C using a constructed ice bath apparatus to prevent the samples from overheating during extraction process.

2. 4. Soxhlet Extraction

To determine all of the phenolic compounds from powdered artichoke leaves, Soxhlet extraction method was used. Three grams of sample was weighed into a Soxhlet cartridge and extraction was carried out in a Soxhlet device using 200 mL of ethanol for 24 hours. The ethanol which contained the bioactive extract was evaporated using a rotary evaporator and after that concentrated extract was recovered using 50 mL ethanol (same as the ratio used for the extraction processes, 3 g sample in 50 mL solvent).²⁷

2. 5. Determination of the Extraction Yield

The antioxidant capacity values of bioactive extracts obtained by UAE processes were compared to the antioxi-

dant 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.²⁷

2. 6. Analysis

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.

2. 6. 1. Determination of Antioxidant Capacity

1.95 mL of DPPH solution at a concentration of 0.1 mM was mixed with 50 μL of extract. The absorbance values of the samples which were kept in dark for 30 minutes were determined at 515 nm wavelength (PG Instruments T80, United Kingdom). The antioxidant capacities of the samples were expressed in mM trolox 100 g dry sample $^{-1}.^{28}$ By application of Soxhlet extraction to the artichoke leaves, the antioxidant capacity value was calculated as 318.69 \pm 2.89 mM trolox 100 g dry sample $^{-1}$.

2. 6. 2. Determination of Total Phenolic Content

Total phenolic contents of the samples were determined using Folin-Ciocalteau method. Total phenolic content was expressed in gallic acid equivalent (mg gallic acid 100 g dry sample⁻¹) after reading the absorbances of the samples at 725 nm wavelength. ¹⁵ As a result of Soxhlet extraction, the total phenolic content of artichoke leaves powder was calculated as 1639.33 ± 18.86 mg gallic acid 100 g dry sample⁻¹.

2. 6. 3. Determination of Total Flavonoid Content

The total flavonoid content of the samples was determined spectrophotometrically using aluminum chloride method. 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. Total flavonoid content of the artichoke leaves powder was calculated as 1522.27 \pm 10.29 mg quercetin 100 g dry sample by Soxhlet extraction.

2. 7. Statistical Analysis

One-sample t-test, comparison of the analysis results of the samples and determination of the Pearson coefficients were carried out using SPSS 22.0 (IBM, USA) package program. The regression analysis which was used to

Extraction yield (%) = $\frac{\text{Antioxidant capacity of the extracts obtained by CE or UAE}}{\text{Antioxidant capacity of the extracts obtained by Soxhlet extraction}} \times 100$

(1)

determine the effects of the independent process variables on the extraction yield, response surface graph and optimization study was done using Design Expert 7.0 (Stat-Ease Inc., USA) package program. For the UAE process, 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.

3. Results and Discussion

TThe results obtained by the CE process are given in Table 1. Extraction yields, antioxidant capacity values, total phenolic and total flavonoid contents of the samples mixed with magnetic stirrer for different periods were determined. It was determined that as the ET increased, the extraction yield increased up to the 22nd hour and there was no increase for the extraction yield at the 24th hour (p < 0.05) (Table 1). When the results for all analyzes were examined, it was found that there was an approximately 4-fold difference between the 2nd hour and 24th hour of ET. It is thought that the reason why the values obtained by Soxhlet extraction cannot be reached in the CE process is that the process takes place at room temperature and the magnetic stirring process cannot be effective enough to reveal some of the antioxidant compounds from the cells. In addition, since only pure water is used as solvent in the CE process and the mechanical effect is insufficient, the extraction yield could not reach the values higher than 79%. In the study, it is seen that the extraction yield increased with the increase in total phenolic and total flavonoid contents (Table 1). It was determined that there is a

positive correlation between extraction yield-total phenolic content and extraction yield-total flavonoid content and the correlation coefficients were calculated as 0.998 and 0.997, respectively (p < 0.05).

The extraction yields, antioxidant capacity values, total phenolic and total flavonoid contents obtained according to the D-Optimal design applied for the UAE process are shown in Table 2. Similar to the CE process, there is a positive correlation between extraction yield-total phenolic content and extraction yield-total flavonoid content of bioactive extracts, and the correlation coefficients were determined as 0.996 and 0.986, respectively (p < 0.05). Same results were obtained in literature by several researchers. Lou et al.³⁰ reported that there was a positive correlation between antioxidant activity and total phenolic content of the kumquat extracts. Likewise, Chlopicka et al.31 revealed that DPPH and total phenolic compounds of breads showed significant and positive correlation. Ibrahimi and Hajdari³² studied the flavonoid content and antioxidant activity of honey and they reported that the flavonoid content and antioxidant activity values were highly correlated (Pearson correlation coefficient of 0.881).

According to the design, the extraction yield of 37% even at the lowest ET and UA value shows the positive effect of the ultrasonication process. While the extraction yield obtained in the CE process in 2 hours was 17%, in the UAE process, two times higher extraction yield was obtained at the lowest UA value (30%) and in six times shorter ET. UAE process showed better results at shorter ET when compared with CE. This phenomenon was explained with the effect of cavitation bubbles created by ultrasound on the tissue of the sample and made it easier to release phenolic compounds present in the cells by breaking down the cell walls.³³ In a recent study, Stumpf et al.³⁴ optimized the extraction procedure for determination of phenolic ac-

Table 1. Extraction yield, antioxidant capacity, total phenolic and total flavonoid contents for CE processes

ET (min)	Extraction yield (%)	Antioxidant Capacity (mM trolox 100 g dry sample ⁻¹)	Total Phenolic Content (mg gallic acid 100 g dry sample ⁻¹)	Total Flavonoid Content (mg quercetin 100 g dry sample ⁻¹)
120	16.77 ± 2.98^{k}	53.43 ± 9.48^{k}	307.30 ± 3.14^{k}	360.92 ± 14.41^{j}
240	23.26 ± 0.91^{j}	74.13 ± 2.89^{j}	443.52 ± 3.59^{j}	426.41 ± 16.47^{i}
360	28.79 ± 0.32^{i}	91.76 ± 1.03^{i}	534.97 ± 7.18^{i}	518.10 ± 2.06^{h}
480	39.95 ± 0.58^{h}	127.33 ± 1.86^{h}	678.49 ± 5.39^{h}	668.00 ± 24.70^{g}
600	44.75 ± 0.26^{g}	142.63 ± 0.82^{g}	807.41 ± 2.69^{g}	$800.43 \pm 18.52^{\rm f}$
720	$50.93 \pm 0.58^{\rm f}$	$162.31 \pm 1.86^{\rm f}$	$880.12 \pm 2.25^{\rm f}$	929.96 ± 12.35^{e}
840	61.31 ± 0.78^{e}	195.39 ± 2.47^{e}	1063.65 ± 4.94^{e}	1014.36 ± 4.12^{d}
960	67.48 ± 0.84^{d}	215.07 ± 2.68^{d}	1124.62 ± 2.25^{d}	1126.43 ± 14.41^{c}
1080	73.06 ± 0.45^{c}	232.85 ± 1.44^{c}	1179.86 ± 1.35^{c}	1178.82 ± 10.29^{b}
1200	75.81 ± 0.06^{b}	241.59 ± 0.21^{b}	1250.36 ± 3.14^{b}	1257.40 ± 6.17^{a}
1320	78.14 ± 0.39^{a}	249.03 ± 1.24^{a}	1271.31 ± 2.25^{a}	1263.23 ± 10.29^{a}
1440	78.46 ± 0.45^{a}	250.05 ± 1.44^{a}	1278.93 ± 2.25^{a}	1264.68 ± 16.47^{a}

ET: Extraction time (min)

⁽a-k) Means with uncommon superscripts within a column are significantly different (p < 0.05).

ids and flavonoids in artichoke leaves. They reported that UAE proved to be more effective than the standard protocol of European Pharmacopoeia (Ph. Eur.) and UAE method can be recommended to be as the standard protocol in the long term. Similarly, Carrera et al. 35 used UAE and CE processes to extract phenolic compounds from grapes and compared the methods in terms of total phenolic content of samples. In the UAE process, it was reported that 8 mg g⁻¹ grape of phenolic compounds were extracted in 6 minutes of application, and 6.4 mg g⁻¹ grape of phenolic compounds were extracted in 60 minutes in the CE process. Considering the simplicity and high efficiency of the method, it has been demonstrated that UAE is more effective than CE. When our data are examined, it is seen that the extraction yield increases as the ET increases at low UA values. On the other hand, it was determined that the extraction yield decreases with the increase of the ET, especially at 68% and 80% UA values. Very high amplitude values may cause agitation of the solvent rather than cavitation and it is important to optimize amplitude value in UAE processes.¹¹ Moreover, this can be explained by the fact that high-level sonication partially degrades the antioxidant-effective components as the ET increases.³⁶

The total phenolic and total flavonoid contents of the obtained extracts by UAE process are shown in Table 2. At the optimum point which was determined as 20.05 minutes of ET and 65.02% of UA, the total phenolic content was determined as 1601.79 \pm 12.11 mg gallic acid 100 g dry sample $^{-1}$ and the total flavonoid content was 1515.57 \pm

4.51 mg quercetin 100 g dry sample⁻¹. In a study, total phenolic content of artichoke leaves was determined as 4.39 \pm 0.81 mg gallic acid 100 g dry sample⁻¹ in 4 hours at 40 °C by using 80% ethanol with CE method.⁵ Another study of Gouveia and Castilho³⁷, in which they used UAE of 35 kHz and 200 W for 60 min at room temperature, revealed the total phenolic content of methanolic extract of artichoke leaves as 233.6 mg gallic acid 100 g dry sample⁻¹. On the other hand, in a different study in which the CE process was used, the total phenolic content of artichoke leaves was determined as 1836 mg gallic acid 100 g dry sample⁻¹.²⁹ In a recent study, Rudić et al.³⁸ valorized the artichoke leaves dust, which were obtained after industrial processing of tea blends by using microwave assisted extraction of polyphenols. They reported that the total flavonoid content at the optimum point was 7975 ± 112 mg quercetin 100 g dry sample⁻¹. Antioxidant capacity, total phenolic and total flavonoid content of the artichoke plant can vary depending on the artichoke species and its different organs. Kollia et al.33 studied the antioxidant activity of different artichoke species using UAE and CE and they revealed that cardoon's head extract of the cardoon and globe artichoke had the highest antioxidant activity when compared with the leaves and stems of these different species. On the other hand, Wang et al.³⁹ analyzed the antioxidative phenolic compounds present in the C. scolymus L and it was observed that the leaves had the highest total phenolic compounds when compared with artichoke hearts. Likewise, Falleh et al. 40 pointed that leaves of the

Table 2. Extraction yield, antioxidant capacity, total phenolic and total flavonoid contents for UAE processes

ET (min) (X ₁)	UA (%) (X ₂)	Extraction yield (%)	Antioxidant Capacity (mM trolox 100 g dry sample ⁻¹)	Total Phenolic Content (mg gallic acid 100 g dry sample ⁻¹)	Total Flavonoid Content (mg quercetin 100 g dry sample ⁻¹)
20	30	36.58	126.60 ± 2.89 ^k	599.74 ± 2.69^{kl}	774.24 ± 14.41 ^f
20	30	37.01	121.20 ± 0.62^{l}	606.73 ± 7.63^{k}	702.92 ± 4.12^{g}
20	30	35.81	123.97 ± 1.24^{kl}	587.04 ± 6.29^{l}	628.70 ± 14.42^{h}
40	30	40.65	$140.15 \pm 1.44^{\text{j}}$	666.42 ± 4.94^{j}	678.18 ± 6.17^{gh}
60	30	46.58	144.67 ± 0.82^{i}	$763.59 \pm 7.18^{\rm h}$	$804.80 \pm 37.05^{\rm f}$
60	30	45.03	139.13 ± 1.24^{j}	738.19 ± 3.59^{i}	$775.69 \pm 57.63^{\rm f}$
40	43	67.54	$221.04 \pm 1.65^{\rm f}$	$1107.15 \pm 4.94^{\rm f}$	1107.51 ± 12.35^{e}
20	55	86.02	267.83 ± 1.44^{e}	1410.07 ± 4.49^{c}	1363.64 ± 16.47^{c}
40	55	91.59	302.95 ± 1.24^{bc}	1501.52 ± 2.68^{b}	1436.41 ± 16.44^{b}
40	55	91.90	305.72 ± 0.62^{b}	1506.60 ± 2.69^{b}	1449.51 ± 30.87^{b}
40	55	91.32	293.48 ± 1.86^{d}	$1497.07 \pm 4.95^{\mathrm{b}}$	1368.01 ± 6.17^{c}
60	55	91.63	301.49 ± 1.24^{c}	1502.15 ± 2.22^{b}	1430.59 ± 32.93^{b}
30	68	96.28	312.57 ± 0.82^a	1578.36 ± 2.25^{a}	1515.00 ± 8.23^{a}
50	68	80.94	264.91 ± 1.44^{e}	1326.88 ± 3.14^{e}	1325.81 ± 28.81^{cd}
20	80	95.78	309.51 ± 1.03^{a}	1570.10 ± 0.90^{a}	1372.38 ± 28.88^{c}
20	80	83.30	267.10 ± 1.24^{e}	1365.62 ± 1.80^{d}	1372.38 ± 4.12^{c}
40	80	81.48	265.49 ± 2.27^{e}	1335.77 ± 1.35^{e}	1296.70 ± 16.47^{d}
60	80	64.98	200.35 ± 2.89^{h}	1065.24 ± 4.94^{g}	1066.76 ± 16.74^{e}
60	80	65.95	203.84 ± 2.06^{g}	1081.11 ± 7.18^{g}	1087.13 ± 37.05^{e}

ET: Extraction time, UA: Ultrasonic amplitude

⁽a-k) Means with uncommon superscripts within a column are significantly different (p < 0.05).

globe artichoke (C. cardunculus L.) had two times higher TPC (1479 mg gallic acid 100 g dry sample⁻¹) than that of artichoke heart flowers (696 mg gallic acid 100 g dry sample⁻¹). Sihem et al.⁴¹ revealed that TPC values and antioxidant activity of Tunisian globe artichoke leaves were higher than the bracts and floral stems. These differences can be explained with the origin of the artichoke, cultivation conditions, climate and the harvesting time. According to the results obtained in our study and the results found in the literature, it is seen that the total phenolic and total flavonoid contents of the artichoke leaves are affected by factors such as genetic diversity and harvest time.⁵ Garcia-Castello et al.42 extracted flavonoids from grapefruit solid wastes by UAE and they reported that total phenolic content and antioxidant capacity of the UAE extracts were 50% and 66% higher than that of CE at lower extraction times, respectively. They found optimum process conditions as 25°C extraction temperature, 40% ethanol concentration and 55 minutes of extraction time which yielded total phenolic content of 80.0 mg gallic acid g dry weight⁻¹ and antioxidant capacity of 38.3 mmol trolox g dry weight-1. They also reported that UAE extracts obtained using only distilled water had 75.3 mg gallic acid g dry weight⁻¹ and 31.9 mmol trolox g dry weight⁻¹, which were similar to the values found at the optimum process conditions. Usage of the distilled water as the solvent in the UAE can ensure economic and environmental process, which was presented in our study as well.

For UAE, the effect of process variables on extraction yield is given by ANOVA table (Table 3). The quadratic model created for the extraction yield is statistically significant at the 99% level (p < 0.01) and the lack of fit is statis-

tically insignificant at the 95% confidence level (p > 0.05) (Table 3). According to the results, the process variable that has the most significant effect on the model is the UA value. In addition to the linear and quadratic effect of the UA, it was determined that the linear effect of the ET and the ET-UA interaction had a significant effect on the model (p < 0.05) (Table 3). Ghafoor et al. 43 optimized the UAE of polyphenols from grapeseed and it was reported that antioxidant capacity of the extracts was significantly affected by linear and quadratic terms of ET. On the other hand, the quadratic effect of the ET does not have a statistically significant effect on the model (p > 0.05) (Table 3). In addition to lack of fit values, to understand what extent the obtained model for the extraction process by UAE meets the experimental data R2, adjusted R2 (adj-R2), adequate precision, predicted residual error sum of squares (PRESS) and coefficient of variation C.V. (%) were determined (Table 3). According to the results, the obtained model was suitable to predict extraction yield values (R² > 0.95). On the other hand, as new terms that can be added to the model always tend to increase the R2 value, it is recommended to use adj-R² values in the expression of model fit. 44 Results showed that R2 and adj-R2 values for the model were very close to each other (< 1.6%) (Table 3), and this reveals that the model does not contain statistically insignificant terms.

The second-order polynomial model in terms of coded factors obtained for the extraction process using UAE and used for the optimization study is given by Equation (2). In addition, the 3D response surface graph including isohips curves showing the effect of the ET and UA on the extraction yield and the relationship between the

Table 3. ANOVA table representing the effect of linear, quadratic and interaction terms on extraction yield for UAE model and statistical parameters

Source	DF	Sum of Squares	F Value	p – Value
Model	5	9198.99	60.11	< 0.0001
X_1	1	158.90	5.19	0.0402
X_2	1	3726.18	121.74	< 0.0001
X_1X_2	1	597.20	19.51	0.0007
X_1^2	1	26.27	0.86	0.3711
X_2^2	1	2531.55	82.71	< 0.0001
Residual	13	397.90		
Lack of Fit	6	297.69	3.47	0.0644
Pure Error	7	100.22		
Total	18	9596.90		
Parameter	Value			
$\overline{\mathbb{R}^2}$	0.9585			
adj- R ²	0.9426			
Adequate Precision	19.113			
PRESS	972.67			
C.V. (%)	7.77			

 X_1 : Extraction time (min), X_2 : Ultrasonic amplitude (%), DF: Degrees of freedom, Adj- R^2 : Adjusted R^2 , PRESS: Predicted residual error sum of squares, C.V. (%): Coefficient of variation

experimental extraction yields and the extraction yields estimated from the model are shown in Figure 1. When Figure 1(a) is examined, linear isohips curves show the interaction between ET and UA. Moreover, the greater the slope for the UA indicates that the UA has the most significant effect for the model. It has been visually demonstrated that the extraction yield decreases due to the increasing ET, especially at high UA values, and the effect of ET is lower at low UA values (Figure 1a). In Figure 1(b), the experimental extraction yields (x axis) and the extraction yields estimated from the model (y axis) were plotted and a linear equation of was obtained. The linear equation showed that predicted and experimental values of extraction yield are very close to each other proving that the model is appropriate.

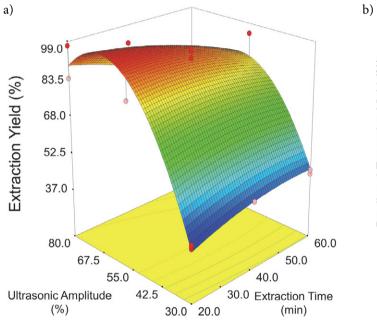
Extraction yield (%) =
$$+91.85-3.75X_1+$$

+ $17.99X_2-8.18X_1X_2-29.44X_2^2$ (2)

was determined by the single sample t-test and it was seen that there was no statistical difference between the two values (p > 0.05).

4. Conclusions

In this study, bioactive extracts having antioxidant properties were obtained from artichoke leaves which can be categorized as agricultural waste using only distilled water as solvent. The UAE and CE were used as extraction processes and they were compared in terms of extraction yield and time. Results showed that bioactive extracts with high antioxidant capacity were obtained at short times and at the room temperature by UAE application. Also, by UAE process, higher extraction yield and shorter extraction time were ensured when compared with CE. Thus, the study presents that utilization of a waste product which is



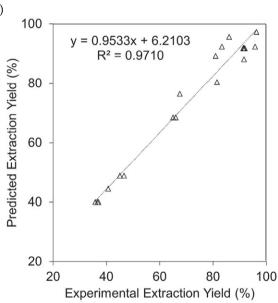


Figure 1. (a) Effect of process parameters on extraction yield and (b) relationship between experimental and predicted extraction yields.

Numerical optimization study was carried out for UAE process to determine the optimum point. 19 solutions with values close to each other were calculated by program and the solution which had the highest 'desirability' value was chosen as the optimum point. The extraction yield was calculated as 98.46% at the optimum point which was having 20.05 minutes of ET and 65.02% of UA. The average experimental extraction yield at the optimum point was determined as 98.77 \pm 0.12% according to the optimum point verification trials performed in triplicate. Whether there was a statistically significant difference between the estimated and experimental extraction yields

a natural antioxidant source can be done by a novel and green extraction technique. Even though ultrasonic systems have high capital cost, in the long term, UAE process can be advantageous for obtaining bioactive extracts from artichoke leaves due to short extraction and high extraction yield.

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Povzetek

V raziskavi smo primerjali učinkovitost uporabe ultrazvočne in klasične ekstrakcije z destilirano vodo za izolacijo bioaktivnih komponent iz artičokovih listov, ki predstavljajo kmetijski odpadek. Določevali smo antioksidacijsko sposobnost in vsebnost celokupnih fenolov ter flavonoidov ekstrahiranih bioaktivnih komponent in primerjali učinkovitost ter trajanje ekstrakcije. Z uporabo D-optimalnega načrtovanja eksperimentov in kriterija »zaželjene« funkcije smo določili pogoje maksimalnega izkoristka ultrazvočne ekstrakcije (čas in moč ultrazvoka). Eksperimenti so pokazali, da lahko z ultrazvočno ekstrakcijo dosežemo višje izkoristke bioaktivnih komponent z visoko antioksidativno sposobnostjo v krajšem času kot pri klasični ekstrakciji. Najvišji izkoristek 98.46 % smo dosegli z 20.05 minutno ekstrakcijo in 65.02 % amplitudo ultrazvoka.



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