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OPTIMIZATION OF ULTRASOUND-ASSISTED EXTRACTION OF POLYPHENOLIC COMPOUNDS FROM CORIANDER SEEDS USING RESPONSE SURFACE METHODOLOGY

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Coriandrum sativum L. (coriander) seeds (CS) were used for preparation of extracts with high content of biologically active compounds. In order to optimize ultrasound-assisted extraction process, three levels and three variables of Box-Behnken experimental design (BBD) in combination with response surface methodology (RSM) were applied, yielding maximized total phenolics (TP) and flavonoids (TF) content and antioxidant activity (IC_{50} and EC_{50} values). Independent variables were temperature (40-80^oC), extraction time (40-80 min) and ultrasonic power (96-216 W). Experimental results were fitted to a second-order polynomial model with multiple regression, while the analysis of variance (ANOVA) was employed to assess the model fitness and determine optimal conditions for TP (79.60^oC, 49.20 min, 96.69 W), TF (79.40^oC, 43.60 min, 216.00 W), IC_{50} (80.00^oC, 60.40 min, 216.00 W) and EC_{50} (78.40^oC, 68.60 min, 214.80 W). On the basis of the obtained mathematical models, three-dimensional surface plots were generated. The predicted values for TP, TF, IC_{50} and EC_{50} were: 382.68 mg GAE/100 g CS, 216 mg CE/100 g CS, 0.03764 mg/mL and 0.1425 mg/mL, respectively.

KEY WORDS: coriander seeds, ultrasound-assisted extraction, optimization, response surface methodology

INTRODUCTION

Nowadays, aromatic and medicinal plants are attracting an increasing amount of attention due to their potential application in various industry fields and for health benefits. One of these plants is coriander (*Coriandrum sativum* L.), which belongs to the *Apiaceae* botanical family, and is widely cultivated and distributed in Mediterranean countries (1). Seeds contain up to 1% of essential oil with the main component of monoterpenoid linalool (>50%) (2), while limonene, camphor and geraniol are also present in significant quantity (3). Coriander seeds also contain vegetal oil with a high concentration of monounsaturated fatty acids, particularly petroselinic acid (4). Coriander has also been recognized as a medical agent which has been used against worms, rheumatism and pain in the joints (2). Several studies have demonstrated hypoglycemic action and effect

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on carbohydrate metabolism (2, 5). It has been reported that volatile components in essential oil from seeds and leaves of coriander have antimicrobial activity (1, 2, 6, 7), as well as anticancerous and antimutagenic (5, 8), antioxidant (2, 8-10) and antidiabetic (8, 11) activities.

Recently, reports on the application of ultrasound as a method for extraction of biological active compounds form plant material have been published. It has been showed that ultrasound-assisted extraction (UAE) technique can be especially suitable due to low equipment requirements and its high economic efficiency (12), offering at the same time high reproducibility, simplified manipulation, and reduced solvent and energy consumption (13). Beside these advantages, the usage of ultrasound for extraction diminishes the danger of thermal degradation of desired compounds and reduces significantly the time needed for the process itself (14). Cavitation, which occurs in the solvent due to the creation, growth and implosion of gas bubbles (15) and mechanical effect of ultrasound which provides a greater penetration of solvent into cellular material (12, 14), are the main benefits of using this method. Like many other processes, this process also possesses certain disadvantages. They are manifested in the form of the effect causing the changes in chemical composition and degradation of desired compounds, as well as in formation of free radical species inside of gas bubbles (16).

Optimization of any technological process is a very important task, aiming to gain maximum of the process with minimal losses at the same time. In the case of UAE there is a need for optimization of the process temperature, time and ultrasonic power. The most frequent technique employed for optimization of these parameters is the response surface methodology (RSM), which represent a collection of statistical and mathematical methods suitable to perform this important task (17). This technique is based on the influence of several different variables on the response of interest, aiming at the optimization of the described response (18).

The aim of this study was to evaluate the influence of extraction time, temperature and ultrasonic power on the extraction process of coriander seeds. After evaluation, UAE was optimized applying the RSM in order to obtain the liquid extracts with the highest phenolics content and antioxidant activity.

EXPERIMENTAL

Plant material

Coriander seeds were acquired from the Institute of Field and Vegetable Crops, Novi Sad, Republic of Serbia (year 2013). Plant material was air-dried and stored at room temperature. Dried seeds were milled in the blender, and mean particle size (0.6493 mm) was determined by CISA Cedaceria Industrial sieve set (Spain).

Chemicals

1,1-Diphenyl-2-picryl-hydrazyl-hydrate (DPPH), Folin-Ciocalteu reagent, gallic acid and (±)-catechin were purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals and reagents were of analytical reagent grade.

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Ultrasound-assisted extraction procedure

In all experimental runs, 10.0 g of powdered coriander seeds were mixed with 100 mL of 63% ethanol (ratio 1:10) in 300 mL glass flasks. Ultrasound-assisted extraction was performed in sonication water bath (EUP540A, Euinstruments, France) at a fixed frequency of 40 kHz. Ultrasonic power, temperature and extraction time were controlled from the panel of the instrument. Flasks were always positioned at the same distance from the transducer, and no agitation was applied. After extraction, the extracts were immediately filtered through filter paper under vacuum. The extracts were collected into glass flasks and stored at 4^{o}C until the analysis. After filtration, 10 mL of liquid extract were used for the extraction yield determination by removing the solvent and drying to the constant mass, to measure mass of the dry residue. The solvent was also removed from the rest of the extract on a rotary vacuum evaporator (Devarot, Elektromedicina, Ljubljana, Slovenia), and dried at 110^{o}C to constant mass. The total extraction yield was expressed as gram of dry extract per 100 grams of coriander seeds (g/100 g CS).

Total phenolics content

The total phenolics (TP) content in the obtained coriander extracts was determined by Folin-Ciocalteu procedure (19, 20), using gallic acid as a standard. Absorbance was measured at 750 nm. Content of phenolic compounds was expressed as milligrams of gallic acid equivalent (GAE) per 100 grams of *C. sativum* seeds (mg GAE/100 g CS). All experiments were performed in three replicates, and the results were expressed as mean values.

Total flavonoids content

The total flavonoids (TF) content was determined using aluminum chloride colorimetric assay (21). The results were expressed as milligrams of catechin equivalents (CE) per 100 g of coriander seeds (mg CE/100 g CS). All experiments were performed in three replicates and the results were expressed as a mean value.

DPPH assay

The free radical scavenging activity of coriander liquid extracts was determined as described by Espín et al. (22). A certain volume of diluted liquid extract was mixed with 95% methanol and 90 μM solution of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) in order to obtain different final concentrations of extract. The blank probe was prepared by using proper extraction solvent instead of sample. After 60 min of incubation at room temperature, the absorbance was measured at 515 nm and the result was expressed as radical scavenging capacity (%RSC), which was calculated by the following equation:

$$\%RSC=100-\frac{(A_{sample}\times 100)}{A_{blank}} \quad [1]$$

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where: A_{sample} is the absorbance of sample solution and A_{blank} is the absorbance of the blank probe. Antioxidant activity was expressed as the inhibition concentration at RSC value 50% (IC_{50}), which represents the concentration of the test solution required to obtain 50% of radical scavenging capacity expressed as mg per mL (mg/mL). All experiments were performed in three replicates, and the results were expressed as a mean value.

Reducing power assay

Reducing power of the samples was determined according to the assay based on the reduction of Fe^{3+} by polyphenol antioxidants (23). 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. The tubes were incubated on 50°C for 20 min. After incubation, 10% trichloroacetic acid solution (1 mL) was added to the reaction mixture. Then, the solution was centrifuged at 3000 rpm for 10 min, and the 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. Reducing power was expressed as the EC_{50} value (concentration in mg/mL at the absorbance of 0.5), which caused the reduction of 50% Fe^{3+} ions in the reaction mixture. The EC_{50} value was determined from the generated curve which represented the relationship between the sample concentration and the absorbance. All experiments were performed in triplicate, and the results were expressed as a mean value.

Experimental design

The RSM was employed to evaluate the effects of extraction parameters and optimize conditions for various responses administering the Box-Behnken experimental design (BBD) with three numeric factors on three levels. The design consisted of seventeen randomized runs with five replicates at the central point. Independent variables used in the experimental design were the temperature (X_1, 40-80°C), extraction time (X_2, 40-80 min) and ultrasonic power (X_3, 96-216 W). The ranges of variables were determined according to the available literature data (15, 24). In order to normalize the 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 (18). The variables were coded according to the following equation (25):

$$X=\frac{X_i-X_0}{\Delta X} \qquad [2]$$

where X is the coded value, X_i is the corresponding actual value, X_0 is the actual value in the center of the domain, and ΔX is the increment of x_i corresponding to the variation of 1 unit of X. The natural and coded values of independent variables used in BBD are presented in Table 1.

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Table 1. Natural and coded levels of independent variables used in the RSM design

Variable	Coded levels		
	-1	0	1
	Natural levels		
Temperature (^oC)	40	60	80
Extraction time (min)	40	60	80
Ultrasonic power (W)	96	156	216

The response variables were fitted to the following second-order polynomial model, which is generally able to describe the relationship between the responses and the independent variables (26):

$$Y=\beta_0+\sum_{i=1}^{3}\beta_i X_i+\sum_{i=1}^{3}\beta_{ii}X_i^2+\sum\sum_{i<j=1}^{3}\beta_{ij}X_iX_j \quad [3]$$

where Y represents the measured response; β_0 is a constant, b_j, b_{jj}, b_{ij} are the linear, quadratic and interactive coefficients of the model, respectively; X_i and X_j are the levels of the independent variables. Optimal extraction conditions were determined considering TP and TF contents, radical scavenging activity and reducing power as responses. The experimental design and multiple linear regression analysis were performed using Design-Expert v.7 Trial (Stat-Ease, Minneapolis, Minnesota, USA). The results were statistically tested by the analysis of variance (ANOVA) with the significance levels of 0.05. The adequacy of the model(s) was evaluated by the coefficient of multiple determination (R^2), coefficient of variance (CV) and *p*-values for the model and lack of fit testing.

RESULTS AND DISCUSSION

Model fitting

The influence of temperature (40-80^{o}C), extraction time (40-80 min), and ultrasonic power (96-216 W) on investigated responses (TP, TF, and antioxidant activity determined by DPPH and reducing power) was optimized using RSM. The results of the experimentally obtained responses using the Box-Behnken experimental design are presented in Table 2. The analysis of variance (ANOVA) was used for determining regression coefficients of the linear, quadratic and interaction terms for each response, and the results are presented in Table 3. The influence of each term was described as statistically significant ($p < 0.05$) and insignificant ($p > 0.05$). The coefficient of multiple determination (R^2) was used as first indicator of the model adequacy, together with ANOVA, and the calculated statistical parameters are presented in Table 4. The relatively high values of R^2 (> 0.90) for TP and TF, indicate that the second-order polynomial model represents a good approximation of the experimental results. On the other hand, R^2 for the antioxidant activity parameters (IC_{50} and EC_{50}) was slightly lower (0.829 and 0.834, respecti-

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vely). The ANOVA was used to provide detailed information about statistical signify-cance of the investigated models. The experimental results of all investigated responses showed good fitting with mathematical models, since the regression for the model was significant ($p < 0.05$), and the lack of fit testing was insignificant ($p > 0.05$) (Table 4). Therefore, the regression equations could be successfully used as predictors of these responses in the investigated experimental domain.

Table 2. Experimental conditions for the Box-Behnken design including natural and coded levels and experimentally obtained values of measured responses, including yield, TP, TF, IC_{50} and EC_{50} values

Independent variables			Measured responses				
X_1 Temperature (°C)	X_2 Time (min)	X_3 Ultrasonic power (W)	Y (g/100 g CS)	TP (mg GAE/ 100 g CS)	TF (mg CE/ 100 g CS)	IC_{50} (mg/mL)	EC_{50} (mg/mL)
0	0	0	8.80	287.76±15.89	150.40±0.00	0.04344±0.0056	0.1523±0.0064
0	-1	1	8.58	307.28±4.23	203.40±2.76	0.04516±0.0045	0.1582±0.0084
1	-1	0	11.06	350.82±28.03	203.90±23.33	0.05398±0.0030	0.1651±0.0084
0	0	0	8.37	282.67±1.56	160.40±4.31	0.04798±0.0030	0.1483±0.0076
0	1	1	9.14	296.61±10.09	162.904.03	0.05105±0.0098	0.1577±0.0095
-1	1	0	7.19	222.32±2.11	124.60±3.32	0.05392±0.0098	0.1634±0.0093
1	0	1	10.55	364.74±15.91	192.60±7.70	0.03569±0.0085	0.1448±0.0063
-1	-1	0	6.52	221.53±2.64	126.90±1.91	0.05094±0.0014	0.1545±0.0011
0	0	0	8.69	288.17±6.90	164.70±2.05	0.04990±0.0008	0.1566±0.0016
-1	0	1	8.21	260.05±11.14	145.70±0.99	0.04820±0.0021	0.1552±0.0050
0	0	0	8.85	265.60±24.32	155.40±7.71	0.04889±0.0019	0.1584±0.0089
0	-1	-1	9.68	326.05±2.64	190.50±2.76	0.05212±0.0021	0.1650±0.0089
1	0	-1	10.91	374.25±13.30	198.70±7.70	0.04862±0.0022	0.1474±0.0060
0	1	-1	9.29	310.64±12.20	165.01±1.77	0.05252±0.0022	0.1583±0.0075
-1	0	-1	7.34	240.85±4.20	138.90±1.27	0.04881±0.0000	0.1483±0.0024
1	1	0	11.18	372.10±3.72	199.50±5.80	0.04868±0.0001	0.1436±0.0020
0	0	0	8.59	287.46±1.04	149.30±1.77	0.04886±0.0001	0.1464±0.0060

Table 3. Estimated regression coefficients of the fitted second-order polynomial model for all investigated responses

Coefficient	Response			
	TP	TF	IC_{50}	EC_{50}
β_0	282.24	156.04	0.048	0.150
Linear				
β_1	64.64	32.32	$-1.863\cdot10^{-3}$	$-2.563\cdot10^{-3}$
β_2	-0.50	-9.09	$4.963\cdot10^{-4}$	$-2.475\cdot10^{-3}$
β_3	-2.89	1.44	$-2.746\cdot10^{-3}$	$-3.875\cdot10^{-4}$
Interaction				
β_{12}	5.12	-0.52	$-2.070\cdot10^{-3}$	$-7.600\cdot10^{-3}$
β_{13}	-7.18	-3.22	$-3.080\cdot10^{-3}$	$-2.375\cdot10^{-3}$
β_{23}	1.19	-3.75	$1.373\cdot10^{-3}$	$1.550\cdot10^{-3}$

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Table 3. Continuation

Coefficient	Response			
	TP	TF	IC_{50}	EC_{50}
Quadratic				
β_{11}	4.64	-1.90	$-4.083 \cdot 10^{-4}$	$-3.313 \cdot 10^{-3}$
β_{22}	4.81	9.58	$4.474 \cdot 10^{-3}$	$7.562 \cdot 10^{-3}$
β_{33}	23.09	14.83	$-2.076 \cdot 10^{-3}$	$-1.625 \cdot 10^{-4}$

Table 4. Analysis of the ANOVA of the fitted second-order polynomial models

Source	Sum of Squares	DF	Mean square	*F*-value	*p*-value
Total phenols content					
Model	36378.25	9	4042.03	25.94	0.0001
Residual	1090.77	7	155.82		
Lack of fit	725.40	3	241.80	2.65	0.1852
Pure error	365.37	4	91.34		
Total	37469.02	16			
$R^2 = 0.971$					
Total flavonoids content					
Model	10516.00	9	1168.44	9.30	0.0038
Residual	879.17	7	125.60		
Lack of fit	707.52	3	235.84	5.50	0.0666
Pure error	171.65	4	42.91		
Total	11395.17	16			
$R^2 = 0.923$					
IC_{50}					
Model	$2.516 \cdot 10^{-4}$	9	$2.795 \cdot 10^{-5}$	3.76	0.0474
Residual	$5.207 \cdot 10^{-5}$	7	$7.439 \cdot 10^{-6}$		
Lack of fit	$2.631 \cdot 10^{-5}$	3	$8.771 \cdot 10^{-6}$	1.36	0.3743
Pure error	$2.576 \cdot 10^{-5}$	4	$6.441 \cdot 10^{-6}$		
Total	$3.037 \cdot 10^{-4}$	16			
$R^2 = 0.829$					
EC_{50}					
Model	$6.426 \cdot 10^{-4}$	9	$7.140 \cdot 10^{-5}$	3.91	0.0429
Residual	$1.278 \cdot 10^{-4}$	7	$1.825 \cdot 10^{-5}$		
Lack of fit	$2.131 \cdot 10^{-5}$	3	$7.102 \cdot 10^{-6}$	0.27	0.8468
Pure error	$1.065 \cdot 10^{-4}$	4	$2.662 \cdot 10^{-5}$		
Total	$7.704 \cdot 10^{-4}$	16			
$R^2 = 0.834$					

Total extraction yield

The experimentally obtained values for the yield are presented in Table 2. They varied in the range of 6.52 to 11.18 g/100 g CS. The highest yield was obtained at 80°C, for

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80 min, and ultrasonic power of 156 W. On the other hand, the lowest yield was obtained under the following conditions: 40°C, 40 minutes, and ultrasonic power of 156 W. As the values were obtained under the same ultrasonic power but at the different values of temperature and time, this indicates the importance of those two independent variables on the yield.

TP content

The values obtained for the TP content are presented in Table 2, and they ranged from 221.53 to 374.25 mg GAE/100 g CS. The previously conducted UAE extraction of CS raffinate obtained after performed the SFE revealed the TP content in the range of 110.53-222.08 mg GAE/100 g of CS (in 70% ethanol as solvent) and 161.82-308.55 mg GAE/100 g CS (water as solvent) (27). These results were lower than those obtained in this study, but indicated the importance of the solvent selection. Comparing to the results obtained by Zeković et al. (28), where the maximal obtained value of TP content was 2629.70 mg GAE/100 g CS, these results present lower content, indicating that subcritical water extraction (SWE) is a more suitable method for extraction of phenolic compound than UAE. On the other hand, Gallo et al. (29) applied the UAE and microwave-assisted extraction (MAE) techniques and obtained 41.81 and 82.09 mg GAE/100 g CS, which presents a lower yield of these compounds comparing to those obtained in this study. The optimization of the MAE extraction process of CS gave the TP content in the range of 136.92-384.54 mg GAE/100g CS (30), which is similar to the results obtained in this study. This indicates the importance of extraction conditions and their significant influence ($p < 0.05$) on the yield of phenolic compounds.

The effects of UAE parameters on TP is presented in Figure 1a, while their significance was determined by RSM influence analysis expressed as regression coefficients from Eq. [3] (Table 3). According to ANOVA, only linear term of temperature and quadratic term of ultrasonic power exhibited significant influence ($p < 0.05$) on TP. The positive influence of the liner term of temperature was rather expected, since temperature affects mass transfer by increasing diffusion, causing degradation of the plant matrix and improving physical solvent properties in terms of penetration and solubility power (15). A negative influence of the linear term of ultrasonic and positive influence of its quadratic term indicate that TP decrease with the increase of ultrasonic power up to a certain value, then TP shows again a slight increase. The predicted second-order polynomial model for TP content is:

$$TP=282.24+64.64X_1-0.50X_2-2.89X_3+5.12X_1X_2-7.18X_1X_3+1.19X_2X_3+ \\ +4.64X_1^2+4.81X_2^2+23.09X_3^2 \quad [4]$$

TF content

The yields of TF are presented in Table 2. The previously performed UAE of CS raffinate obtained after SFE, showed the TF content in the range of 64.50-153.74 mg

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CE/100 g SC (70% ethanol as solvent) and 80.86-297.63 mg CE/100 g SC (water as solvent) (27). The results show that the TF content in the water UAE extract was higher than achieved in this study. Comparing the results for TF obtained in this study with the results obtained by Zeković et al. (28), where TF content in CS extracts varied from 231.15 to 628.00 mg CE/100 g CS, as it was the case with TP content, which indicates once again the superiority of the SWE method over the UAE method. The optimization of MAE process gave the TF content in the range of 94.50-211.83 mg CE/100 g SC (30), which is in accordance with the TF content obtained in this study.

According to the data presented in Table 3, the linear term of temperature and quadratic term of ultrasonic power exhibited a significant influence on the TF content ($p < 0.05$). The presented regression coefficients (Table 3) suggest that the influence of these three parameters on TF content should be similar as for the TP content (Figure 1.b). The predicted second-order polynomial model for the TF content is:

$$TP=156.04+32.32X_1-9.09X_2+1.44X_3-0.52X_1X_2-3.22X_1X_3-3.75X_2X_3-1.90X_1^2+9.58X_2^2+14.83X_3^2 \quad [5]$$

DPPH and reducing power assays

The results obtained assaying DPPH assay are presented in Table 2, and the measured values of IC_{50} ranged from 0.03569 to 0.05398 mg/ml. Comparing these results with those from the previous study (28), reporting the IC_{50} values of CS extracts in the range from 0.01706 to 0.06336 mg/mL, there is no big difference between the obtained values. Namely, the obtained the highest values of antioxidant activity at the high temperature were the same as in this study. Wangensteen et al. (2) reported the antioxidant activity of 0.51000 mg/mL of CS ethanolic extract obtained by solid-liquid extraction technique, which is lower compared to the result obtained in this research. The antioxidant activity of UAE extracts of CS (raffinate after the SFE process) against DPPH was in the range of 0.02478-0.04183 mg/mL (for 70% ethanolic extracts) and 0.07499-0.10770 mg/mL (for water extracts) (27). Ethanolic extracts exhibited higher activity against this radical than the extracts obtained in this study. The extracts obtained during the optimized MAE process of CS showed an activity in the range of 0.03020-0.06650 mg/mL (30), which is higher than in the case of UAE.

In the case of reducing power assay (Table 2), the obtained values ranged between 0.1436 and 0.1651 mg/mL. The highest value of EC_{50} indicates the lowest antioxidant activity while the lowest EC_{50} indicates the highest antioxidant activity, as well as it was the case of IC_{50} values in the DPPH assay. Comparing these results with those obtained during the optimization of MAE process (30), where obtained EC_{50} values were in the range of 0.1153-0.1824 mg/mL, the MAE extracts showed once again higher antioxidant activity.

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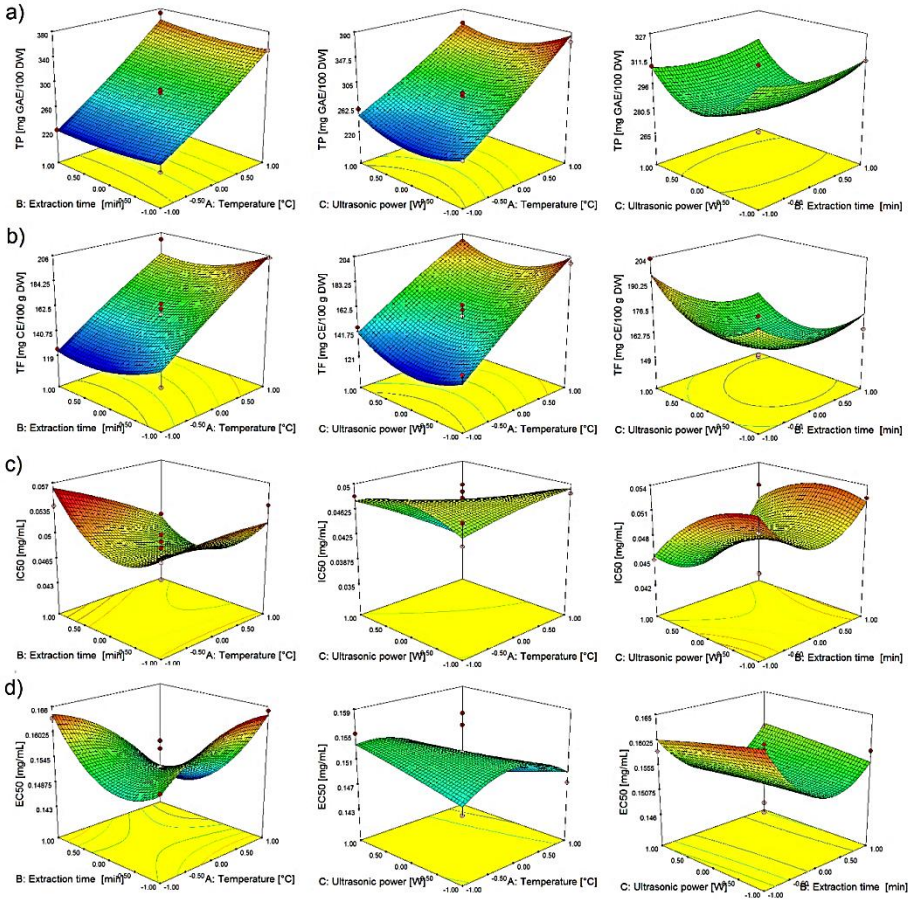

Figure 1. Response surface plots showing combined effects of UAE parameters on: a) TP, b) TF, c) IC_{50} and d) EC_{50}

The influence of the parameters on the DPPH assay is presented in Figure 1c, while the influence on the reducing power is shown in Figure 1d. The predicted second-order polynomial models for DPPH assay (Eq. [6]) and reducing power test (Eq. [7]) are:

$$IC_{50}=0.048-1.863E\text{-}0.03X_1+4.963E\text{-}0.04X_2-2.746E\text{-}0.03X_3-$$
$$-2.070E\text{-}0.03X_1X_2-3.080E\text{-}0.03X_1X_3+1.373E\text{-}0.03X_2X_3-$$
$$-4.083E\text{-}0.04X_1^2+4.474E\text{-}0.03X_2^2-2.076E\text{-}0.03X_3^2 \quad [6]$$

$$EC_{50}=0.15+2.563E\text{-}0.03X_1+2.475E\text{-}0.03X_2-3.875E\text{-}0.04X_3-$$
$$-7.600E\text{-}0.03X_1X_2-2.375E\text{-}0.03X_1X_3+1.550E\text{-}0.03X_2X_3-$$
$$-3.313E\text{-}0.03X_1^2+7.562E\text{-}0.03X_2^2-1.625E\text{-}0.04X_3^2 \quad [7]$$

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In the case of the DPPH assay, the linear term of ultrasonic power and quadratic term of extraction time exhibited a significant influence on the IC_{50} value ($p < 0.05$). With the increase in the ultrasonic power, the IC_{50} value was constant first, to decrease at higher values of ultrasonic power.

This phenomenon is connected with the influence of this parameter on the TP and TF contents. With the increase in ultrasonic power, the TP and TF content decreased in the beginning, while the IC_{50} value was constant. After certain value of ultrasonic power there were increases in the TP and TF contents, while the IC_{50} value sharply decreased, consequently causing an increase in the antioxidant activity. On the other hand, in the beginning of the extraction, the IC_{50} value decreased, and then started to increase. The decrease in the antioxidant activity after certain time could be explained by the heating effect or overexposure to ultrasound irradiation, when decomposition of the antioxidant agent in the extract might occur (31). Similar effects of ultrasonic power and extraction time on DPPH assay have been reported previously (32, 33). In the case of interaction of ultrasound power and time, the IC_{50} value decreased with both extraction time and ultrasonic power in the beginning of the extraction process, and the lowest IC_{50} value (highest antioxidant activity) were achieved at approximately half of the extraction process and ultrasonic power range (Figure 1c).

As for the reducing power, significant influence exhibited only the quadratic term of time and interaction between temperature and time ($p < 0.05$). The influence of the time was the same as in the case of DPPH assay. This means that both antioxidant tests rely on the reaction with the same compounds in the extracts and that their decomposition occurred due to prolonged exposure to high temperature or ultrasonic power.

Pearson's correlation coefficients among Y, TP, TF, IC_{50} and EC_{50} values are presented in Table 5. There was high correlation between Y and TP and between TP and TF ($r > 0.9$). The correlation among Y and TF was good ($r = 0.8710$).

Table 5. Pearson's correlation coefficients among Y, TP, TF, IC_{50} and EC_{50}

r	**Y**	**TP**	**TF**	**IC_{50}**	**EC_{50}**
EC_{50}	−0.1553	−0.2523	−0.0419	0.6024	1
IC_{50}	−0.1906	−0.2956	−0.2291	1	
TF	0.8710	0.9198	1		
TP	0.9671	1			
Y	1				

On the other hand, the correlation among the antioxidant assays (DPPH and RP) was moderate ($r = 0.6024$), while the correlation among Y TP, TF and antioxidant assays was very week ($r < 0.5$) and negative. A good correlation among Y, TP and TF was rather expected, and indicated the increases in TP and TF with the increase in Y.

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Optimization of UAE

As the aim of this research was the optimization id the extraction process to obtain maximal TP and TF contents and maximal antioxidant activity, each of the individual responses was optimized. This optimization was based on previously obtained experimental results and performed statistical analysis. The estimated optimal conditions and predicted values of individual responses are presented in Table 6.

Table 6. Predicted maximal values of individual responses and estimated values of optimal conditions

Optimal conditions	Investigated responses			
	TP (mg GAE/100 g CS)	TF (mg CE/100 g CS)	IC_{50} (mg/mL)	EC_{50} (mg/mL)
Predicted value	382.68	216.06	0.03764	0.1425
Temperature (^oC)	79.60	79.40	80.00	78.40
Time (min)	49.20	43.60	60.40	68.60
Ultrasonic power (W)	96.60	216.00	216.00	214.80

Observing the data from Table 6 it can be noticed that the conditions for both antioxidant activity tests were similar. On the other hand, there were differences in ultrasonic power for TP and TF contents. In the case of TP content, the maximum value required almost minimal ultrasonic power, while it was vice versa in the case of TF content, where maximal ultrasonic power was required. The desirability for all cases was 1.00 except for the IC_{50} value, which was 0.893.

CONCLUSION

Preparation of extracts with high antioxidant activity and maximum content of biological active compound (phenolic and flavonoid compounds) require careful selection of extraction parameters. In order to optimize process parameters, response surface methodology was successfully applied. It was shown that a second-order polynomial model was able to successfully describe the extraction process of polyphenolic compound and antioxidant activity. The obtained results showed that temperature and ultrasonic power had the strongest influence on total phenolics and total flavonoids content, while extraction time was crucial parameter in the case of DPPH and reducing power assays. Regarding the DPPH assay, ultrasonic power appeared to be an important factor for the optimization process. The temperature and time profiles were similar for all four measured responses, but in the case of ultrasonic power maximum value was predicted for all responses with exception of total phenolics content, where minimum of ultrasonic power was required.

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ОПТИМИЗАЦИЈА УЛТРАЗВУЧНЕ ЕКСТРАКЦИЈЕ ПОЛИФЕНОЛНИХ ЈЕДИЊЕЊА ИЗ СЕМЕНА КОРИЈАНДЕРА МЕТОДОМ ОДЗИВНЕ ПОВРШИНЕ

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Ултразвучном екстракцијом добијени су екстракти семена коријандера са високим садржајем биолошки активних једињења. Како би се оптимизовао процес екстракције, употребљена је метода одзивне површине (RSM) у циљу добијања екстраката са максималним садржајем укупних фенолних (TP) и флавоноидних (TF) једињења, као и максималном антиоксидативном активношћу (IC_{50} и EC_{50}). Независне промењиве у овом случају биле су температура (40-80^{o}C), време трајања екстракције (40-80 min) и снага ултразвука (96-216 W). Добијени експериментални резултати су фитовани полиномним моделом другог реда са вишеструком регресијом, а анализа варијанси (ANOVA) је примењена ради процене модела и добијања оптималних услова за максималан принос TP (79,60^{o}C, 49,20 min, 96,69 W), TF (79,40^{o}C, 43,60 min, 216,00 W), IC_{50} (80,00^{o}C, 60,40 min, 216,00 W) и EC_{50} (78,40^{o}C, 68,60 min, 214,80 W). Предвиђене максималне вредности при оптималним условима за TP, TF, IC_{50} и EC_{50} биле су: 382,68 mg GAE/100 g CS, 216 mg CE/100 g CS, 0,03764 mg/ml и 0,1425 mg/ml.

Кључне речи: семе коријандера, ултразвучна екстракција, оптимизација, метода одзивне површине

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