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Microwave-assisted extraction of antioxidant phenolic compounds from artichoke (*Cynara scolymus* L. cv Bayrampasa): optimisation and kinetic modelling

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Abstract

The objective of the present work was to optimise an efficient alternative technique for the extraction of total phenolics and antioxidants from Bayrampasa variety artichoke by-products, and to evaluate the potential role of artichoke as a source of health-promoting phenolic compounds and antioxidants. In the present work, microwave-assisted extraction (MAE) was used in order to obtain phenolic compounds and antioxidants from artichoke by-products such as leaves and bracts. The obtained phenolic compounds and antioxidants were assessed in terms of total phenolic content (TPC), 2,2-diphenyl-1-picryl-hydrazine (DPPH) antioxidant activity, and cupric-reducing antioxidant capacity (CUPRAC). The highest TPC and CUPRAC values were obtained at 4 min, and the highest DPPH activity was observed at 6 min and 80°C for leaf and bract extracts; also, the TPC, DPPH, and CUPRAC values of bract extracts were significantly lower than that of leaf extracts. Modelling of MAE for the artichoke leaves and bracts mixture using the central composite design was examined for determination of solvent/solid ratio (v/w), time, and solvent/water ratio (v/v). Additionally, second order and Peleg's kinetic models proved to be the most suitable in describing the MAE kinetics for artichoke leaves and bracts mixture.

Introduction

Artichoke (*Cynara scolymus* L.), widely found in the Southern Europe and around the Mediterranean Sea, is of the Asteraceae family, and multi-year herbaceous plant which represents an important role in the Mediterranean diet. Artichoke is a rich source of minerals, polyphenolics, and antioxidants (Ruiz-Aceituno *et al.,* 2016). Artichoke cultivation produces large amounts of agricultural waste or by-products which comprise about 80 - 85% total biomass of the plant, and consist mainly of leaves, external parts of the artichoke (bracts), and stems which are not suitable for human diet but could be used as a source of cynarine, inulin, phenolics, and antioxidants. In the last decade, the possibility to recover the by-products (artichoke leaves, external bracts, and stems) produced by agricultural cultivation has been proposed. Adding value to the agro-industrial by-products has gained attention due to the economic and environmental concerns. Furthermore, nutritional and pharmaceutical properties of both artichoke leaves and bracts include high levels of polyphenolic compounds, antioxidants, cynarine, and inulin (Gaafar and Salama, 2013). Therefore, various studies have shown that artichoke leaves and bracts extracts have major medicinal properties including antimicrobial, anti-inflammatory, diuretic, and choleretic activities (Gaafar and Salama, 2013; Ruiz-Aceituno *et al.,* 2016). In this context, these discarded leaves, bracts, and stems are a valuable sources for the production of functional extracts.

Extraction is the most important process for separation of bioactive compounds from plant materials. Many factors such as solvent type, extraction temperature, extraction time, and solvent/solid ratio can significantly influence the extraction yield, phenolic content, and antioxidant activity. In the last decade, ultrasound and microwave-assisted extraction (MAE) technologies have been applied conveniently for bioactive compounds instead of the conventional solid-liquid extraction method due to their simple and effective properties. In addition, these new and advanced separation techniques increases extraction yields and decreases extraction time effectively. The MAE has gained great interest in the recent years to obtain bioactive materials from industrial by-products (Ruiz-Aceituno *et al.,* 2016). The MAE is a separation

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process in which microwave energy is used to heat a solvent to extract it from the solid. This method enables the diffusion of the active substance from the solid into solvent phase in a short time, thus increasing the extraction yield and decreasing both solvent consumption and extraction time (Dahmoune *et al.,* 2015).

Conventional artichoke in Turkey is intensively cultivated in the provinces of Izmir, Bursa, Aydın, Antalya, and Adana. In 2018, while 39,477 tons of conventional artichoke production in Turkey were obtained from a total of 3,276 hectares of land, around 28,626 tons of organic artichokes were grown in the provinces of Izmir, Bursa, Aydin, Antalya, and Adana. In Turkey, the most commonly cultivated artichoke variety is Bayrampasa, which has high quality and important economic impact within the Marmara region. This variety which has a very large, tight, and rounded features, also carries big flower head which is suitable for the canning industry.

The objective of the present work was to develop an efficient alternative technique for the extraction of phenolic compounds and antioxidants from Bayrampasa variety artichoke by-products. For this purpose, in the first part of the work, extract phases were obtained from leaves and bracts of artichoke using MAE. The total phenolic content (TPC), 2,2-diphenyl-1-picryl-hydrazine (DPPH) antioxidant activity, and cupric-reducing antioxidant capacity (CUPRAC) values of the extracts were also determined at different extraction temperatures and interval times. In the second part of the work, the effects of solvent/solid ratio (v/w), extraction time, and solvent/water ratio (v/v) for MAE on phenolic content and antioxidants from the extracts of leaves and bracts mixture were examined using central composite design (CCD) in the response surface methodology (RSM) algorithm. Besides that, four models were developed to describe the kinetic mechanisms of phenolic and antioxidant extraction from the mixture, and the best model constants were determined. The present work represents the first example in the literature to perform MAE on by-products of Bayrampasa variety artichokes, and the results offer a reference for its valorisation.

Materials and methods

Artichoke sample preparation

Cynara scolymus L. cv. Bayrampasa was collected from Bursa, Turkey. Leaves and bracts of artichokes were collected and dried in an oven at 50°C, and the samples were milled to 500 μm mesh. The milled leaves and bracts were stored at 4°C until further analyses.

Materials

Folin-Ciocalteu phenol reagent, gallic acid, 2,2-diphenyl-1-picryl-hydrazine, (±)-6-hydroxy-2,5,7,8-tetramethyl chromane-2-carboxylic acid (Trolox), neocuproine, and sodium carbonate were purchased from Sigma-Aldrich. Methanol was purchased from JT Baker Chemicals. Ethanol, copper (II) chloride, and ammonium acetate were purchased from Merck. All reagents were of analytical grade.

MAE of leaves and bracts of artichoke

The extract from artichoke leaves and bracts were obtained using a microwave extraction (MAE) system. MAE was carried out in a MARS 6 (CEM, NC, USA) device. This system allows for simultaneous irradiation of up to 24 extraction vessels by applying 1,800 W of microwave energy at 100% power. One of the vessels is a reference vessel to check for heat. The temperature probe is fibre optic with a phosphorous sensor which allows temperatures to be selected in the range of 20 - 200°C. Extraction conditions such as percentage power input and temperature can be varied accordingly. The samples were placed into lined Teflon PFA vessels with a volume of 100 mL. The vessels are located in a carousel which rotates for 360° during the operation. Every time a vessel rotates past the temperature probe, the temperature is accurately measured in real time. This allows the MAE equipment to automatically make power adjustments to ensure a successful extraction.

For each extraction, 1 g of dried leaves and 1 g of dried bracts were placed separately in 100 mL Teflon PFA-lined extraction vessels, and 15 mL ethanol was added to each vessel. The extraction times (3, 4, 6, 8, and 10 min) and extraction temperatures (60, 70, and 80°C) were varied during the MAE process. The microwave power was set at 320 W. After MAE, the extract phases were filtered through Whatman No. 1 filter paper, and crude extracts were stored at -18°C in amber flasks until TPC, DPPH, and CUPRAC analyses (Apak *et al.,* 2004; Thaipong *et al.,* 2006). All MAE experiments were performed in triplicate.

MAE of artichoke leaves and bracts mixture

According to the preliminary MAE experiments for leaf and bract extracts, the highest values of TPC, DPPH, and CUPRAC analyses were obtained with a mixture of 70% (w/w) of leaves and

30% (w/w) of bracts fractions. The same extraction procedure was applied for the mixture. TPC, DPPH, and CUPRAC analyses were determined. All MAE experiments were performed in triplicate. The yield of MAE was calculated using Eq. 1:

$$\text{Yield } \% = (E_M / E_{Ph.\ Eur}) \times 100 \qquad \text{(Eq. 1)}$$

where, E_M = TPC, DPPH, CUPRAC antioxidant activity using MAE, and $E_{Ph.\ Eur}$ is the TPC, DPPH, CUPRAC antioxidant activity using extraction procedure according to Ph. Eur (Stumpf *et al.,* 2019).

Total phenolic content (TPC) analysis

Total phenolic content of artichoke leaf and bract extracts were assessed spectrophotometrically using the Folin-Ciocalteu procedure. For this, 150 µL of extract samples, 2,400 µL of distilled water, and 150 µL of 0.2 N Folin-Ciocalteu reagents were transferred into a 15 mL test tube, and mixed for 1 min using a vortex mixer. Then, 300 µL of 1 N Na_2CO_3 in distilled water was added to the mixture, and stirred for 2 h. The absorbance of the samples was determined at 725 nm (Thaipong *et al.,* 2006). The TPC in leaf and bract extracts were determined by comparing the absorbance with that of gallic acid standard solutions. TPC values were expressed as mg gallic acid equivalents (GAE) per 100 g of dry weight. All TPC analyses were performed four times.

2,2-diphenyl-1-picryl-hydrazine (DPPH) antioxidant activity analysis

DPPH antioxidant activities of artichoke leaf and bract extracts were assessed spectrophotometrically as previously reported by Thaipong *et al.* (2006). The stock DPPH solution was prepared using 24 mg of DPPH and 100 mL of methanol which was then stored at -20°C until further use. The solution was obtained by mixing 10 mL of the stock solution with 45 mL of methanol. Then, 150 µL of extract samples and 2,850 µL of DPPH solution were allowed to react in the dark for 24 h. The absorbance of the samples was determined at 515 nm. DPPH values were expressed as mg Trolox equivalent (TE) per 100 g of dry wight. All DPPH analyses were performed four times.

Cupric-reducing antioxidant capacity (CUPRAC) analysis

CUPRAC antioxidant capacities of artichoke leaf and bract extracts were assessed spectrophotometrically as previously reported by Apak *et al.* (2004). For this, 1 mL of extracts were placed in a cuvette, and allowed to react at 23°C for 1 h by adding 1 mL of distilled water, 1 mL of 1 M ammonium acetate, 1 mL of 10 mM $CuCl_2$, and 1 mL of 7.5 mM neocuproine. The absorbance of the samples was determined at 450 nm. CUPRAC antioxidant capacity values were expressed as mg Trolox equivalent (TE) per 100 g of dry weight. All CUPRAC analyses were performed four times.

Statistical analysis of MAE for artichoke leaves and bracts

The data are presented as mean ± the standard deviation (SD). One-way analysis of variance (ANOVA) with Tukey's test was performed by using Statgraphics software (Statistical Graphics Corp., USA) in order to determine the significant differences among the TPC, DPPH, and CUPRAC results for leaf and bract extracts. Differences were considered significant at $p < 0.05$ (Patosz *et al.,* 2020).

Modelling of the MAE process for artichoke leaves and bracts mixture using CCD

Optimum predicting functions of CCD in RSM (Sahin *et al.,* 2020) were investigated for the determination of optimum conditions (solvent/solid ratio (v/w), time (min), and solvent/water ratio (v/v)) for phenolic content and antioxidants of artichoke leaves and bracts mixture. Five levels and three independent variables were applied to optimise the MAE process for the mixture. The coded and actual levels of experimental variables were determined to be time (2 - 6 min), solvent/solid ratio (5/1 - 25/1, v/w), and solvent/water ratio (0/100 - 100/0, v/v). The Design Expert software (Version 11.0.0, Stat-Ease, Inc., Minneapolis, MN) program was employed for the modelling and optimisation of the MAE process (Saponjac *et al.,* 2020).

The experimental design matrix for the actual process variables and responses are listed in Table 1. Adjusted-R^2, predicted-R^2, probability value at 95% confidence interval, coefficient of variation, lack-of-fit, and analysis of variance (ANOVA) were used as statistical indicators.

Kinetic modelling of the MAE process for artichoke leaves and bracts mixture

In the present work, four kinetic models were used to evaluate the experimental data from the TPC, DPPH, and CUPRAC analyses and the entire MAE process for artichoke leaves and bracts mixture. The first-order kinetic model, second-order kinetic model, Peleg's model, and Page's model were examined, and the kinetic models were compared in order to determine which one would best fit the

experimental data.

The first-order kinetic model can be used in extraction kinetics for plants (Harouna-Oumarou *et al.,* 2007). Therefore, the model was used to describe MAE kinetic behaviour of the TPC, CUPRAC, and DPPH values of the mixture extracts. A kinetic approach based on Fick's law can be described using Eq. 2:

$$dC_t / dt = k(C_e - C_t) \quad \text{(Eq. 2)}$$

where, C_t, C_e, k, and t = solute concentration at t (min, mg/g), solute concentration at equilibrium (mg/g), mass transfer coefficient (1/min), and extraction time (min), respectively.

The second-order kinetic model equation can be described for solid-liquid extraction (Harouna-Oumarou *et al.,* 2007), and the rate equation can be described using Eq. 3:

$$dCt / d_t = k(C_e - C_t)^2 \quad \text{(Eq. 3)}$$

where, dC_t/dt, k, C_t, and C_e = rate of extraction (mg/g min), model constant of extraction process (g/mg min), solute concentration at t (min, mg/g), solute concentration at equilibrium (mg/g), and extraction time (min), respectively. The initial extraction rate of h was equal to $k.C_e^2$.

The Peleg model can be used to explain kinetic behaviour of the solid-liquid extraction (Kaderides *et al.,* 2019), and can be described using Eq. 4:

$$C_t = t/(k_1+k_2t) \quad \text{(Eq. 4)}$$

where, C_t, k_1, and k_2 = concentration of solute (mg/g) at t (min), Peleg's rate constant (min g/mg), and Peleg's capacity constant (g/mg), respectively.

Page's model is commonly used for the solid-liquid extraction process (Kaderides *et al.,* 2019), and can be described using Eq. 5:

$$C_t = \exp(-kt^n) \quad \text{(Eq. 5)}$$

where, k (mg/g) and n (mg/g^{-1}) = Page's constants, and t = extraction time (min), respectively.

Results and discussion

MAE of artichoke leaves and bracts

The chemical components of artichoke leaves and bracts have been studied extensively, and found to be rich sources for polyphenols. The data obtained from HPLC analysis suggested that the non-edible parts of artichoke could represent a good source of polyphenols. Also, based on the HPLC results, chlorogenic acid, cynarine, luteolin 7-glycoside, and apigenin 7-o-neohesperidoside were found in the artichoke leaf and bract extracts. It can be safely stated that the discarded and non-edible parts such as leaves and bracts are important sources for the production of functional extracts. For this purpose, the TPC, DPPH, and CUPRAC values were determined for leaf and bract extracts of the Bayrampasa variety of artichoke.

In the preliminary MAE experiments, different temperatures (60 - 80°C) and time intervals (3 - 10 min) were investigated for determination of the highest TPC, DPPH, and CUPRAC values. The extractability of phenolic compounds and antioxidants from artichoke leaves, bracts, and extraction yields were determined.

Quantitatively, the highest TPC, DPPH, and CUPRAC values were determined in artichoke leaf extract. The highest TPC and CUPRAC values were obtained at 4 min, and DPPH activity was observed at 6 min. The TPC values were determined to be 172.22, 172.36, and 239.22 mg/100 g GAE at 60, 70, and 80°C, respectively, for 3 min. The Folin-Ciocalteu procedure was used to determine the TPC of extracts from Bayrampasa variety artichoke. The Folin-Ciocalteu procedure is a colorimetric method based on electron transfer reactions between the Folin-Ciocalteu reagent and polyphenolic compounds in the extracts. However, the Folin-Ciocalteu procedure is not specifically only for TPC determinations. It is known that ascorbic acid and reducing sugar that may be present in high abundance in plant food extracts can also affect the Folin-Ciocalteu reagent and the results of TPC. The plant extracts may contain interfering substances that can react with the Folin-Ciocalteu reagent, thus skewing the results for TPC determinations. Among these reducing compounds, ascorbic acid, dehydroascorbic acid, and reducing sugars have the highest impact in terms of hampering the accuracy of the procedure (Isabelle *et al.,* 2010; Sanchez-Rangel *et al.,* 2013). The highest TPC, DPPH, and CUPRAC values were found as 299.93 mg/100 g GAE (91% yield), 287.84 mg TE/100 g (92% yield), and 1,989.77 mg TE/100 g (94% yield), respectively.

Based on the analyses, the TPC, DPPH, and CUPRAC of the leaf extract were obtained in two stages according to time. A rapid increase in TPC, DPPH, and CUPRAC values at the beginning of the extraction (4 - 6 min) was observed with a sharp decrease during further progress (6 - 10 min). The stability of the phenolic compounds and antioxidants at the highest temperatures (80°C) might be related to

the relatively short extraction time (4 - 6 min). Choosing a suitable extraction time is not only a very important parameter which allows for the achievement of MAE, but also for stability of phenolic compounds and antioxidants. In the present work, the extraction time was set to 4 and 6 min. The highest amounts of TPC and CUPRAC values from leaf extracts were obtained in 4 min, and the highest DPPH value was determined in 6 min. Extending the extraction time from 4 or 6 min to 10 min did not seem to have a significant influence on the TPC, DPPH, and CUPRAC values of artichoke leaf extracts.

Furthermore, the highest TPC, DPPH, and CUPRAC antioxidant activity was obtained at 80°C for leaf extracts at 238.83 mg/100 g GAE, 242.04 mg TE/100 g, and 1472.70 mg TE/100 g, respectively. At 70 and 60°C, the TPC values decreased from 239.03 to 193.6 mg/100 g GAE (86 to 84% yield) in 4 min, while the DPPH values decreased from 192.86 to 186.61 mg TE/100 g (75 to 74% yield) in 6 min. The extraction yields (%) for the TPC analyses were 84% for 60°C; 86% for 70°C; and 91% for 80°C after 4 min extraction. Similar trend was observed for DPPH and CUPRAC analyses where the extraction yields were 74 and 73% for 60°C, 75 and 79% for 70°C, and 86 and 94% for 80°C. The highest extraction efficiency for TPC and antioxidant activities was observed at the extraction temperature of 80°C in the previous studies (Hodzic *et al.,* 2009; Carciochi *et al.,* 2018). Lower temperatures enabled poor recovery of the TPC, DPPH, and CUPRAC with the lowest temperature causing the least efficiency in that aspect (80 > 70 > 60°C).

Temperature is an important parameter that is associated with microwave power which controls the quantity of energy converted to heat for the extraction. Higher temperatures increase the extraction yield and decrease the reaction time in general, but if it is not chosen suitably, it can also cause degradation, thus hampering the extraction yield (Dragovic-Uzelac *et al.,* 2012). Higher temperature had a positive influence on the TPC, DPPH, and CUPRAC values of artichoke leaf extract, thus indicating that these compounds are relatively stable at high temperature.

The TPC, DPPH, and CUPRAC values of artichoke bract extract were lower than those of leaf extracts. The highest TPC, DPPH, and CUPRAC values determined were 241.43 mg/100 g GAE (88% yield), 187.04 mg TE/100 g (87% yield), and 1,250.74 mg TE/100 g (90% yield), respectively, at 4 min. The TPC, DPPH, and CUPRAC values of bract extract were significantly lower (up to 1.2-fold for TPC, 1.5-fold for DPPH, and 1.78-fold for CUPRAC) than those of leaf extract.

The highest TPC, DPPH, and CUPRAC of bract extract were again found at 80°C; the average values for TPC, DPPH, and CUPRAC antioxidant activity were 152.30 mg/100 g GAE, 135.23 mg TE/100 g, and 999.09 mg TE/100 g, respectively. A significantly higher amount of TPC and antioxidant capacities was observed from bract extract obtained at 80°C as compared to their counterparts obtained at 60 and 70°C. These results represented the significant effect of temperature as an MAE parameter with respect to TPC, DPPH, and CUPRAC values.

The highest amount of TPC, DPPH, and CUPRAC of bracts were obtained with 4 min extraction. On the other hand, increasing the microwave extraction time above 4 min did not have a significant effect on TPC and antioxidants of the bract extract. This can be explained by Fick's second law of mass transfer. This approach accepts equilibrium between the polyphenols and the solvent after a certain microwave extraction time (Chew *et al.,* 2011).

Extended microwave extraction time can cause the loss of phenolic compounds and antioxidants. This was observed in the extraction of phenolic compounds and antioxidants from the bracts, as the extraction yields for TPC, DPPH, and CUPRAC declined with the increasing microwave extraction time, and thus 4 min was set to be an optimum condition for MAE (Ahmad and Langrish, 2012). During the MAE, extended extraction time can cause thermal degradation and oxidation, thus resulting in the decreased extraction yield of phenolic compounds and antioxidants (Khoddami *et al.,* 2013). Shorter microwave extraction time in the microwave system is one of the major potential benefits of MAE, as it reduces the risk of decomposition and oxidation of chemicals.

From the preliminary results of the TPC, DPPH, and CUPRAC analysis of leaf and bract extracts, the extraction temperature and extraction time had significant effect on the artichoke extracts. The highest TPC, DPPH, and CUPRAC values of leaf and bract extracts were obtained when MAE was performed at 80°C, microwave power of 320 W, and the longest extraction time of 4 and 6 min. It was obvious that MAE could be a potential method for the extraction of phenolic compounds and antioxidants from artichoke leaves and bracts. The TPC, DPPH, and CUPRAC values found in the leaf and bract extracts were very high under the determined optimum conditions (80°C, 4 and 6 min).

Therefore, it was appropriate to mix the leaves and bracts by phases, and analyse the TPC, DPPH, and CUPRAC values for MAE extract of the mixture again at optimum temperatures. According to Zhang *et al.* (2013), optimisation of the extraction parameters is helpful to understand the interaction between independent factors and obtaining the optimal parameters for extraction. In addition, the MAE efficiency depends on several variables due to the nature of the obtained bioactive compounds, making it necessary to select and optimise the extraction conditions. Therefore, the effects of solvent/solid ratio (v/w), extraction time, and solvent/water ratio (v/v) were assessed to investigate the optimal MAE extraction parameters for artichoke leaves and bracts mixture in the second part of the present work. However, the effects of extraction factors on the yield of MAE have not been studied; thus, no information is available for kinetic modelling of artichoke leaves and bracts mixture. So, four kinetic models were applied to the TPC, DPPH, and CUPRAC analyses from the mixture in order to determine the behaviour of the MAE process.

Modelling of the MAE process for artichoke leaves and bracts mixture using CCD

Based on the CCD experimental design matrix, the adequacy of linear, two factors interactive (2FI), quadratic, and cubic models are described in Table 2 (Sahin *et al.,* 2020). The models were adapted to MAE experiments, and the results of the TPC, DPPH, and CUPRAC analyses of leaves and bracts mixtures were used to determine the regression statistics model.

It was found that the fit of the data representing quadratic models for the TPC, DPPH, and CUPRAC were statistically significant with sequential *p*-values of the quadratic models < 0.0001, and R^2 of the models were acceptable for the MAE of leaves and bracts mixtures. The results suggest that the quadratic model was statistically significant for MAE, and the model described the relationship between responses and independent process variables. The adjusted-R^2 values were determined to be 0.99 for TPC, 0.96 for DPPH, and 0.97 for CUPRAC (Table 2). The differences between the adjusted-R^2 and predicted-R^2 values of quadratic models were significantly lower than that of the cubic models; therefore, the quadratic model was selected as suitable for modelling MAE from artichoke. Furthermore, the cubic models for the TPC and antioxidant capacities were determined as aliased.

The statistical significance of the regression equations was examined by ANOVA and Fisher's *F*-test value (*f*-value) for RSM (Table 3). The relationship between the independent variables and the responses were expressed in quadratic models (Eq. 6 for TPC, Eq. 7 for DPPH, and Eq. 8 for CUPRAC):

TPC=561.48+88.19A-16.31B+20.94C+27.13AB+ 22.88AC-32.37BC-71.89A^2-31.01B^2-111.14C^2 (Eq. 6)

DPH=799.52+114.62A-11.00B+58.12C-7.00AB+55 .25AC-114.25BC-91.36A^2-35.49B^2-162.74C^2 (Eq. 7)

CPRAC=1010.59+98.75A+21.62B+56.62C-22.50A B+79.75AC-176.00BC-125.08A^2-67.08B^2-194.9 5C^2 (Eq. 8)

where, A, B, and C = solvent/solid ratio (v/w), extraction time (min), and solvent/water ratio (v/v), respectively (Table 3); AB, AC, BC, and A2, B2, C2 = interaction of two independent variables and squared effect of the independent variables, respectively. Eq. (6), (7) and (8) show the effect of the independent variables, interactions of two independent variables, and squared effects of independent variables on the TPC, DPPH, and CUPRAC profiles from artichoke leaves and bracts mixture.

The TPC, DPPH, and CUPRAC results of leaves and bracts mixture were evaluated with ANOVA, and the analyses checked the statistical significance of the quadratic models by calculating the *p*- and *f*-value. In ANOVA, a model with *p*-value < 0.05 and high *f*-value can be accepted as suitable (Sahin *et al.,* 2020). The results of ANOVA for the quadratic models with *p*- and *f*-values for the TPC, DPPH, and CUPRAC are given in Table 3. It was found that all *p*-values were smaller than 0.003, except for extraction time (B), interaction of the solvent/solid ratio (v/w), and extraction time (AB) effect for DPPH and CUPRAC results. Furthermore, all *f*-values were higher than 7.0 for the TPC, DPPH, and CUPRAC models. The low *p*-values and high *f*-values showed that the quadratic models were significant, and suggested a good relationship between response and independent variables for the TPC, DPPH, and CUPRAC data. Also, the lowest calculated *p*-value or the highest calculated *f*-value for the model variables showed the most effective variables on the response. Hence, A, B, C, A2, B2, C2, AB, AC, and BC were remarkable variables for

Table 1. Experimental design matrix and data.

Run	Actual value of variable			Response					
	Solvent/ solid ratio (v/w)	Extraction time (min)	Solvent/ water ratio (v/v)	TPC (mg GAE/ 100 g)	DPPH (mg TE/ 100 g)	CUPRAC (mg TE/ 100 g)	Yield % (TPC)	Yield % (DPPH)	Yield % (CUPRAC)
	(A)	(B)	(C)						
1	15	4	50	563	801	2,663	77	85	91
2	15	4	50	573	833	2,354	79	88	81
3	10	3	25	249	223	668	34	24	23
4	15	4	50	571	722	2,359	79	76	81
5	15	2	50	486	716	1,851	67	76	63
6	20	5	25	432	563	1,876	59	60	64
7	5	4	50	117	213	768	16	23	26
8	20	3	25	339	357	907	47	38	31
9	25	4	50	446	685	1,722	61	72	59
10	15	4	50	544	845	2,402	75	89	82
11	15	4	100	157	214	761	22	23	26
12	10	5	75	176	308	995	24	33	34
13	20	3	75	499	870	2,478	69	92	85
14	20	5	75	466	635	1,793	64	67	61
15	15	4	50	561	781	2,561	77	83	88
16	10	3	75	321	531	1,524	44	56	52
17	15	4	50	572	845	2,456	79	89	84
18	15	6	50	404	629	1,771	56	67	61
19	10	5	25	237	473	1,920	33	50	66
20	15	4	0	92	113	366	13	12	13

TPC; A, C, AC, BC, A2, B2, and C2 for DPPH; and A, C, AC, BC, A2, B2, and C2 for CUPRAC. Furthermore, based on *f*-values, the most effective model variables for the TPC, DPPH, and CUPRAC were solvent/solid ratio (v/w) (A) and solvent/water ratio (v/v) (C). The most effective variables on the response were C2 > A2 > A > B2 > BC > C > AB = AC > B for TPC; C2 > A2 = A > BC > C > B2 > AC for DPPH; and C2 > A2 > BC > A > B2 > C = AC for CUPRAC, in that order, for results from the leaves and bracts mixture.

Response surface methodology (RSM)

Three-dimensional RSM graphs are necessary in order to observe the interaction of independent variables on the TPC, DPPH, and CUPRAC responses. These graphs (Figure 1a, 1b, and 1c) were obtained based on Eqs. 6, 7, and 8 (Saponjac *et al.,* 2020).

The effect of extraction time (min) and the solvent/solid ratio (v/w) on TPC and effect of solvent/water ratio (v/v) and solvent/solid ratio (v/w) on DPPH and CUPRAC values are shown in Figure 1a and Table 1. The TPC value was found to be 486 mg GAE/100 g (67% yield) for 2 min, 573 mg GAE/100 g (79% yield) for 4 min, and 404 mg GAE/100 g (56% yield) for 6 min at 15/1 (v/w) and 50/50 (v/v) solvent/water ratio. It can be said that extraction time affected the TPC response during 4 min, and a significant decrease in this value was observed after 4 min. Figure 1a also shows the effects of solvent/water ratio (v/v) and solvent/solid ratio (v/w) on DPPH and CUPRAC responses, while keeping the other factors at the centre level. DPPH and CUPRAC values decreased sharply from 801 and 2,663 mg TE/100 g for 50% ethanol in water,

Table 2. Regression statistics models of the MAE for leaves and bracts mixture of Bayrampasa variety artichoke.

Source	Sequential *p*-value	Lack of fit *p*-value	Adjusted R^2	Predicted R^2	
TPC					
Linear	0.1799	< 0.0001	0.1175	-0.1718	
Interactive (2FI)	0.8851	< 0.0001	-0.0350	-0.3072	
Quadratic	***< 0.0001***	***0.1037***	***0.9905***	***0.9668***	***Suggested***
Cubic	0.3839	0.0461	0.9913	0.6718	Aliased
DPPH					
Linear	0.2402	0.0005	0.0800	-0.2577	
Interactive (2FI)	0.5620	0.0004	0.0274	-0.2880	
Quadratic	***< 0.0001***	***0.2354***	***0.9650***	***0.9266***	***Suggested***
Cubic	0.2167	0.2859	0.9605	0.4180	Aliased
CUPRAC					
Linear	0.5243	0.0002	-0.0367	-0.3884	
Interactive (2FI)	0.3776	0.0002	-0.0143	-0.2497	
Quadratic	***< 0.0001***	***0.3407***	***0.9645***	***0.9664***	***Suggested***
Cubic	0.1844	0.9845	0.8760	0.8963	Aliased

Table 3. ANOVA analysis and *F*-test data of the MAE for leaves and bracts mixture of Bayrampasa variety artichoke.

Source	df	TPC			DPPH			CUPRAC		
		Sum of squares	*f*-value	*p*-value	Sum of squares	*f*-value	*p*-value	Sum of squares	*f*-value	*p*-value
Model	9	5.26E + 05	220.7	< 0.0001	1.15E + 06	37.31	< 0.0001	9.797E + 06	58.39	< 0.0001
A	1	1.24E + 05	470.2	< 0.0001	2.10E + 05	61.5	< 0.0001	9.288E + 05	49.411	< 0.0001
B	1	4257.56	16.09	0.0025	1936	0.57	0.4691	44838.06	2.39	0.1535
C	1	7014.06	26.5	0.0004	54056.25	15.81	0.0026	3.050E + 05	16.22	0.0024
AB	1	5886.13	22.24	0.0008	392	0.11	0.7419	24090.13	1.28	0.2840
AC	1	4186.13	15.82	0.0026	24420.5	7.14	0.0234	3.030E + 05	16.12	0.0025
BC	1	8385.12	31.69	0.0002	1.04E + 05	30.55	0.0003	1.475E + 06	78.40	< 0.0001
A2	1	1.30E + 05	490.97	< 0.0001	2.10E + 05	61.39	< 0.0001	2.306E + 06	124.68	< 0.0001
B2	1	24180	91.37	< 0.0001	31666	9.26	0.0124	6.547E + 05	35.89	0.0001
C2	1	3.11E + 05	1173.48	< 0.0001	6.66E + 05	194.79	< 0.0001	5.631E + 06	302.65	< 0.0001
Residual	10	2646.38			34185.07			1880E + 05		

214 mg and 761 mg TE/100 g for 100% ethanol, and 113 and 366 mg TE/100 g for 100% water, respectively, when the solvent/solid ratio was 15/1 (v/w) and extraction time was 4 min. The TPC, DPPH, and CUPRAC values increased significantly from 249 mg GAE/100 g, 223 mg TE/100 g, and 668 mg TE/100 g for 10/1 (v/w) to 339 mg GAE/100 g, 357 mg TE/100 g and 907 mg TE/100 g for 20/1 (v/w), respectively, when solvent/water ratio was 25/75 (v/v) and the extraction time was 3 min. Moreover, increases in the TPC, DPPH, and CUPRAC values were observed when the solvent/solid ratio (v/w) increased from 5/1 to 15/1 (v/w) with a constant value of solvent/water ratio of 50/50 (v/v) and extraction time of 4 min. The TPC, DPPH, and CUPRAC values increased effectively from 117 mg GAE/100 g (16% yield), 213 mg TE/100 g (23% yield), and 768 mg TE/100 g (26% yield) for 5/1 (v/w) to 563 mg GAE/100 g (77% yield), 801 mg TE/100 g (85% yield), 2,663 mg TE/100 g (91 % yield) for 15/1 (v/w), respectively (Table 1). The values did not change significantly after 15/1 (v/w). This situation can be explained with mass transfer principles, since a higher solvent/solid ratio implies higher concentration gradient between the solid and the bulk of the solvent, thus resulting in a greater driving force for diffusion of compounds into the solvent.

Figure 1b shows the effect of solvent/water ratio (v/v) and solvent/solid ratio (v/w) on TPC and the effect of solvent/water ratio (v/v) and extraction time (min) on DPPH and CUPRAC values. An increase in solvent volume in water from 25/75 (v/v) to 75/25 (v/v) produced a positive effect on the TPC response. The TPC value increased from 432 mg GAE/100 g (59% yield) for 25/75 (v/v) solvent/water ratio (v/v) to 466 mg GAE/100 g (64% yield) for 75/25 (v/v) solvent/water ratio (v/v), respectively, when solvent/solid ratio was 20/1 (v/w) and extraction time was 5 min (Figure 1b and Table 1). Besides, DPPH and CUPRAC values increased from 223 and 668 mg TE/100 g for 3 min to 473 and 1,920 mg TE/100 g for 10/1 (v/w) for 5 min at 25/75 (v/v) solvent/water ratio, respectively. This could be explained by different solvent/water ratios (v/v) affecting the polarity of the solvent system, thereby changing the solubility of the phenolic compounds from the extracts.

Figure 1c shows the effect of solvent/water ratio (v/v) and extraction time (min) on TPC response. TPC values increased from 486 mg GAE/100 g (67% yield) to 563 mg GAE/100 g (77%

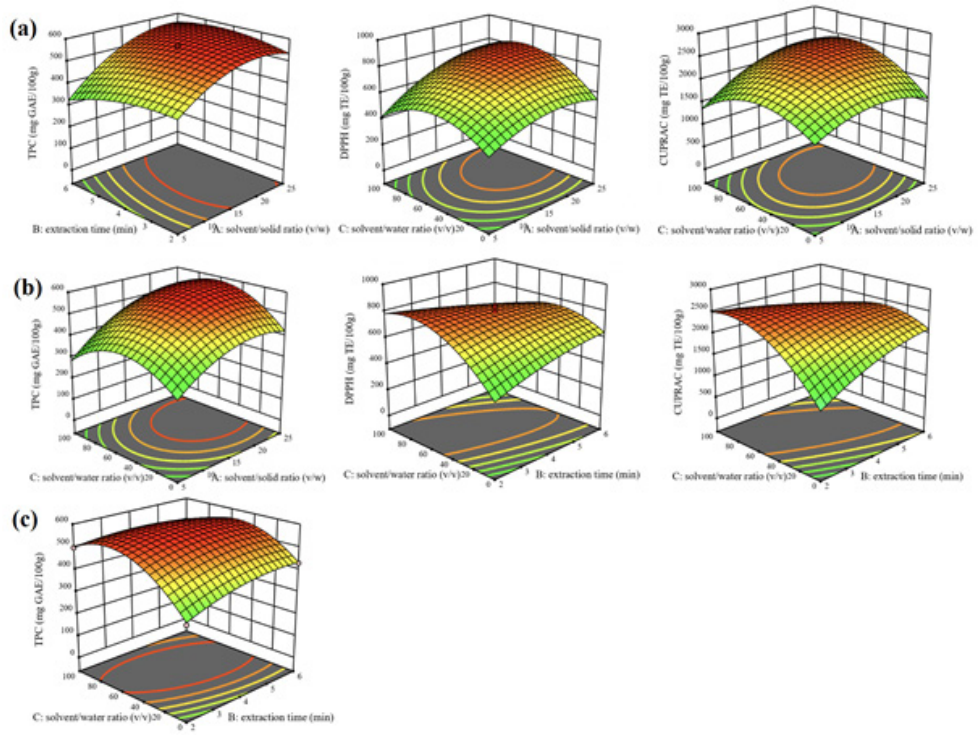

Figure 1. (a) Effect of extraction time (min) and solvent/solid ratio (v/w) on TPC, and effect of solvent/water ratio (v/v) and solvent/solid ratio (v/w) on DPPH and CUPRAC values; (b) effect of solvent/water ratio (v/v) and solvent/solid ratio (v/w) on TPC, and effect of solvent/water ratio (v/v) and extraction time (min) on DPPH and CUPRAC values; and (c) effect of solvent/water ratio (v/v) and extraction time (min) on TPC.

yield) when the extraction time increased from 2 to 4 min for 15/1 (v/w) solvent/solid ratio for 50% ethanol in water.

Kinetic modelling of the MAE process for leaves and bracts mixture

Four kinetic models were created to fit the MAE process for artichoke leaves and bracts mixture extracts and the highest value of R^2 was chosen for the best of fit. Table 4 shows the model parameters and R^2 values of the models. Among these, the second-order and Peleg's models fit best with the results with the highest values of R^2.

The plots of $\ln(C_e/(C_e - C_t))$ versus t had a linear form based on the first-order model. From the slope and the intercept of the plots, k, C_e, and R^2 values were calculated (Table 4). k values of TPC, DPPH, and CUPRAC were determined as 1.55, 1.35, and 1.05 1/min, respectively. $C_{e,experimental}$ and $C_{e,calculated}$ values were determined as 621 and 12,217 mg GAE/100 g; 891 and 3,248 mg TE/100 g; and 2,450 and 3,510 mg TE/100 g for TPC, DPPH, and CUPRAC, respectively. $C_{e,calculated}$ values were determined to be different from $C_{e,experimental}$; therefore, this result showed that first-order kinetics did not fit the characterisation of the MAE process for artichoke leaves and bracts mixture. Besides, these results had generally low coefficients of determination. Furthermore, the linearisation was better in the first 4 min of the MAE than in later stages. Thus, the MAE process did not follow the first-order kinetic model entirely, although it can be safely stated that the beginning of the MAE fitted this model. It can be explained that maximum phenolic compounds were dissolved and extracted effectively in the first 4 min of the MAE. Also, only the remaining phenolic compounds were extracted from the artichoke between 4 and 10 min, and the extraction capacities were generally worse in this period.

The TPC, DPPH, and CUPRAC values of artichoke leaves and bracts mixture extracts were analysed by using the second-order kinetic model. The equilibrium extraction capacity, C_e; the initial extraction rate, h; the extraction rate constant, k; and the coefficient of determination, R^2 are given in Table 4. h values were calculated as 416.7, 1,000, and 1,428.5 mg/g min for TPC, DPPH, and CUPRAC, respectively. k values were also found to be 9 × 10-5 g/mg min for DPPH and 1.4 × 10-3 g/mg min for CUPRAC. When compared with the first-order model, the second-order model showed very high R2 (> 0.95). Besides, $C_{e,experimental}$ and $C_{e,calculated}$ values were determined as 621 and 871 mg GAE/100 g for TPC; 891 and 1,296 mg TE/100 g for DPPH; and 2,450 and 2,000 mg TE/100 g for CUPRAC. These results indicated that the MAE of leaves and bracts mixture was characterised better by the second-order kinetic model than the first-order model.

Table 4. Kinetic parameters of the MAE for leaves and bracts mixture of Bayrampasa variety artichoke.

1st order kinetic model	**k (1/min)**	**C_e (mg/g)**	***R^2***	
TPC	1.55	12217	0.83	
DPPH	1.35	3248	0.82	
CUPRAC	1.05	3510	0.74	
2nd order kinetic model	**h (mg/g min)**	**C_e (mg/g)**	**k (g/mg min)**	***R^2***
TPC	416.7	871	3×10^{-4}	0.95
DPPH	1000	1296	9×10^{-5}	0.99
CUPRAC	1428.5	2000	1.4×10^{-3}	0.99
Peleg's kinetic model	**k_1 (g min/mg)**	**k_2 (g/mg)**	***R^2***	
TPC	0.0024	0.0009	0.95	
DPPH	0.0007	0.0010	0.99	
CUPRAC	0.0013	0.0007	0.92	
Page's kinetic model	**k (mg/g)**	**n (mg/g)**	***R^2***	
TPC	-6.00	0.108	0.76	
DPPH	-6.55	0.044	0.84	
CUPRAC	-6.51	0.092	0.81	

The kinetic parameters of Peleg's model for the TPC, DPPH, and CUPRAC are presented in Table 4. It is known that low k_1 values represent a faster rate of MAE, and low k_2 values represent the maximum yield of MAE. k_1 and k_2 values were calculated to be 0.0024 g min/mg and 0.0009 g/mg for TPC; 0.0007 g min/mg and 0.0010 g/mg for DPPH; and 0.0013 g min/mg and 0.0007 g/mg for CUPRAC. Peleg's model showed high R^2 values (> 0.92), better than the first-order and Page's kinetic models. The R^2 values were between 0.76 and 0.84 for Page's model (Table 4). k values were calculated as -6.0, -6.55, and -6.51 mg/g for TPC, DPPH, and CUPRAC, respectively, from artichoke leaves and bracts.

Conclusion

In the present work, MAE of phenolic compounds and antioxidants from leaves, bracts, and leaves-bracts mixture of Bayrampasa variety artichoke were investigated. In the first part of the work, the MAE process for 4 min with ethanol at 80°C was required to separate phenolic compounds and antioxidants with 91 - 94% extraction yields for leaves and 87 - 90% extraction yields for bracts of Bayrampasa artichoke. The TPC, DPPH, and CUPRAC antioxidant activities were determined to be 299 mg GAE/100 g, 273 mg TE/100 g, and 1,989 mg TE/100 g, respectively, for leaves; and 241 mg GAE/100 g, 187 mg TE/100 g, and 1,250 mg TE/100 g, respectively, for bracts. In the second part of the work, solvent/solid ratio (v/w), extraction time (min), and solvent/water ratio (v/v) were optimised using CCD for MAE of the TPC, DPPH, and CUPRAC antioxidants in the artichoke leaves and bracts mixture. Furthermore, the first-order, second-order, Peleg's, and Page's kinetic models were examined to describe the kinetic mechanisms of polyphenolic compound and antioxidant extraction. The MAE process for 4 min with solvent/water ratio 50:50 (v/v), solvent/solid ratio 15/1 (v/w), and temperature 80°C is necessary to separate phenolic compounds from leaves and bracts mixture with 77 - 91% extraction yields. The TPC, DPPH, and CUPRAC antioxidant activities were determined to be 563 mg GAE/100 g, 801 mg TE/100 g, and 2,663 mg TE/100 g, respectively, in these conditions. Therefore, it can be concluded that MAE has considerable efficiency for the separation of polyphenolic compounds from discarded leaves and bracts of Bayrampasa variety artichoke for the production of functional extracts.

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