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# Optimization of Microwave-Assisted Extraction of Polyphenolic Compounds from *Ocimum basilicum* by Response Surface Methodology

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**Abstract** Microwave-assisted extraction (MAE) was optimized by response surface methodology in order to enhance the extraction of polyphenols from basil (*Ocimum basilicum L*). Box–Behnken experimental design on three levels and three variables was used for optimization. Influence of ethanol concentration (50, 70, and 90%); microwave power (400, 600, and 800 W); and extraction time (15, 25, and 35 min) on each response were investigated. Experimental results were fitted to a second-order polynomial model, and multiple regression analysis and analysis of variance were used to evaluate model fitness and optimal conditions. Considering the maximum content of extracted total phenols, total flavonoids, and antioxidant activity, the optimal conditions for all investigated response were ethanol concentration of 50%, microwave power of 442 W, and extraction time of 15 min. Under the optimal conditions, obtained basil liquid extract contained 4.299 g gallic acid equivalents/100 g dry weight (DW) of total polyphenols, 0.849 g catechin equivalents/100 g DW of total flavonoids, and $IC_{50}$ and $EC_{50}$ values of 9.602 and 82.889 μg/mL, respectively. The development of simultaneous MAE procedure for extraction of total phenols, total flavonoids, and potential antioxidants from basil, represented valorization of basil as valuable source of bioactive compounds.

**Keywords** Basil (*Ocimum basilicum* L.) · Polyphenols · Flavonoids · Antioxidant activity · Response surface methodology

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## Introduction

Food industries are interested in the use of antioxidants or food rich in antioxidant compounds, as food preservatives or as dietetic supplements (Gil-Chavez et al. 2013). Nowadays, great attention is on a natural antioxidants or herbal extracts rich in antioxidants, from plant sources, in particular aromatic herbs and spices. Basil (*Ocimum basilicum L.*) belongs to the Lamiaceae family, highly aromatic with pleasant taste used mostly in culinary. It is native to Asia, Africa, South America, and the Mediterranean but widely cultivated in many countries. Basil is economically important for essential oil (EO) production. The EO of basil extracted via steam distillation from the leaves and flowering tops are used to flavor foods, dental and oral products, fragrances, and medicines (Simon et al. 1999). Basil EO possess antiviral (Sanchez et al. 2010), antimicrobial (Carocho et al. 2016; Nebedum et al. 2009; Ngueface et al. 2004), anti-inflammatory, insecticidal (Keita et al. 2000; Umeria et al. 1998) and antioxidant activities (Filip et al. 2016; Pripdeevech et al. 2010). It is known that basil is a rich source of polyphenolic compounds and flavonoids such as cinnamic acid, caffeic acid, rosmarinic acid, chicoric acid, quercetin, kaempherol, etc. (Grayer et al. 2002; Javanmardi et al. 2002; Jayasinghe et al. 2003; Lee and Scagel 2009; Tada et al. 1996). Additionally, its phenolic compounds and flavonoids have shown to be powerful antioxidants, free radical scavengers, and metal chelators (Flanigan and Niemeyer 2014; Jayasinghe et al. 2003; Pietta 2000). Moreover, phytochemicals such as phenolic compounds from plants, are of great interest due to their health-benefitting antioxidant properties and possible protection against inflammation, cardiovascular diseases, and certain types of cancer.

Extracts from natural plants, as carriers of antioxidant compounds and antioxidant activity, could be obtained by different extraction techniques. Conventional extraction techniques

including maceration, percolation, Soxhlet extraction, and soaking, have certain drawbacks, such as long extraction time, huge amount of solvent and heating, with risk of thermal degradation of thermolabile active compounds. Therefore, various novel extraction techniques have been introduced and investigated, most of which were claimed to be better in terms of efficiency, solvent consumption, and extraction time. The novel techniques available are microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), and pressurized solvent extraction (PSE). MAE has drawn significant research attention in various fields, in particular medicinal plant research, due to its special heating mechanisms, moderate capital cost, and its good performance under atmospheric conditions (Chan et al. 2011; Filly et al. 2014; Veggi et al. 2013). Microwave consists of electromagnetic field which oscillates in frequency range from 0.3 to 300 GHz. Microwave can penetrate into herbal materials and interact with the polar compounds, causing heat generation. The heating of microwave energy acted directly on molecules by ionic conduction and dipole rotation (Zhang et al. 2011). Based on these principals, compounds with high and low dipolar moment can be extracted in various proportions by microwave extraction.

Considering the maximization of extraction yield or target compound(s), with respect to the quality of extracts, optimization of microwave power and extraction time, and other potential operating parameters are usually performed by response surface methodology (RSM). This methodology has been used to optimize the extraction processes of polyphenols from various plant materials through conventional and non-conventional extraction techniques. RSM is an often-used approach on the process optimization where influence of independent variables on response is investigated one by one, while all other factors are kept under constant values (Baş and Boyacı 2007). This experimental methodology combines mathematical and statistical techniques useful for developing, improving, and optimizing process. Analyzing the effect of the independent variables on response variables, this experimental methodology allows generation of mathematical model which could describe the process in investigated experimental domain.

So far, microwave treatment has been widely employed to extract total phenolics from different plant materials (Bouras et al. 2015; Milutinović et al. 2014; Simić et al. 2016; Zeković et al. 2016). To the best of our knowledge, the influence of MAE for the recovery of polyphenols from *O. basilicum* has not been reported yet. The objective of this study was to employ RSM to assess the effect of different combination of ethanol concentration as extraction solvent (50–90%), irradiation power of MAE (400–800 W), and extraction time (15–35 min) on the total phenols content (TP), total flavonoids content (TF), and antioxidant activity of basil liquid extracts obtained by MAE. For this purpose, this research aims to be a useful engineering tool to develop an extraction process from basil, considering the difficulty of obtaining natural plant extracts with specific compounds

## Materials and Methods

### Plant Material

Basil was cultivated at the Institute of Field and Vegetable Crops, Novi Sad, Alternative Crops Department, Bački Petrovac, Serbia, in 2013. The collected plant material (leaves and flowering tops) were air dried and stored at room temperature. The dried basil was grounded in a domestic blender and the mean particle size (0.363 mm) of material was determined using sieve sets (CISA, Cedaceria Industrial, Spain). Moisture content (10.824%) of basil was analyzed using standard procedure, i.e., by drying the plant sample at 105 °C until constant weight. This analysis was performed in three replicates.

### Chemicals

Folin-Ciocalteu reagent, (±)-catechin and 1,1-diphenyl-2-picryl-hydrazyl-hydrate (DPPH) were purchased from Sigma (Sigma-Aldrich GmbH, Steinheim, Germany). Gallic acid was purchased from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical grade.

### MAE Procedure

Mono-mode microwave-assisted extraction (MAE) was performed in homemade setup consisting of microwave oven (NN-E201W, Panasonic, Japan) and appropriate glass apparatus with round flask and condenser (Zeković et al. 2016). Seventeen experimental MAE runs on different extraction time, ethanol concentration and irradiation power, were performed. In each experimental run, 5.0 g of sample were mixed with 50 mL of extraction solvent in 500-mL round flasks. Flasks were then placed in MAE apparatus and extractions were performed on fixed frequency. Flasks were always positioned on the same distance from the magnetron and no additional agitation was applied. After the extraction, crude extracts were immediately filtered through filter paper (4–12 μm pore size, Schleicher and Schuell, Germany) under vacuum. Extracts were collected into glass flasks and stored at 4 °C until further analysis.

### Screening of Variables

Large number of parameters could affect on the investigated extraction system, therefore, it is of great importance to select those parameters with major effects. The factors that may

influence on MAE are solvent nature, solvent to solid ratio, extraction time, microwave power, temperature, sample characteristics, extraction cycles, etc. It is important to understand the effects and interactions of these factors on the MAE process.

Selection of the most suitable solvent was performed as initial step. There are many reports suggesting that ethanol is the most appropriate solvent for the extraction of various polyphenolic compounds from the herbal materials (Do et al. 2014; Zeković et al. 2014). Ethanol concentration was chosen based on the total phenolics, flavonoids, and antioxidant activity in obtained basil extracts prepared by maceration, previously reported by Vidović et al. (2012). Selection of conditions ranges for the MAE was based on the literature data (Zeković et al. 2016). Influence of the microwave power was in the range of 400 to 800 W, and for extraction time in the range of 15 to 35 min. Details of analysis are presented in Table 1.

### Total Phenolics Content

Total phenolics content (TP) in obtained basil liquid extracts was determined using Folin-Ciocalteu procedure (Kähkönen et al. 1999; Singleton and Rossi 1965). Gallic acid was used as standard for preparation of standard curve, and absorbance of the samples was measured at 750 nm (6300 Spectrophotometer, Jenway, UK). Content of phenolic compounds was expressed as grams of gallic acid equivalents (GAE) per 100 g dry weight (DW). All experiments were prepared in triplicate, and results are expressed as mean values.

### Total Flavonoids Content

Total flavonoids content (TF) was determined using aluminum chloride colorimetric assay (Harborne 1984). Catechin was used for preparation of standard curve and absorbance was measured at 510 nm. Results were expressed as grams of catechin equivalents (CE) per 100 g DW. All experiments were prepared in triplicate, and results are expressed as mean values.

**Table 1** Experimental domain used in Box–Behnken design (BBD)

| Symbols | Independent variables | Coded levels | | |
|---|---|---|---|---|
| | | −1 | 0 | +1 |
| $X_1$ | Ethanol concentration [%] | 50 | 70 | 90 |
| $X_2$ | Extraction time [min] | 15 | 25 | 35 |
| $X_3$ | Microwave power [W] | 400 | 600 | 800 |

### Antioxidant Assay—DPPH Test

The free radical scavenging activity of basil extracts was determined using simple and fast spectrophotometric method as described by Espin et al. 2000. Briefly, prepared extracts were mixed with methanol (96%) and 90 μM 2,2-diphenyl-1-picryl-hydrazyl (DPPH) to give different final concentrations. After 60 min at room temperature, the absorbance of samples was measured at 515 nm. Radical scavenging capacity (%RSC) was calculated by the following equation:

$$\%\mathrm{RSC} = 100-\left(A_{\mathrm{sample}} \times 100\right)/A_{\mathrm{blank}} \quad (1)$$

where $A_{\mathrm{sample}}$ is the absorbance of sample solution and $A_{\mathrm{blank}}$ is the absorbance of control. Antioxidant activity was also expressed as $IC_{50}$ which represent the concentration of test (extract solution) required for obtaining the 50% of radical scavenging capacity, expressed as micrograms per milliliter. All experiments were prepared in triplicate, and results are expressed as mean values.

### Reducing Power Assay

Reducing power of the samples was determined according to assay based on the reduction of $Fe^{3+}$ by polyphenol antioxidants, previously described by Oyaizu (1986). Different dilutions of liquid extract (1 mL) were mixed with phosphate buffer (1 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide (1 mL) in glass tubes. Tubes were incubated on 50 °C for 20 min. After incubation, 10% trichloroacetic acid solution (1 mL) was added to the reaction mixture. Tubes were then centrifuged at 3000 rpm for 10 min and supernatant (2 mL) was further mixed with double-distilled water (2 mL) and 0.1% $FeCl_3$ solution (0.4 mL). Absorbance was measured at 700 nm. The blank probe was prepared by using proper extraction solvent instead of sample. Antioxidant activity was further expressed as $EC_{50}$ value (μg/mL), which causes reduction of 50% $Fe^{3+}$ ions in reaction mixture. All experiments were prepared in triplicate, and results are expressed as mean values.

### Experimental Design and Statistical Analysis

The RSM was used for investigation of effects of extraction parameters and optimized the conditions for various responses. A Box–Behnken design (BBD) with three variables (Box and Behnken 1960) was used to determine the response pattern and then to establish a model. The complete design was carried out in random order and consisted of 17 combinations including five replicates at central point .The three independent variables used in this study were ethanol concentration ($X_1$, 50–90%); extraction time ($X_2$, 15–35 min); and irradiation power ($X_3$, 400–800 W). In order to normalize

parameters, each of the coded variables was forced to range from −1 to 1, so that they all affect the response more evenly, and so the units of the parameters are irrelevant (Baş and Boyacı 2007). Variables were coded according to the following equation (Wang et al. 2013):

$X = (x_i - x_0)/\Delta x$ 1).

where $X$ is the coded value, $x_i$ is the corresponding actual value, $x_0$is the actual value in the centre of the domain, and $\Delta x$is the increment of $x_i$ corresponding to a variation of 1 unit of $X$. The coded values of the experimental factors and their levels for the BBD are shown in Table 1.

The response variables were fitted to the following second-order polynomial model (Eq. (2)) which is generally able to describe relationship between the responses and the independent variables:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum \sum_{i<j=1}^{3} \beta_{ij} x_i x_j \quad (2)$$

where $Y$ represents the response variable; $X_i$and$X_j$are the independent variables affecting the response; and$\beta_0$ , $\beta_i$ , $\beta_{ii}$ , and $\beta_{ij}$are the regression coefficients for intercept, linear, quadratic, and interaction terms, respectively.

Optimal extraction parameters were obtained for four different responses, TP, TF, radical scavenging activity, and reducing power. Treatment of multiple responses and selection of optimal conditions were based on desirability function, $D$ (Derringer 1980; Myers et al. 2009). The experimental design and multiple linear regression analysis were performed using Design-Expert v.7 Trial (Stat-Ease, Minneapolis, MN, USA). The results were statistically tested by analysis of variance (ANOVA) with the 0.05 significance levels. The adequacy of the models was evaluated by the coefficient of determination ($R^2$), coefficient of variance (CV), and $p$ values for the model and lack of fit testing.

## Results and Discussion

### Fitting the Models

A Box–Behnken experimental design was employed to optimize tree important operating variables (ethanol concentration, extraction time, and microwave power) in order to achieve maximal content of particular groups of compounds (TP and TF) and maximal antioxidant activity ($IC_{50}$ and $EC_{50}$). Obtained experimental results are presented in the Table 2. Multiple regression coefficients from Eq. (2) were generated for all responses using statistical approach called the method of least square (MLS) which represents a multiple regression technique used to fit a mathematical model to a set of experimental data generating the lowest residual possible (Bezerra et al. 2008). The regression coefficients of the model for each response are summarized in Table 3, and analysis of variance (ANOVA) results are presented in the Table 4.

**Table 2** Box–Behnken experimental design with natural and coded MAE conditions and experimentally obtained values of total phenols content (TP), total flavonoids content (TF), antioxidant activity ($IC_{50}$) and reducing power ($EC_{50}$)

| Run order | Independent variable | | | Investigated responses | | | |
|---|---|---|---|---|---|---|---|
| | Ethanol concentration [%] | Extraction time [min] | Microwave power [W] | TP [g GAE/ 100 g DW] | TF [g CE/ 100 g DW] | $IC_{50}$ [μg/ mL] | $EC_{50}$ [μg/ mL] |
| 1 | 70 (0) | 25 (0) | 600 (0) | 2.526 | 1.0202 | 13.709 | 113.461 |
| 2 | 70 (0) | 15 (−1) | 800 (1) | 2.762 | 1.3570 | 13.173 | 130.855 |
| 3 | 90 (1) | 15 (−1) | 600 (0) | 1.925 | 0.3309 | 13.734 | 134.360 |
| 4 | 70 (0) | 25 (0) | 600 (0) | 2.840 | 0.8912 | 14.160 | 104.779 |
| 5 | 70 (0) | 35 (1) | 800 (1) | 2.992 | 0.9807 | 13.845 | 108.138 |
| 6 | 50 (−1) | 35 (1) | 600 (0) | 4.580 | 1.0220 | 9.954 | 112.068 |
| 7 | 90 (1) | 25 (0) | 800 (1) | 1.712 | 0.3585 | 16.491 | 130.465 |
| 8 | 50 (−1) | 15 (−1) | 600 (0) | 4.636 | 1.0004 | 9.458 | 106.230 |
| 9 | 70 (0) | 25 (0) | 600 (0) | 2.397 | 0.8912 | 15.364 | 113.421 |
| 10 | 50 (−1) | 25 (0) | 800 (1) | 4.176 | 1.0936 | 16.170 | 95.531 |
| 11 | 70 (0) | 25 (0) | 600 (0) | 2.728 | 0.8661 | 14.277 | 103.406 |
| 12 | 70 (0) | 15 (−1) | 400 (−1) | 2.677 | 0.9377 | 11.229 | 100.050 |
| 13 | 90 (1) | 25 (0) | 400 (−1) | 1.869 | 0.3627 | 14.841 | 128.590 |
| 14 | 70 (0) | 35 (1) | 400 (−1) | 2.760 | 0.8876 | 10.872 | 123.244 |
| 15 | 50 (−1) | 25 (0) | 400 (−1) | 4.074 | 1.0757 | 15.006 | 85.542 |
| 16 | 90 (1) | 35 (1) | 600 (0) | 1.223 | 0.3559 | 11.37 | 120.778 |
| 17 | 70 (0) | 25 (0) | 600 (0) | 2.245 | 0.8768 | 14.155 | 118.756 |

**Table 3** Corresponding $p$ values of linear, interaction, and quadratic terms of regression coefficients, obtained for selected response variables

| Term | Response | | | |
|---|---|---|---|---|
| | TP | TF | $IC_{50}$ | $EC_{50}$ |
| Linear | | | | |
| $X_1$ | <0.0001 | < 0.0001 | 0.0690 | 0.0010 |
| $X_2$ | 0.6184 | 0.2399 | 0.5865 | 0.7400 |
| $X_3$ | 0.7679 | 0.1184 | 0.0251 | 0.2332 |
| Interaction | | | | |
| $X_{12}$ | 0.3202 | 0.9875 | 0.1813 | 0.2345 |
| $X_{13}$ | 0.6809 | 0.9188 | 0.8081 | 0.6034 |
| $X_{23}$ | 0.8146 | 0.1627 | 0.6098 | 0.0179 |
| Quadratic | | | | |
| $X_{11}$ | 0.0481 | 0.0010 | 0.8830 | 0.7855 |
| $X_{22}$ | 0.2334 | 0.4249 | 0.0002 | 0.1141 |
| $X_{33}$ | 0.7021 | 0.1260 | 0.0352 | 0.6436 |

$p < 0.01$ highly significant; $0.01 \leq p < 0.05$ significant; $p \geq 0.05$ not significant

According to particularly high values of coefficient of multiple determination ($R^2$) for TP, TF, and $IC_{50}$ (0.960, 0.948, and 0.908), applied polynomial model were in accordance with experimental results. Although, $R^2$ was relatively high in case of $EC_{50}$ (0.869), it was significantly lower compared to other responses. Furthermore, coefficient of variance (CV) which represents dispersion degree of the data, is low (≤10%) for almost all responses, indicating good reproducibility of investigated system. Complete information about the model adequacy could be provided by model and lack-of-fit testing. According to statistically significant values of $p$ values (<0.05) for all investigated responses (Table 4), it was possible to conclude that applied model provides proper representation of experimental results. Also, assumption of the constant variance was satisfied since lack-of-fit testing was insignificant ($p > 0.05$). Acquired experimental data was used for creation of response surface three-dimensional plots for each response.

## Influence of the Extraction Parameters on Total Phenolics Content

The TP content in obtained basil liquid extracts varied from 1.223 to 4.636 g GAE/100 g DW. The lowest yield of TP was obtained on higher level of ethanol concentration (90%) and extraction time (35 min), and middle level of microwave power (600 W), while TP was found to be highest on lower level of ethanol concentration (50%) and extraction time (15 min), and middle level of microwave power (600 W). Generally, low TP yields (1.223–1.925 g GAE/100 g DW) were obtained with 90% ethanol as a solvent, while higher TP yields (4.074–4.636 g GAE/100 g DW) were obtained using 50% ethanol as extraction solvent.

According to $p$ values of regression coefficients (Table 3), only linear term of ethanol concentration had highly significant ($p < 0.0001$) influence, while quadratic term of ethanol concentration had significant influence ($p < 0.05$) on TP. All other effects are insignificant. Linear term of ethanol concentration and extraction time exhibited negative influence, except microwave power. The positive effects of independent variables revealed that their positive changes can cause an increase in the response value (Yang et al. 2009).

The second-order polynomial model used to express the TP content as a function of independent variables is shown below:

$$\begin{aligned} TP = {} & 2.55 - 1.34X_1 - 0.056X_2 \\ & + 0.033X_3 - 0.16X_1X_2 - 0.065X_1X_3 + 0.037X_2X_3 \\ & + 0.35X_1^2 + 0.19X_2^2 + 0.059_3^2 \end{aligned} \quad (3)$$

Response surface plots (Fig. 1a) shows that TP of basil extracts decreased with increase ethanol concentration from 50 to 90%. Moreover, significant negative effect of quadratic term of ethanol concentration shows that TP reaches maximum near lower level of ethanol concentration (50%), then rapidly starts to decrease with increase of ethanol concentration. From previous, it can be concluded that ethanol concentration significantly affected extraction of phenolic compounds from basil. In our study, we used ethanol as a solvent because of its extraction potency and safety for human consumption and environment. A combination of alcohol with water is more effective in extracting polyphenols than alcohol alone, because the extraction and the separation of polyphenols depend largely on the polarity of solvents and the compounds (Prasad et al. 2011). Presence of certain amount of water probably causes the swelling of plant material, which causes the increase of the contact area between the solvent and plant matrix, and has a direct impact on the extraction efficiency. Moreover, in order to get maximum content of target compounds, extraction can be carried with the best solvent mixture ratio basis on the chemical composition and polarity of target compound(s). So, the solvent chosen in MAE needs to be able to absorb the microwave energy and convert this energy in heat, which depends on the solvent dielectric properties (Zhang et al. 2011).

The graphs in Fig. 1 also shows that the TP content slightly increased with the increase of microwave power from 400 to 600 W, and then slightly decrease with increasing microwave power until 800 W. This could be explained with following phenomenon: the higher microwave power caused an increase of the system temperature and enhanced the extraction of components other than polyphenols, which caused a relative

**Table 4** Analysis of variance (ANOVA) of the fitted second-order polynomial model for TP, TF, $IC_{50}$, and $EC_{50}$ values

| Source | Sum of squares | Degrees of freedom | Mean square | *F* value | *p* value |
|---|---|---|---|---|---|
| Total phenolics content | | | | | |
| Model | 15.31 | 9 | 1.70 | 18.66 | 0.0004 |
| Residual | 0.64 | 7 | 0.091 | | |
| Lack of fit | 0.41 | 3 | 0.14 | 2.32 | 0.2167 |
| Pure error | 0.23 | 4 | 0.058 | | |
| Total | 15.95 | 16 | | | |
| $R^2 = 0.960$ | | | | | |
| CV = 10.67% | | | | | |
| Total flavonoids content | | | | | |
| Model | 1.39 | 9 | 0.15 | 14.17 | 0.0010 |
| Residual | 0.08 | 7 | 0.011 | | |
| Lack of fit | 0.06 | 3 | 0.020 | 5.09 | 0.0749 |
| Pure error | 0.02 | 4 | $3.969 \times 10^{-3}$ | | |
| Total | 1.47 | 16 | | | |
| $R^2 = 0.948$ | | | | | |
| CV = 12.42% | | | | | |
| $IC_{50}$ value | | | | | |
| Model | 64.26 | 9 | 7.14 | 7.69 | 0.0068 |
| Residual | 6.50 | 7 | 0.93 | | |
| Lack of fit | 4.98 | 3 | 1.66 | 4.38 | 0.0939 |
| Pure error | 1.52 | 4 | 0.38 | | |
| Total | 70.75 | 16 | | | |
| $R^2 = 0.908$ | | | | | |
| CV = 7.19% | | | | | |
| $EC_{50}$ value | | | | | |
| Model | 2584.26 | 9 | 287.14 | 5.15 | 0.0209 |
| Residual | 390.12 | 7 | 55.73 | | |
| Lack of fit | 221.86 | 3 | 73.95 | 1.76 | 0.2937 |
| Pure error | 168.26 | 4 | 42.06 | | |
| Total | 2974.37 | 16 | | | |
| $R^2 = 0.869$ | | | | | |
| CV = 6.58% | | | | | |

decrease of the total polyphenols in obtained basil extracts (Milutinović et al. 2014).

One of the advantages of the MAE compared with the conventional extractions is that the time required for the extraction of phytochemicals from plant sources is significantly reduced. The content of polyphenols in basil extracts decreased when extraction time is longer than 15 min (see Fig. 1a, c). Longer exposure of the sample to the microwave irradiation and the solvent, probably caused extraction of chemical compounds from basil other than polyphenols, such

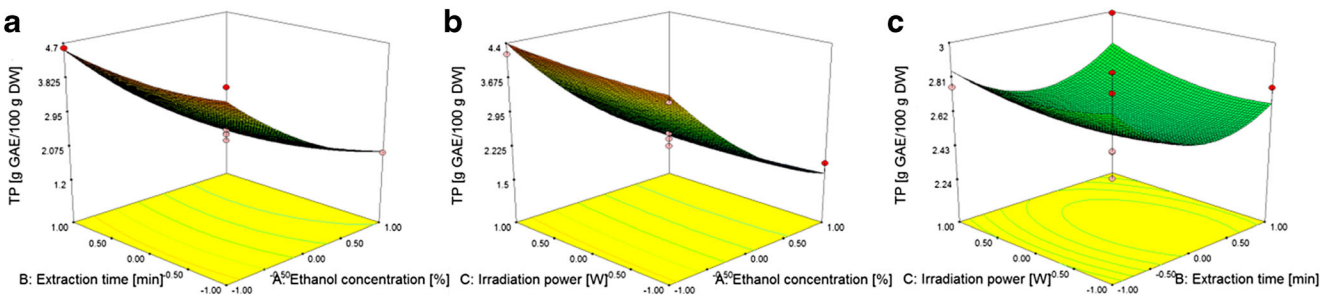


**Fig. 1** Response surface plots showing the effects of investigated variables on total phenolics content

as minerals and carbohydrates (Pavlović et al. 2013). Our results were in line with other researches reports (Milutinović et al. 2014, 2015; Simić et al. 2016).

Previously, microwave treatment has been widely employed to extract phenolic compounds from different plant materials using various operating conditions and similar effect of same MAE process parameters on TP content were observed (Zeković et al. 2016; Milutinović et al. 2014). To our knowledge, there have been no previous attempts of polyphenols extraction from basil using MAE method. Other authors used different techniques for extraction of polyphenols from basil. TP observed in basil MAE extracts was significantly higher than TP obtained by solid-liquid extraction (10.39–17.99 mg GAE/100 g dry extract), during 90 min with different combination of ethanol concentration (30–70%) and temperature (40–70 °C) (Vidović et al. 2012). Juliani and Simon (2002) obtained 55.7 mg GAE/g DW in *O. basilicum* extract obtained by maceration with 80% ethanol and Rafat et al. (2010) in Malaysian *O. basilicum* obtained 32.23 mg GAE/g of dry sample obtained by maceration with 95% ethanol during 72 h. The main differences in TP can be explained in using solvent with different concentrations and variations in basil raw material (Flanigan and Niemeyer 2014; Kwee and Niemeyer 2011), and our findings are in accordance with these results. On the other hand, TP obtained in this research was higher than TP observed in basil extracts (8.72–83.0 mg GAE eq/g dry extract weight) obtained by UAE (MeOH, 0–100%; *T*, 20–60 °C; and *t*, 20–70 min) (Izadiyan and Hemmateenejad 2016). Comparing to conventional solid-liquid extraction and UAE, MAE provides high content of polyphenols in obtained extracts in a shorter extraction time.

## Influence of the Extraction Parameters on Total Flavonoids Content

Experimental results of TF obtained under different MAE conditions are presented in Table 2. The highest value of TF content (1.3570 g CE/100 g DW) was obtained with 70% ethanol, 800 W, and 15 min. However, the lowest content of TF (0.3309 g CE/100 g DW) was observed using 90% ethanol, 600 W, and 15-min extraction. Generally, lower content of TF (0.3309–0.3627 g CE/100 g DW) were obtained using 90% ethanol as extraction solvent and the same was in case with total phenolics content.

Table 3 shows that the linear term of ethanol concentration ($p < 0.0001$) had highly significant effect, while quadratic term of ethanol concentration had significant influence ($p < 0.05$) on TF. On the other hand, linear and quadratic terms of microwave power and extraction time, as well as terms of interaction were insignificant.

The second-order polynomial model that predicts the content of TF from basil is given with next equation:

$$\begin{aligned} TF = {} & 0.91 - 0.35X_1 - 0.047X_2 + 0.066X_3 + 8.5 \\ & \cdot 10^{-4} X_1X_2 - 5.525 \\ & \cdot 10^{-3} X_1X_3 - 0.082X_2X_3 - 0.27X_1^2 + 0.043X_2^2 \\ & + 0.088X_3^2 \end{aligned} \quad (4)$$

Equation (4) shows that linear and quadratic terms of ethanol concentration exhibited negative effect on TF content in basil extracts. The same effects was observed for TP extraction, which indicated that similar factors and their interactions affected total flavonoid content in extracts. Since the flavonoids represent a subgroup of polyphenols, this result could be expected (Milutinović et al. 2015; Zeković et al. 2016) and is confirmed by significant correlation ($p = 0.002$) between the total flavonoids and the total polyphenols content in the extracts (Table 5). In our study, TF correlated lower with TP (Table 5), but still good enough ($r = 0.691$) indicating that similar MAE parameters provide relatively good extraction of both groups of compounds. The moderate negative correlation was observed between TF, TP, and antioxidant activity parameters ($IC_{50}$ and $EC_{50}$), which means that $IC_{50}$ and $EC_{50}$ decrease, i.e., increase in TP and TF leads to an increase in antioxidant activity.

The 3D response surfaces, which are the graphical representations of the quadratic polynomial regression Eq. (4) are shown in Fig. 2.

## Influence of the Extraction Parameters on Antioxidant Activity

In this study, the antioxidant activity of the basil extracts was evaluated by using in vitro tests, DPPH radical scavenging assay, and reducing power assay. In complex system, such as natural extracts with different polyphenol compounds, it is useful to make a comparative analyze of antioxidant activity using different methods. In the DPPH radical scavenging assay, the radical could be quenched by the antioxidants via hydrogen atom transfer, while reducing power assay is based

**Table 5** Pearson's correlation coefficients ($r$) and $p$ values for TP, TF, $IC_{50}$, and $EC_{50}$

| Investigated response | TP | TF | $IC_{50}$ |
|---|---|---|---|
| TF | 0.691[a] | | |
| | 0.002[b] | | |
| $IC_{50}$ | −0.303[a] | −0.165[a] | |
| | 0.237[b] | 0.527[b] | |
| $EC_{50}$ | −0.649[a] | −0.533[a] | −0.018[a] |
| | 0.005[b] | 0.028[b] | 0.946[b] |

[a] Pearson's correlation coefficient ($r$)

[b] $p$ value ($p < 0.05$ significant)

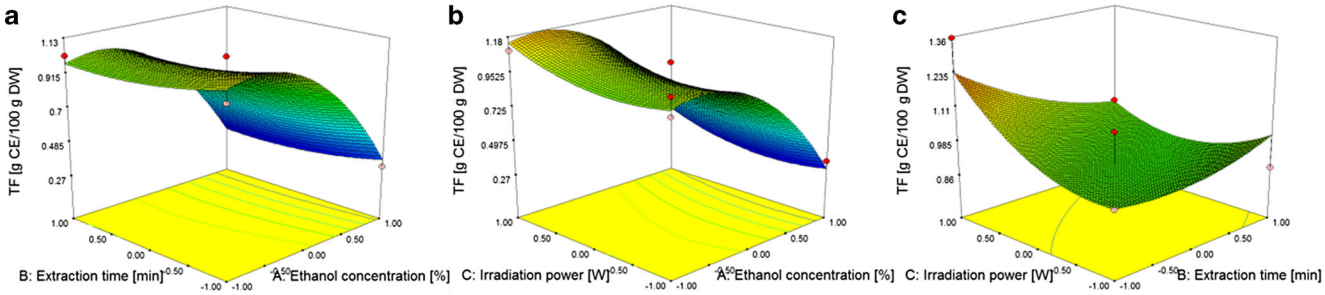


**Fig. 2** Response surface plots showing the effects of investigated variables on total flavonoids content

on the ability of polyphenols to reduced $Fe^{3+} \rightarrow Fe^{2+}$ via electron transfer. The DPPH radical scavenging is express as $IC_{50}$ value, while reducing power towards $Fe^{3+}$ ions and basil extracts is express as $EC_{50}$ value.

The $IC_{50}$ value for basil extracts obtained by MAE was in the range from 9.458 to 16.491 μg/mL (Table 2). The lowest $IC_{50}$ value (9.458 μg/mL), i.e., highest antioxidant activity was observed on lower level of ethanol concentration (50%) and extraction time (15 min), but middle level of microwave power (600 W). Antioxidant activity of ethanolic basil extracts obtained by MAE was slightly higher than antioxidant activity determined in basil extract obtained using supercritical carbon dioxide at 100 bar and 60 °C (18.93 μg/mL), previously reported by Filip et al. (2016). On the other hand, antioxidant activity in this work was higher than antioxidant activity of ethanolic basil extract (3.90 mg/mL), reported by Vardapetyan et al. (2014) and methanolic extract (41.80 μg/mL) preparing using Soxhlet extraction, reported by Albayrak et al. (2013). Comparing the $IC_{50}$ value obtained in our extracts and $IC_{50}$ value of well-known antioxidant compounds, it can be concluded that antioxidant activity of basil extracts obtained by MAE was similar to antioxidant activity of quarcetin (10.5 μg/mL) (Perez–Rozes et al. 2008) and synthetic antioxidant BHA (11.08 μg/mL) (Kaurinović et al. 2011).

The $EC_{50}$ value for basil extracts obtained by MAE ranged from 85.54 to 134.36 μg/mL (Table 2). The lowest $EC_{50}$ value (85.54 μg/mL), i.e., highest antioxidant activity was obtained using 50% ethanol concentration, 400 W microwave power, during 25 min of MAE, indicating that increase of ethanol concentration and microwave power cause decrease of reducing power. The reducing power of bioactive compounds may also serve as a significant indicator of its antioxidant capacity. Comparing our results with $EC_{50}$ of ascorbic acid (40 μg/mL) (Msaada et al. 2014), it can be concluded that basil liquid extracts exhibit two times lower activity. On the other hand, the reducing power of ethanolic basil extracts obtained by MAE was slightly higher than in coriander (0.1153–0.1824 mg/mL), obtained with the same extraction technique (Zeković et al. 2016).

The second-order polynomial model that predicts the antioxidant activity of basil extracts express as $IC_{50}$ and $EC_{50}$ values are presented with following equations:

$$IC_{50} = 14.33 + 0.73X_1 - 0.19X_2 + 0.97X_3 - 0.72X_1X_2 + 0.12X_1X_3 + 0.26X_2X_3 + 0.072X_1^2 - 3.28X_2^2 + 1.22X_3^2 \quad (5)$$

$$EC_{50} = 110.76 + 14.35X_1 - 0.91X_2 + 3.44X_3 - 4.86X_1X_2 - 2.03X_1X_3 - 11.48X_2X_3 + 1.03X_1^2 + 6.57X_2^2 - 1.76X_3^2 \quad (6)$$

The DPPH radical scavenging was significant influenced by linear term of microwave power, and quadratic terms of extraction time and microwave power ($p < 0.05$) (Table 3). Equation (5) shows positive effects of the linear and quadratic terms of microwave power, while quadratic term of extraction time had negative effect on DPPH radical scavenging activity.

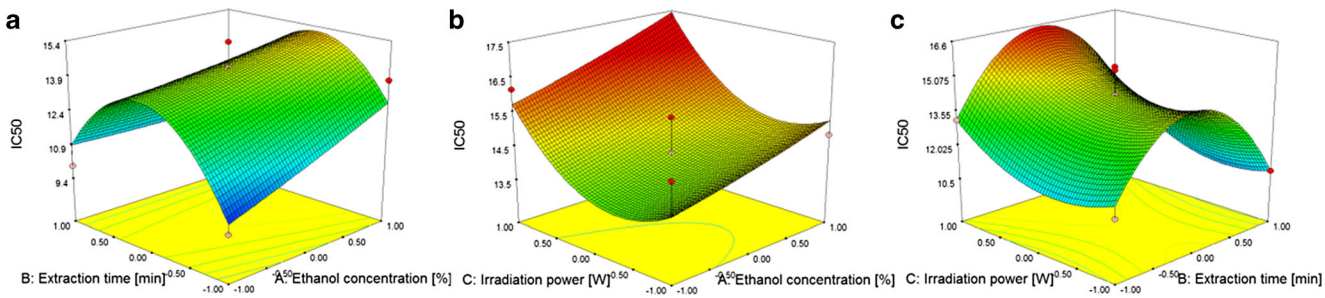


**Fig. 3** Response surface plots showing the effects of investigated variables on $IC_{50}$ value and their interaction

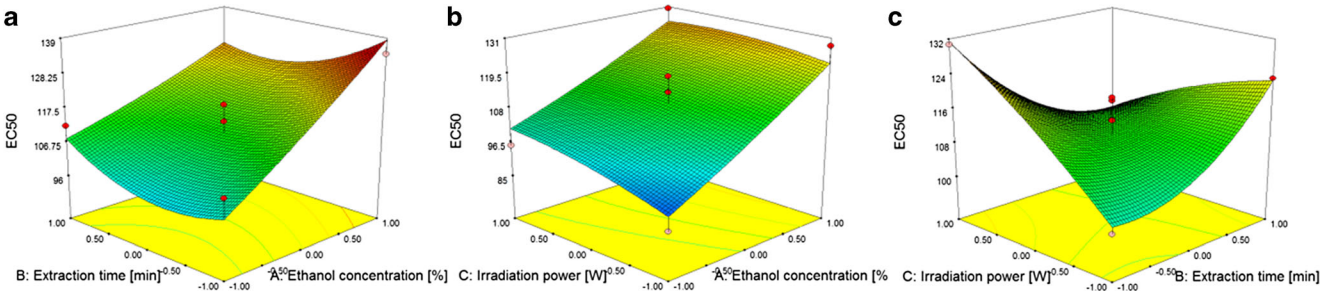


**Fig. 4** Response surface plots showing the effects of investigated variables on $EC_{50}$ value and their interaction

That means that $IC_{50}$ increased when microwave power and extraction time increase from 400 to 800 W, and 15–25 min, respectively, and then rapidly decrease at longer extraction time (Fig. 3c). Additionally, better antioxidant activity has basil extracts obtained by MAE on lower ethanol concentration and extraction time, but middle microwave power (Fig. 3a, c).

The antioxidant activity, evaluated by reducing power method, was significantly influenced by linear term of ethanol concentration and interaction between microwave power and extraction time. Equation (6) shows positive effects of the linear terms of ethanol concentration, suggesting that reducing power will be decreased on higher levels of this variable (Fig. 4b). On the other hand, interaction between microwave power and extraction time shows negative effects, indicating that minimum of $EC_{50}$ is on the lower levels of these variables (Fig. 4c).

### Optimization of Basil MAE and Validation of the Models

The main objective of this research was to find the best MAE conditions for obtaining the basil extracts with high polyphenol and flavonoid contents and antioxidant activity. Considering the maximum content of extracted total phenolics and total flavonoids, minimum of $IC_{50}$ and $EC_{50}$ values, the optimal conditions for all four investigated responses were: ethanol concentration of 50%, microwave power of 442 W and extraction time of 15 min. In Table 6, the predicted content of TP, TF, $IC_{50}$, and $EC_{50}$ values are presented. Determination of optimal conditions and predicted values was based on desirability function which for multi-response optimization was 0.856. In order to verify predictive mathematical model of the investigated process, MAE was performed on estimated optimal conditions for all four investigated responses. Comparison between observed experimental and predicted results showed that experimental values of all responses were within the 95% confidence interval for the predicted model. The good correlation between results confirmed the suitability of the used model and the success of RSM in optimizing the investigated MAE conditions.

## Conclusion

Response surface methodology was successfully applied for optimization of the conditions for MAE of total phenolics, total flavonoids and antioxidant activity of basil extracts. Analysis of variance (ANOVA) shows that second-order polynomial model provided adequate mathematical description of the MAE of polyphenols with high antioxidant activity. Concerning TP and TF, ethanol concentration had dominant negative influence on MAE, indicating lower ethanol concentration for maximum polyphenol content in obtained basil extracts. On the other hand, in case of antioxidant activity,

**Table 6** Predicted and observed values of each individual response at optimal conditions

| Optimal conditions | Investigated response | | | |
|---|---|---|---|---|
| | TP [g GAE/ 100 g DW] | TF [g CE/ 100 g DW] | $IC_{50}$ [μg/mL] | $EC_{50}$ [μg/mL] |
| Ethanol concentration = 50% | Predicted[a] | | | |
| Extraction time = 15 min | 4.315 ± 0.74 | 1.008 ± 0.25 | 10.177 ± 2.36 | 85.539 ± 18.33 |
| Irradiation power = 442 W | Observed[b] | | | |
| | 4.299 ± 0.36 | 0.849 ± 0.12 | 9.602 ± 1.56 | 82.889 ± 12.58 |

[a] Mean ± CI (95% confidence interval)

[b] Mean ± SD (standard deviation; $n = 4$)

ethanol concentration and microwave power, i.e., ethanol concentration-microwave power interactions had significant influence, suggesting that maximum antioxidant activity would be observed on low level of MAE parameters. In addition, the optimal conditions for all four investigated responses were ethanol concentration of 50%, microwave power of 442 W and extraction time of 15 min. These results show that under low microwave power, using relatively low ethanol concentration and during short extraction time, a potent natural extracts can be obtained. Moreover, MAE as an environmental friendly process for production phenolic-rich extracts from basil could be of great interest for application in cosmetic and food industries.

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**Compliance with Ethical Standards**

**Funding** Not applicable.

**Conflict of Interest** Snežana Filip declares that she has no conflict of interest. Branimir Pavlić declares that he has no conflict of interest. Senka Vidović declares that she has no conflict of interest. Jelena Vladić declares that she has no conflict of interest. Zoran Zeković declares that he has no conflict of interest.

**Ethical Approval** This article does not contain any studies with human or animals subjects.

**Informed Consent** Not applicable.

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