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A novel technology for extraction of phenolic antioxidants from mandarin (*Citrus deliciosa* Tenore) leaves: Solvent-free microwave extraction

Selin Şahin†

Engineering Faculty, Department of Chemical Engineering, Istanbul University, 34320 Avcılar, Istanbul, Turkey
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Abstract−Solvent-free microwave extraction (SFME) of polyphenols and flavonoids from mandarin (*Citrus deliciosa* Tenore) leaves was investigated. A face central composite design (FCCD) through response surface methodology (RSM) was applied to study the effects of extraction time (30-90 sec), microwave irradiation power (250-350 W) and solid mass (2.5-7.5 g), and to optimize the extraction process. The optimum conditions were: extraction time, 53.155 sec; microwave power, 339.190 W; and solid mass, 2.500 g. Under the optimum conditions, 0.8610 mg-GAE/g-DL of total phenolic content (TPC) and 0.2440 mg-CE/g-DL of total flavonoid content (TFC) were extracted. The antioxidant activity of the extracts was assessed by cupric ion reducing antioxidant capacity (CUPRAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) methods, respectively. Antioxidant values were expressed as mg trolox equivalent antioxidant capacity per gram of dried leaf (mg-TEAC/g-DL). CUPRAC values were highly correlated with both TPC and TFC (r=0.9282/0.8842, P=0.05) in the extracts, whilst DPPH (r=0.7717/0.7435, P=0.05) and ABTS (r=0.6814/0.7072, P=0.05) were relatively less correlated with the same responses.

Keywords: *Citrus deliciosa* Tenore Leaves, Solvent-free Microwave Extraction, Optimization, TPC, TFC, Antioxidant Activity

INTRODUCTION

Citrus is one of the most important horticultural crops produced approximately 80 million tons per year, mostly in areas including southern China, the Mediterranean basin (including southern Spain), South Africa, Australia, the southernmost United States, Mexico and parts of South America [1]. This industry also produces large quantities of peels, leaves and seed residue, representing almost half of the total fruit weight [2-4]. Citrus industry by-products have been found to contain higher amounts of total phenolics compared to the edible portions [5]. Researches have pointed out that they are rich in biologically active compounds such as vitamin C, phenolic acids and flavonoids [1,6-12].

Extraction of those fine chemicals will favor the price of product from agricultural wastes in addition to ecological phenomenon. The best separation method is being searched to obtain those biologically active, fine compounds from the cheapest and the most abundant plants or/and plant waste with the most efficiency and the least time consumption for ages [13-15]. Microwave-assisted extraction (MAE) is a novel technology having potential applications of various extractions of biologically active components in plants. Pan et al. [16] showed that time of heat reflux extraction, ultrasonic extraction and extraction at room temperature was, respectively, about 10, 20 and 300-folds of time of extraction with MAE for the same extraction of tea polyphenols [16]. Li et al. [17] investigated MAE of phenolic compounds from tomatoes, and found it more efficient for greater antioxidant activities and higher total phenolic contents than solvent extraction [17]. Hong et al. [18] developed an MAE technique to optimize the extraction of polyphenols from grape seed in order to perform fast and efficient extractions [18]. Singh et al. [19] studied on developing an MAE process for the extraction of antioxidants from potato peels [19]. Their results indicated that MAE had a better performance than conventional methods. Ballard et al. [20] proved that total polyphenols extracted from peanut skins by MAE was higher than that obtained by solid-liquid extraction procedures reported in previous works [20]. MAE is fast enough to require only minutes, eliminating degradations due to oxidative damage by reducing the extraction time with both severe heating conditions and intensive mechanical disruption [18, 21]. On the other hand, solvent-free microwave extraction (SFME) is more environmentally friendly as it uses no solvent, just under microwave influence and earth gravity at atmospheric pressure. With respect to SFME, there are studies on the extraction of essential oils from *Origanum vulgare* [22], *Rosmarinus officinalis* L. [23], *Elletaria cardamomum* L. [24], *Citrus Paradisi.* L. peels [25], *Schisandra chinensis* [26], and *Dryopteris fragrans* [27]. However, studies are very scarce regarding the SFME of polyphenols in plant materials with the exception of flavonoid extraction from onions [21], and antioxidant extraction from sea buckthorn by-products [28] and berries [29].

Mandarin (Rutaceae family, *Citrus* genus) is the most common citrus crop grown in the Aegean and Mediterranean regions with the temperate summers and mild winters of Turkey [30]. Moreover, polyphenolic substances, especially flavonoids, are plentiful in this species [31]. As far as is known, there is a lack of literature on the flavonoid and phenolic contents of the mandarin leaves. Hayat

†To whom correspondence should be addressed.
E-mail: selins@istanbul.edu.tr
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et al. [6,32,33] evaluated the effects of microwave treatment on the phenolic compounds and antioxidant capacity of citrus mandarin pomace, but not leaves [6,32,33]. On the other hand, Kırbaşlar and Kırbaşlar [34] used steam distillation to extract mandarin (*Citrus reticulata* Blanco) leaves for oil, but not for phenolic components [34]. There exists no study on the extraction of citrus mandarin leaves assisted by microwave without using any solvent, as it is well known. Therefore, the present study was conducted to provide useful information to scale-up processes, by developing mathematical models that are able to describe and control the processes.

MATERIALS AND METHODS

1. Materials

1-1. Plant Material

Citrus deliciosa Tenore leaves were collected during the harvesting period in October 2013 by Batı Akdeniz Agricultural Research Institute (BATEM), located in Antalya, Turkey. They were picked randomly from the same tree grown in Mediterranean area of Turkey, presenting Mediterranean climate. The leaves were both dried and stored at ambient temperature in the dark. Before extraction processes, they were ground into particles whose average diameters were between 0.9-2.0 mm.

1-2. Chemicals and Reagents

Ethanol and methanol were provided from Merck (Darmstadt, Germany) and were of >99.5% and >99.8% mass fraction purity, respectively. 2,2-diphenyl-1-picrylhydrazyl (DPPH•), 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), 2,9-dimethyl-1,10-phenanthroline (neocuproine), folin-ciocalteu reagent, (+)-catechin, sodium carbonate, gallic acid, hydrocloric acid and sodium hydroxide were purchased from Sigma-Aldrich (Steinheim, Germany). Potassium persulfate, sodium nitrite and aluminium chloride were also obtained from Merck. 8 mΩ deionized water from a Millipore Milli-Q water purification system was used to prepare mixtures analyses.

2. Solvent-free Microwave Extraction

The extraction was conducted in a microwave apparatus (NEOS-GR, Milestone Srl, Italy) operating at 2.45 GHz and maximum power of 900 W. The device with specific software is also equipped with a video camera. This environmentally friendly technology is an original "upside down" microwave alembic combining microwave heating and earth gravity. The physical phenomenon, hydrodiffusion, allows the extract diffused outside the plant material, to drop by earth gravity out of the microwave reactor and fall through a perforated Pyrex disc.

Even though the direct interaction of microwaves with biological water favors the release of compounds trapped inside the cells of plant material, water was used as a pretreatment in order to favor the process in this study. The extraction procedure was performed at atmospheric pressure. The leaves (2.5-7.5 g) were placed in a 1 L Pyrex® glass beaker and placed in the micowave oven without addition of any solvent under various power (250-300-350 W) conditions for a certain period of time (30-90 sec). The extract drained by gravity on a condenser outside the microwave irradiation cavity cooled down to room temperature [35]. Then, the mixture was centrifuged (Nüve, CN 180) at 5,000 ×g for 25 min. After centrifugation, the supernatant was filtered through a 0.45 μm syringe filter and stored at −80 °C until analysis for the biochemical measurements.

3. Biochemical Measurements

3-1. Determination of Total Phenolic Content

The concentration of total phenols in the extracts was measured by UV-spectrophotometry (PG Instruments, T60/Leicestershire, England), based on calorimetric oxidation/reduction reaction. The total phenolic content was determined according to the Folin-Ciocalteu method by following the procedure of Malik and Bradford [36]. Folin-Ciocalteu reagent was used as oxidizing agent. To 10 Folin-Ciocalteu reagent of extract, 190 μL of water was added. 1 mL of Folin-Ciocalteu reagent and 800 μL of Na_2CO_3 (75%, w/v) were added. The samples were incubated for 30 min. The absorbance was measured at 760 nm. The amount of total phenolic content was expressed in gallic acid equivalent per g of dried leaf (mg-GAE/g-DL).

3-2. Determination of Total Flavonoid Content

Total flavonoid content was determined by using a slightly modified calorimetric method described by Sakanaka et al. [37]. Briefly, 1.375 mL of distilled water was added into the 0.125 mL of the extract. Then, 75 μL of 5% sodium nitrite solution was added into the mixture. 150 μL of 10% aluminium chloride was added into this solution after 6 minutes. In the next 5 min, 0.5 mL of 1 M sodium hydroxide solution and 275 μL of distilled water were put in the final mixture and mixed. The absorbance was measured by UV-spectrophotometry at 510 nm against a blank. The standart curve was made by using catechin as a standart solution. The amount of total flavonoid content was expressed in mg (+)-catechin equivalent per g of dried leaf (mg-CE/g-DL).

3-3. Determination of Antioxidant Capacity by DPPH Assay

Free radical scavenging activity on DPPH was done according to the method of Yu et al. [38] with some modifications. Samples were tested individually at a final concentration of 100 μM by diluting a methanolic solution of DPPH radical (500 μM). The mixtures were vigorously mixed and allowed to stand in the dark for 30 min at 25 °C, as stated previously [39,40]. The absorbance was measured at 517 nm against a blank sample without DPPH. Results were expressed as mg trolox equivalent antioxidant capacity per gram of dried leaf (mg-TEAC/g-DL).

3-4. Determination of Antioxidant Capacity by ABTS Assay

Free radical scavenging activity of the extracts by ABTS assay was done according to the modified method of Re et al. [41]. After addition of 150 μL of sample solution to 2850 μL of diluted ABTS solution, absorbance was measured at 10^{th} minute at 734 nm against a blank sample without ABTS. Results were expressed as mg trolox equivalent antioxidant capacity per gram of dried leaf (mg-TEAC/g-DL).

3-5. Determination of Antioxidant Capacity by CUPRAC Assay

Cupric ion reducing antioxidant capacity (CUPRAC) method of Apak et al. [42] was also applied to the mandarin leaf extracts exactly the same as reported [42]. The absorbance of the extract samples was measured at 450 nm against a blank sample without CUPRAC. Results were expressed as mg trolox equivalent antioxidant capacity per gram of dried leaf (mg-TEAC/g-DL).

4. Experimental Design

A face central composite design (FCCD) was performed with

Table 1. Values of the independent variables and their coded forms with their symbols employed in RSM for optimization of mandarin leaves through SFME

Independent variables	Units	Symbol of the variables	Coded levels		
			−1	0	1
Extraction time	Sec	X_1	30	60	90
Microwave irradiation power	W	X_2	250	300	350
Solid mass	G	X_3	2.5	5	7.5

three variables to explore the effect of variables on the response (Table 1). Total phenolic content (Y_1) and total flavonoid content (Y_2) were the responses, respectively. Extraction time (X_1), microwave irradiation power (X_2) and solid mass (X_3) were independent variables, selected based on the preliminary experiments including three levels. To apply the FCCD, Design-Expert 8.0.7.1 software (trial version) was used. Twenty experiments were conducted with six replications at the center values to evaluate the pure error sum of squares.

Experimental data were fitted to the quadratic model. The quadratic model proposed is shown as follows in Eq. (1):

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii} X_i^2 + \sum_{i=1}^{3}\sum_{j=i+1}^{3}\beta_{ij} X_i X_j + e \quad (1)$$

where Y is the response, β_0 is the constant coefficient often described as intercept, X_i(i=1-3) is the non-coded variable, β_i is the linear, and β_{ii} is the quadratic, and β_{ij} (i and j=3) is the second order interaction coefficients [43].

5. Statistical Analysis

Three replicate extractions were carried out for each of the samples followed by a minimum of three spectrophotometric measurements from each extract. The analysis of variance (ANOVA) test was applied to identify the interaction between the variables and the response using Design-Expert program. Furthermore, statistical analysis on the means of antioxidant activity assays was carried out using the ANOVA procedure of the InStat® software, version 3.0 (GraphPad, San Diego, CA, USA). Tukey's test of significance between means was used for exhibition of significance in the values of each antioxidant activity method. InStat® software was also used for the Pearson correlation coefficient (r) determination to quantify the correlation between TPC/TFC and antioxidant activity values obtained by each assay.

RESULTS AND DISCUSSION

Possible interactions among process operating parameters should be considered to optimize an extraction process. Those independent parameters can be counted as microwave irradiation power, extraction time, temperature, particle size of the solid material, moisture of the solid material and solid mass in the case of SFME. The present article evaluates some of these parameters depending on the preliminary investigations. The three factors and the levels of factors (lower, middle and upper) for RSM are given in Table 1 with their coded values. Experimental conditions of FCCD runs designed with Design Expert 8.0.7.1 are shown in Table 2. It indicates the influence of extraction time (30-90 sec), irradiation power (250-350 W) and solid mass (2.5-7.5 g) on the TPC and TFC in mandarin leaf extract obtained by SFME.

Table 2. FCCD of the independent variables (X_1, X_2, X_3) and experimental results for the TFC and TPC*

Run number	Independent variables			Responses	
	X_1 (sec)	X_2 (W)	X_3 (g)	TPC (mg-GAE/g-DL)	TFC (mg-CE/g-DL)
1	30	250	2.5	0.4431±0.2600	0.1229±0.0601
2	90	250	2.5	0.5183±0.1615	0.1388±0.0412
3	30	350	2.5	0.7689±0.2722	0.2155±0.0817
4	90	350	2.5	0.5449±0.0334	0.1478±0.0115
5	30	250	7.5	0.2365±0.3109	0.0632±0.0934
6	90	250	7.5	0.4423±0.0901	0.1280±0.0235
7	30	350	7.5	0.4812±0.0446	0.1378±0.0141
8	90	350	7.5	0.5724±0.2434	0.1595±0.0712
9	30	300	5	0.2839±0.3114	0.0802±0.0814
10	90	300	5	0.3259±0.4101	0.0892±0.1126
11	60	250	5	0.3636±0.4400	0.1211±0.1138
12	60	350	5	0.5212±0.0933	0.1533±0.0136
13	60	300	2.5	0.8034±0.0134	0.2188±0.0212
14	60	300	7.5	0.6239±0.1142	0.1904±0.0833
15	60	300	5	0.5661±0.3012	0.1547±0.0813
16	60	300	5	0.5797±0.1923	0.1648±0.0423
17	60	300	5	0.5566±0.0843	0.1738±0.0613
18	60	300	5	0.5679±0.2211	0.1687±0.0300
19	60	300	5	0.5689±0.4510	0.1703±0.0412
20	60	300	5	0.5712±0.2901	0.1712±0.1001

*Data are expressed as the mean (n=9) ±S.D.

1. Model Fitting

ANOVA for the quadratic equations of Design Expert 8.0.7.1 for the TPC and TFC responses are given in Tables 3 and 4, respectively. Regression analyses were done at 95% of confidence interval. According to Table 3, the model derived for TPC was found significant (p<0.0001) to display the relationship between the response and independent variables with an F value of 21.02. The ANOVA result also showed that the experimental data had correlation coefficient (R^2) of 0.9498 with the calculated model, accounting for the 94.98% of the results. As for TFC, the model was also significant (p<0.0001) with an F value of 20.82 based on the ANOVA for the quadratic model (Table 4). According to the high correlation coefficient (R^2=0.9493), the RSM approach applied in this study indicates to be convenient for the TFC of mandarin leaves obtained by means of SFME.

Using response surface methodology from the software, quadratic models for TPC and TFC were derived, respectively. The equations are Eq. (2) and Eq. (3):

$$\begin{aligned} Y = &-2.42618 + 0.033825X_1 + 0.018537X_2 - 0.41546X_3 \\ &- 3.44833E\text{-}005X_1X_2 + 7.43E\text{-}004X_1X_3 + 2.24E\text{-}005X_2X_3 \\ &- 2.21343E\text{-}004X_1^2 - 2.46836E\text{-}005X_2^2 + 0.033527X_3^2 \end{aligned} \quad (2)$$

Table 3. ANOVA for the quadratic equations of Design Expert 8.0.7.1 for the SFME of TPC in mandarin leaves

Source	Sum of squares	df	Mean square	F value	P-value Prob>F
Model	0.36	9	0.04	21.02	<0.0001
X_1-time	3.618E-003	1	3.618E-003	1.88	0.1998
X_2-power	0.078	1	0.078	40.79	<0.0001
X_3-solid mass	0.052	1	0.052	27.18	0.0004
X_1X_2	0.021	1	0.021	11.15	0.0075
X_1X_3	0.025	1	0.025	12.94	0.0049
X_2X_3	6.272E-005	1	6.272E-005	0.033	0.8602
X_1^2	0.11	1	0.11	56.86	<0.0001
X_2^2	0.01	1	0.01	5.46	0.0416
X_3^2	0.12	1	0.12	62.92	<0.0001
Residual	0.019	10	1.919E-003		
Lack of fit	0.019	5	3.782E-003	67.40	0.0001
Pure error	2.806E-004	5	5.611E-005		
Cor total	0.38	19			

Table 4. ANOVA for the quadratic equations of Design Expert 8.0.7.1 for the SFME of TFC in mandarin leaves

Source	Sum of squares	df	Mean square	F value	P-value Prob>F
Model	0.03	9	3.288E-003	20.82	<0.0001
X_1-time	1.910E-004	1	1.910E-004	1.21	0.2972
X_2-power	5.755E-003	1	5.755E-003	36.45	<0.0001
X_3-solid mass	2.719E-003	1	2.719E-003	17.22	0.0020
X_1X_2	2.007E-003	1	2.007E-003	12.72	0.0051
X_1X_3	2.391E-003	1	2.391E-003	15.14	0.0030
X_2X_3	2.531E-006	1	2.531E-006	0.016	0.9018
X_1^2	0.012	1	0.012	74.48	<0.0001
X_2^2	4.570E-004	1	4.570E-004	2.89	0.1197
X_3^2	8.171E-003	1	8.171E-003	51.75	<0.0001
Residual	1.579E-003	10	1.579E-004		
Lack of fit	1.345E-003	5	2.691E-004	5.76	0.0386
Pure error	2.334E-004	5	4.668E-005		
Cor total	0.031	19			

$$Y=-0.58106+0.010879X_1+4.18462E\text{-}003X_2-0.10899X_3 -1.05583E\text{-}005X_1X_2+2.305E\text{-}004X_1X_3+4.5E\text{-}006X_2X_3 -7.26566E\text{-}005X_1^2-5.15636E\text{-}006X_2^2+8.72145E\text{-}003X_3^2 \quad (3)$$

2. Optimization of Conditions for SFME of Mandarin Leaves

As seen in Table 2, twenty experimental runs were chosen randomly by the design expert software in the experiments. Microwave irradiation power (X_2) was found as the most significant ($p<0.0001$) variable on the SFME of both TPC and TFC from mandarin leaves, followed by solid mass (X_3). All the linear, quadratic and interaction parameters were significant, except for the time (X_1) and X_2X_3 at the level of $p>0.05$ (Tables 3 and 4).

To estimate the interaction among the different independent variables and their corresponding effect on the response, three-dimensional response surface plots (Figs. 1-6) were constructed according to Eq. (2) and Eq. (3), respectively. Figs. 1 and 2 show the effect of interaction between irradiation power and time on TPC and TFC of the leaves under a constant material mass (2.5 g). The extraction yields of both TPC and TFC had a tendency to increase by increasing the irradiation power and time until a certain point. With a further increase in the related operation parameters, polyphenol yields started to decrease. Périno-Issartier et al. [28] displayed a similar trend on the antioxidant extraction from sea buckthorn (*Hippophae rhamnoides*) food by-products. They observed high speed of extraction at high powers such as 500-900 W through SFME. However, those conditions resulted in less total dry extract yields due to the degradation of compounds. Hayat et al. [6] also observed that increase in power 100 to 160 W with time, liquid to solid ratio and solvent concentration enhanced the extraction yield when extracting phenolic acids from citrus mandarin peels by means of MAE [6]. However, there was a decrease in the yield over a microwave irradiation power of 160 W. In addition, the results of the pres-

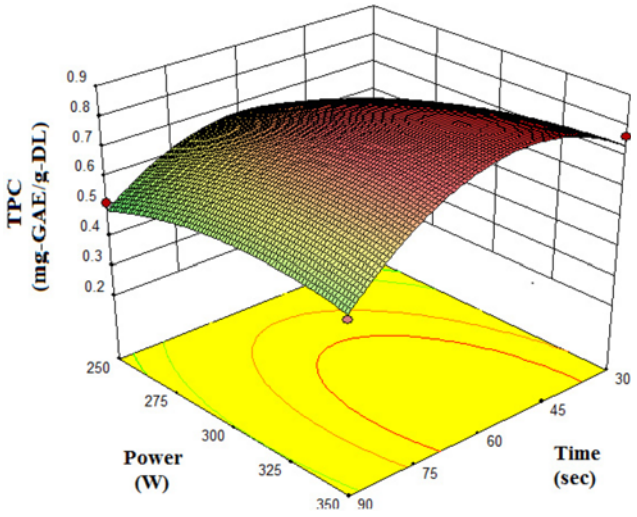

Fig. 1. Response surface plot for the TPC of the mandarin leaf extract as a function of microwave irradiation power to extraction time (solid mass=2.5 g).

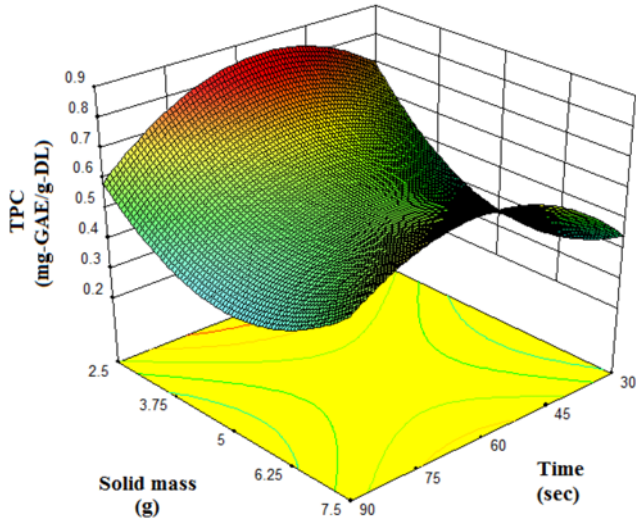

Fig. 3. Response surface plot for the TPC of the mandarin leaf extract as a function of solid mass to extraction time (power =339.190 W).

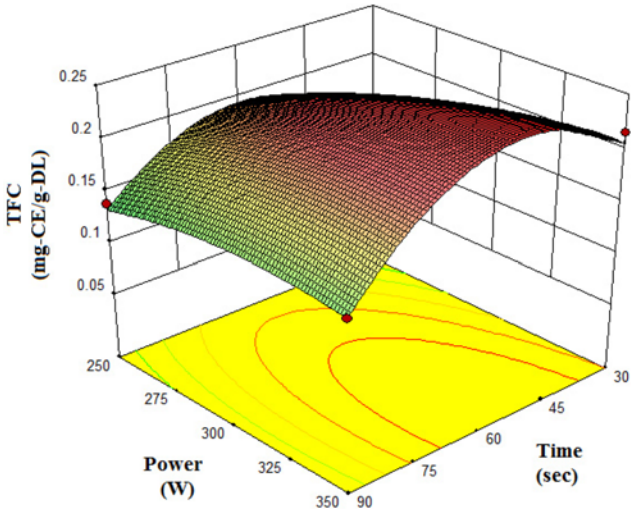

Fig. 2. Response surface plot for the TFC of the mandarin leaf extract as a function of microwave irradiation power to extraction time (solid mass=2.5 g).

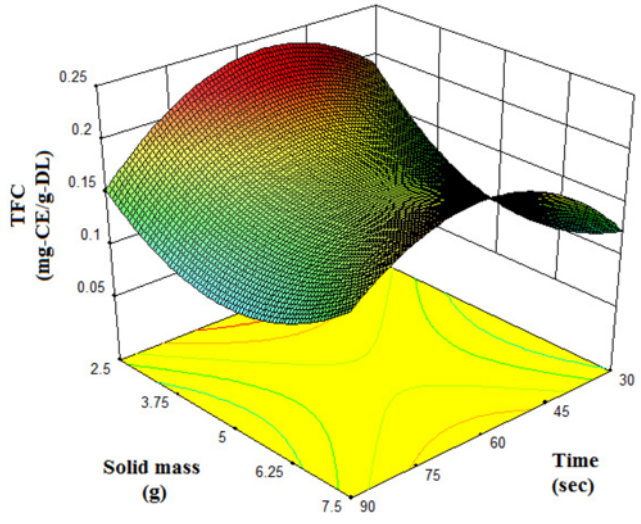

Fig. 4. Response surface plot for the TFC of the mandarin leaf extract as a function of solid mass to extraction time (power =339.190 W).

ent study are also in good agreement with those of Pan et al. [16] and Karabegović et al. [44] with respect to time effect on the MAE process. Pan et al. [16] indicated that the extraction of caffeine from green tea leaves started to decrease by time after 4 min. Karabegović et al. [44] displayed that the extract yield of cherry laurel fruits rapidly with the increase in time from 10 to 25 min. Then, they observed a drop in the yield by increasing the time.

Figs. 3 and 4 exhibit the influence of extraction time and solid mass on phenolic and flavonoid material obtained by SFME (power =339.190 W). Time exhibited the same trend as explained above. Both TPC and TFC decreased by increasing the mass from 2.5 to 5.0 g. There was a significant reduction (approximately 30%) in TPC and TFC yields under the condition of 60 sec and 300 W. Then, the yields began to rise, but still less than that of 2.5 g. The reduction rate in TPC dropped to almost 22% at the solid mass of 7.5 g. In case of TFC, there was almost 13% reduction when the solid mass increased from 2.5 to 7.5 g. This phenomenon is consistent with that of Ballard et al. [20], who extracted phenolic antioxidant compounds from peanut skins [20]. They showed that an increase in the mass of the skins from 1.5 to 3.5 g caused a 35.8% reduction in TPC. This could be explained by the fact that increasing the sample mass decreased the surface area. Increasing the sample mass lowers the surface area available for the extracting agent to penetrate the sample matrix and solubilize the phenolics, thereby causing a reduction in extraction yield of these compounds.

Figs. 5 and 6 are the response surface plots to show the effect of microwave irradiation power and solid mass on the TPC and TFC of the extracts obtained by SFME, based on the equations. The yields

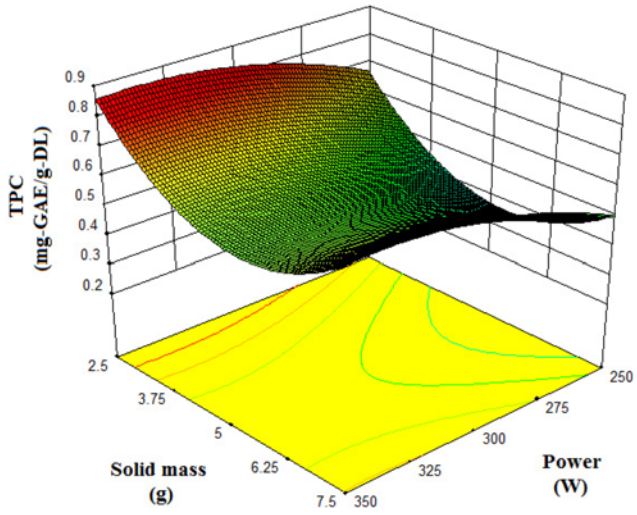

Fig. 5. Response surface plot for the TPC of the mandarin leaf extract as a function of solid mass to microwave irradiation power (time=53.155 sec).

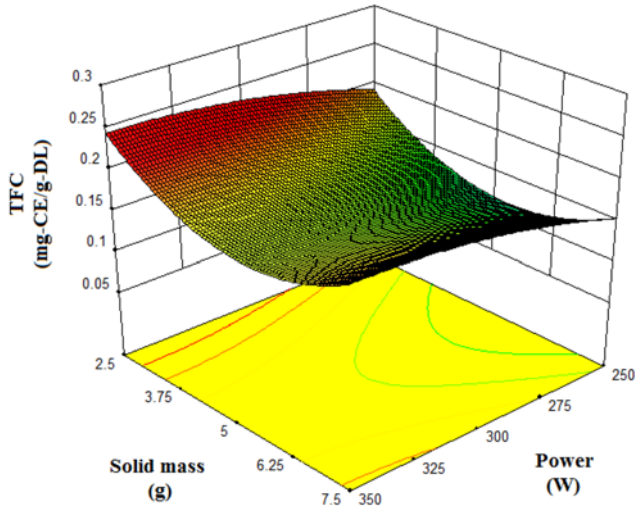

Fig. 6. Response surface plot for the TFC of the mandarin leaf extract as a function of solid mass to microwave irradiation power (time=53.155 sec).

increased by increasing power at a fixed extraction time (53.155 sec). Conversely, they both decreased with mass as stated in the previous paragraph.

3. Antioxidant Capacity of Mandarin Leaves

Three complementary assays such as DPPH, ABTS and CUPRAC were carried out, since antioxidant activity is a complex process occurring through several mechanisms [26]. Table 5 shows the antioxidant activity of the extracts with the statistical analysis of the related experiments. Data were expressed as trolox equivalent with respect to inhibition of DPPH and ABTS radicals, and CUPRAC. Antioxidant capacity measured by CUPRAC method changed between 0.3397±0.40 and 1.1539±0.39 mg-TEAC/g-DL through various conditions of SFME. Generally, the samples extracted under high irradiation power with low mass showed greater antioxidant

Table 5. Antioxidant capacity of the mandarin leaves extracted by SFME measured by various assays (DPPH, ABTS and CUPRAC methods)*

Run number	Independent variables			Antioxidant activity assays		
	X_1 (sec)	X_2 (W)	X_3 (g)	CUPRAC (mg-TEAC/g-DL)	DPPH (mg-TEAC/g-DL)	ABTS (mg-TEAC/g-DL)
1	30	250	2.5	0.6084±0.2800ac	0.0948±0.0501ac	0.0722±0.0114ad
2	90	250	2.5	0.7170±0.1943bc	0.1283±0.0211abc	0.0930±0.0300bd
3	30	350	2.5	1.1539±0.3901ab	0.1708±0.0515a	0.1490±0.0333ac
4	90	350	2.5	0.7873±0.0501abc	0.1205±0.0233ac	0.0883±0.0412ad
5	30	250	7.5	0.3397±0.4000c	0.0660±0.0210c	0.0370±0.0215d
6	90	250	7.5	0.6496±0.1412ab	0.0870±0.0109ac	0.0778±0.0801ad
7	30	350	7.5	0.6766±0.0616ab	0.0981±0.0102ac	0.0928±0.0814a
8	90	350	7.5	0.7573±0.3734ab	0.0942±0.0603ac	0.0946±0.0526a
9	30	300	5	0.4124±0.4723c	0.0773±0.0908c	0.0534±0.0316d
10	90	300	5	0.4623±0.5437c	0.0900±0.0600ac	0.0588±0.0709d
11	60	250	5	0.5056±0.6043c	0.0966±0.1004ac	0.0527±0.0144d
12	60	350	5	0.6708±0.0810abc	0.1001±0.0218ac	0.0864±0.0623a
13	60	300	2.5	1.1217±0.1200ab	0.1957±0.0313b	0.1424±0.0200a
14	60	300	7.5	0.5565±0.3516c	0.0722±0.0623c	0.0773±0.0016a
15	60	300	5	0.7600±0.2516ab	0.1599±0.0200ab	0.1755±0.0414c
16	60	300	5	0.7961±0.2718ab	0.1409±0.0111acb	0.1700±0.0303c
17	60	300	5	0.7856±0.1338ab	0.1531±0.0403acb	0.1781±0.0401c
18	60	300	5	0.7774±0.2224ab	0.1467±0.0805acb	0.1699±0.0200c
19	60	300	5	0.7944±0.0941ab	0.1501±0.0604acb	0.1790±0.0202c
20	60	300	5	0.8080±0.1604ab	0.1488±0.0815acb	0.1767±0.0410c

*Data are expressed as the mean (n=9) ±S.D. Means within the same column not sharing a common letter indicate significant difference at $p<0.05$

capacity, sharing the same values at $p>0.05$. As for DPPH method, antioxidant activity ranged from 0.0660±0.02 to 0.1708±0.05 mg-TEAC/g-DL. Values obtained by ABTS method had the same tendency as those of DPPH method, which were between 0.0370± 0.02 and 0.1790±0.02 mg-TEAC/g-DL.

To explore the the relationships between antioxidant activity and total phenolic and flavonoid contents in mandarin leaves, the correlation must be considered. The positive correlation between phenolic (r=0.6814-0.9282, p=0.05), flavonoid (r=0.7072-0.8842, p=0.05) content and antioxidant activity of the extracts proves that phenolic compounds and flavonoids significantly contribute to the antioxidant capacity of the leaves. The CUPRAC assay showed higher antioxidant activity values than those of DPPH and ABTS, which is in agreement with the results of Li et al. [45,46]. They had higher antioxidant values with CUPRAC compared to DPPH and ABTS methods in grape seed powder and China wines, respectively. A higher correlation between the TPC/TFC and CUPRAC (r=0.9282/0.8842, p=0.05) was observed, compared to DPPH (r=0.7717/0.7435, p= 0.05) and ABTS (r=0.6814/0.7072, p=0.05). Pan et al. [47] explained this phenomenon by the fact that the antioxidant activity of the target compounds was attributed to various mechanisms among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging [47].

CONCLUSIONS

The results of the present study suggest that 53.155 sec of extraction time, 339.190 W of microwave power and 2.5 g of plant material should be employed as optimal operating conditions to extract the greatest TPC (0.8610 mg-GAE/g-DL) and TFC (0.2440 mg-CE/g-DL) from mandarin leaves through SFME. Linear coefficient of microwave irradiation power and square coefficient of time and solid mass were the most significant variables on the SFME of both TPC and TFC from mandarin leaves. TPC and TFC in the leaf extracts were highly correlated with the antioxidant capacity values obtained by CUPRAC method, while they were both less correlated with DPPH and ABTS methods. Most of all, the positive correlation between phenolic and flavonoid contents and antioxidant activity of the extracts measured by various methods proved that phenolic compounds and flavonoids significantly contribute to the antioxidant capacity of the leaves. Finally, as a green, easy to set up and rapid separation method, SFME can be an alternative to other novel extraction techniques to obtain extracts rich in polyphenols with a high antioxidant capacity.

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