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Optimization of subcritical water extraction of antioxidants from *Coriandrum sativum* seeds by response surface methodology

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ABSTRACT

Subcritical water extraction (SWE) of antioxidants from *Coriandrum sativum* seeds (CSS) was optimized by simultaneous maximization of the total phenolics (TP) and total flavonoids (TF) yield and antioxidant activity, using IC_{50} value. Box–Behnken experimental design (BBD) on three levels and three variables was used for optimization together with response surface methodology (RSM). Influence of temperature (100–200 °C), pressure (30–90 bar) and extraction time (10–30 min) on each response was investigated. Experimentally obtained values were fitted to a second-order polynomial model and multiple regression. Analysis of variance (ANOVA) was used to evaluate model fitness and determine optimal conditions. Moreover, three-dimensional surface plots were generated from employed mathematical model. The optimal SWE conditions obtained in simultaneous optimization were temperature of 200 °C, pressure of 30 bar and extraction time of 28.3 min, while obtained values of TP and TF yields and IC_{50} value at this experimental point would be 2.5452 g GAE/100 g CSS, 0.6311 g CE/100 g CSS and 0.01372 mg/ml, respectively. Moreover, good and moderate linear correlation was observed between antioxidant activity (IC_{50} value) and total phenolics content ($R^2 = 0.965$), and total flavonoids content ($R^2 = 0.709$) which indicated that these groups of compounds are responsible for antioxidant activity of *C. sativum* extracts.

1. Introduction

Coriander (*Coriandrum sativum* L.) is aromatic plant which is widely distributed and cultivated in Mediterranean countries. The coriander seeds contain an essential oil (up to 1%) [1], where monoterpenoid linalool is the main compound (>50%), and limonene, camphor and geraniol are present in significant quantity [2,3]. It has the advantage of being more stable and of retaining its agreeable odor longer than any other oil of its class [4]. Leaves and seeds are employed as condiment in food industry, being used to flavor various commercial foods such as liqueurs, teas, meat products and pickles [5]. Besides aromatic, coriander seeds are recognized for their medicinal properties. The seeds and aerial parts of the plant were extensively used in traditional medicine for various ailments such as spasm, neuralgia gastric complaints, dysentery, dyspepsia and giddiness [6]. Studies have demonstrated hypolipidemic action and effects of carbohydrate metabolism of *C. sativum* seeds [7]. Seeds have been also recognized due to their antimicrobial potential against different pathogen bacteria and yeasts [8,9]. Both hydrophilic and lipophilic extracts of coriander have demonstrated significant antioxidant activities in in vitro and in vivo studies [10,11].

Conventional solvent extractions with organic solvents and hydrodistillation have been widely used for the isolation of volatile and nonpolar compounds from plant material. In order to overcome certain disadvantages of conventional techniques such as extraction time, use of organic solvent and thermal degradation, modern extraction techniques have been developed. Supercritical fluid extraction (SFE) with non-toxic carbon dioxide (CO_2) is becoming more common for the extraction of flavours and fragrances, and can often yield more rapid extractions than hydrodistillation, as well as recovering some species that are not recovered by hydrodistillation [12]. More recently, subcritical or superheated water extraction (SWE) has been developed as a new technique based on the use of water, at temperatures between 100 and 374 °C and pressure high enough to maintain the liquid state [13]. Dielectric constant of water which controls solubility of the solute in water is directly connected with temperature which allows modification of water selectivity with change of temperature. The dielectric constant of water is 78.4 at room temperature, however at 200 °C it

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is 35.6, which is enough for quantitative extraction of less polar compounds [14]. SWE demonstrated ability to selectively extract different classes of compounds, with the more polar organics being extracted at lower temperatures and the less polar organics being extracted at higher temperatures [15]. The most important advantages of SWE over conventional extraction techniques are shorter extraction time, higher quality of the extract, lower costs of the extracting agent, and the environmental compatibility [16].

The most common and often used approach on the process optimization uses one-factor-at-a-time, where influence of independent variables on responses are investigated one by one, while all other factors are kept under constant values. This approach could be time-consuming and expensive for certain experiments. Moreover, possible interaction effects between variables may not be evaluated. In order to overcome these disadvantages, response surface methodology (RSM) could be applied. RSM is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes in which a response of interest is influenced by several variables, and the objective is to optimize this response [17]. Analyzing the effects of the independent variables, this experimental methodology generates a mathematical model which describes the chemical processes within the experimental range [18].

The main objectives of present work were to investigate effects of SWE conditions (temperature, extraction time and pressure), and to apply RSM approach in order to optimize these conditions to obtain the highest polyphenolics content and highest antioxidant activity of obtained liquid extracts of dried *C. sativum* seeds.

2. Materials and methods

2.1. Chemicals

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

2.2. Plant material

Coriander (*C. sativum*), i.e. coriander seeds, were produced by the Institute of Field and Vegetable Crops, Novi Sad, Serbia (year 2012). Seeds were air-dried, milled and mean particle size (0.466 mm) was determined by sieve set (CISA Cedaceria Industrial, Spain).

2.3. SWE procedure

Subcritical water extraction (SWE) was performed in batch-type high-pressure extractor (Parr Instrument Company, USA) with internal volume 450 ml and maximum operating pressure of 200 bar and temperature 350 °C, connected with temperature controller (4838, Parr Instrument Company, USA). Extraction procedure was carried out by the scheme from Fig. 1. In all experimental runs, 10.0 g of coriander seeds sample were mixed with 100 ml of water in extractor (1). The operating pressure was reached with the injection of nitrogen in extractor from gas cylinder (2) through valve (3), and measured with pressure indicator (4). Nitrogen was used in order to prevent possible oxidation on high temperatures in the presence of oxygen from air. Extractor vessel was heated with electric heating jacket (5) and temperature was measured and controlled on controller (6), connected with temperature indicator (7). Magnetic stirrer (8) (750 rpm) was used for the stirring in order to increase mass and heat transfer and prevent local overheat on the inner walls of extractor. After the extraction,

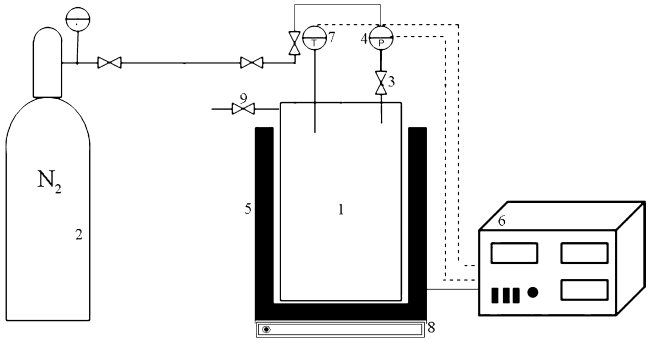

Fig. 1. Schematic diagram of subcritical water extraction system: (1) extractor; (2) nitrogen cylinder; (3) input gas valve; (4) pressure indicator; (5) electric heating jacket; (6) digital controller; (7) temperature indicator; (8) magnetic stirrer; (9) output gas valve.

extractor was immediately cooled in ice-bath at 30 °C, and nitrogen was discharged from extractor through valve (9).

Temperature (100–200 °C), pressure (30–90 bar) and extraction time (10–30 min) were independent variables. Temperature profiles of the extraction on different process temperature are presented on Fig. 2. The first part of all three curves describes approximately linear heating of extractor which lasted for 11, 16 and 21 min for extraction at 100, 150 and 200 °C, respectively. During extraction period, temperature was held constant (stationary phase) for different extraction time depending on experimental run. After the extraction, extractor was cooled in ice-bath during approximately 5 min to reach room temperature. After extraction, extracts were immediately filtered through filter paper under vacuum. Extracts were collected into glass flasks and stored at 4 °C until the analysis.

2.4. Determination of total phenols content

The total phenolics content (TP) in obtained *C. sativum* 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 grams of gallic acid equivalent (GAE) per 100 g of *C. sativum* seeds (g GAE/100 g CSS). All experiments were performed in three replicates.

2.5. Determination of total flavonoids content

The total flavonoids content (TF) was determined using aluminum chloride colorimetric assay [21]. Results were expressed as grams of catechin equivalents (CE) for 100 g of dry *C. sativum*

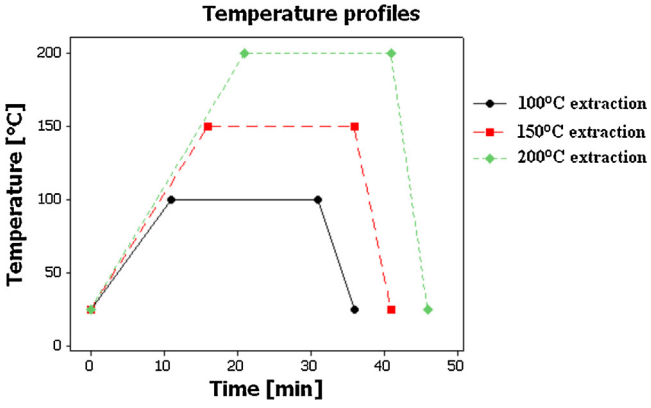

Fig. 2. Temperature profiles of the extraction at different process temperature.

seeds (g CE/100 g CSS). All experiments were performed in three replicates.

2.6. DPPH assay

The free radical scavenging activity of *C. sativum* liquid extracts was determined as described by Espín et al. [22]. A certain volume of diluted *C. sativum* liquid extract was mixed with 95% methanol and 90 μM 2,2-diphenyl-1-picryl-hydrazyl (DPPH) in order to gain different final concentrations of extract. After 60 min at room temperature, the absorbance was measured at 515 nm and result was expressed as radical scavenging capacity (%RSC). %RSC was calculated by following equation:

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

where A_{sample} is the absorbance of sample solution and A_{blank} is the absorbance of blank probe. Antioxidant activity was expressed as the inhibition concentration at RSC value 50% (IC_{50}), which represents the concentration of test solution required to obtain 50% of radical scavenging capacity.

2.7. Experimental design and statistical analysis

The RSM was applied to evaluate the effects of extraction parameters and optimize conditions for various responses. Box–Behnken experimental design (BBD) with three numeric factors on three levels was used. Design consisted of fifteen randomized runs with three replicates at the central point. Independent variables used in experimental design were temperature (T, 100–200 °C), pressure (p, 30–90 bar) and extraction time (t, 10–30 min). 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 [17]. Variables were coded according to the following equation [23]:

$$X = \frac{(x_i - x_0)}{\Delta x} \quad (2)$$

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 Δx is the increment of x_i corresponding to a variation of 1 unit of X. The natural and coded values of independent variables used in BBD are presented in Table 1.

The response variables were fitted to the following second-order polynomial model (Eq. (3)) which is generally able to describe relationship between the responses and the independent variables [24]:

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

where Y represents the response variable, X_i and X_j are the independent variables affecting the response, and β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms. Optimal extraction conditions were determined considering total phenols and total flavonoids content, and antioxidant activity as responses. Treatment of multiple responses and selection of optimal conditions were based on desirability function D [25]. The experimental design and multiple linear regression analysis were performed using Design-Expert v.7 Trial (Stat-Ease, Minneapolis, Minnesota, USA).

Table 1
Experimental domain with natural and coded values of independent variables used in Box–Behnken design (BBD).

Independent variable	Factor levels		
	−1	0	1
Temperature [°C]	100	150	200
Pressure [bar]	30	60	90
Time [min]	10	20	30

3. Results and discussion

Box–Behnken experimental design, developed for the process optimization considering total phenols and total flavonoids content, and antioxidant activity (IC_{50} value) with experimentally obtained values for each response under different SWE conditions, is presented in Table 2.

Experimentally obtained values for total phenols content (TP) varied from 0.5119 to 2.6297 g GAE/100 g CSS (Table 2). It was possible to conclude that temperature was the most significant factor influencing the response since TP did not vary significantly on fixed level of temperatures, i.e. TP contents obtained on 200 °C and different pressure and extraction time were 2.4612–2.6297 g GAE/100 g CSS. TP obtained with SWE at 200 °C in this work was significantly higher comparing to previously reported results of TP obtained with maceration for 24 h using ethanol, methylenechloride, ethyl acetate, butanol and water, which varied from 0.09 g GAE/100 g CSS obtained with methylenechloride to 1.89 g GAE/100 g CSS obtained with ethyl acetate [11]. Even though, modern extraction

Table 2
Box–Behnken experimental design with natural and coded SWE conditions and experimentally obtained values of total phenols content (TP), total flavonoids content (TF) and antioxidant activity (IC_{50}).

Run order	Independent variables			Investigated responses		
	X_1 Temperature [°C]	X_2 Pressure [bar]	X_3 Time [min]	TP [g GAE/100 g CSS]	TF [g CE/100 g CSS]	IC_{50} [mg/ml]
1	150(0)	60(0)	20(0)	0.9119	0.3043	0.05636
2	150(0)	30(−1)	30(1)	1.0240	0.3370	0.04676
3	150(0)	60(0)	20(0)	0.9633	0.3054	0.05392
4	200(1)	30(−1)	20(0)	2.6297	0.5852	0.01795
5	150(0)	90(1)	30(1)	1.1138	0.3728	0.04852
6	200(1)	60(0)	30(1)	2.4141	0.6187	0.01728
7	150(0)	30(−1)	10(−1)	0.8466	0.2789	0.05341
8	200(1)	90(1)	20(0)	2.4612	0.6280	0.01706
9	150(0)	90(1)	10(−1)	0.8151	0.2692	0.05006
10	150(0)	60(0)	20(0)	0.9690	0.3076	0.05313
11	100(−1)	30(−1)	20(0)	0.5344	0.2315	0.05524
12	200(1)	60(0)	10(−1)	2.5915	0.3040	0.01901
13	100(−1)	60(0)	30(1)	0.5636	0.2715	0.06315
14	100(−1)	60(0)	10(−1)	0.5119	0.2408	0.06336
15	100(−1)	90(1)	20(0)	0.5198	0.2460	0.05578

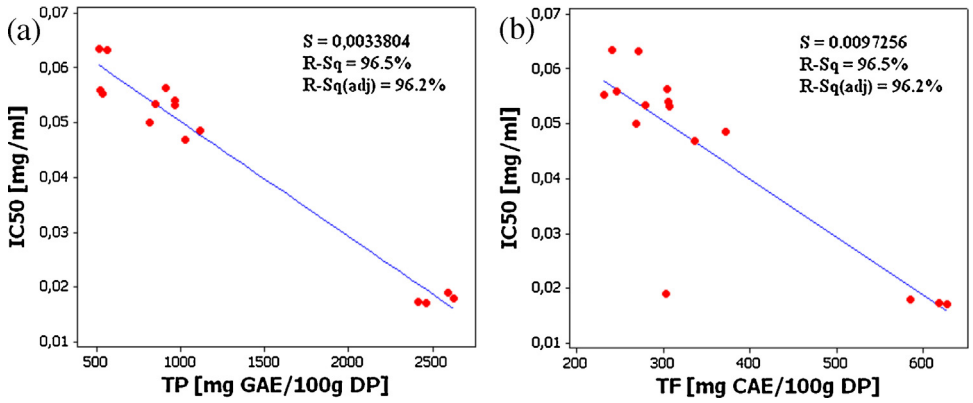

Fig. 3. Correlation between total phenolics content (TP) and IC_{50} value (a) and total flavonoids content (TF) and IC_{50} value (b).

techniques such as ultrasound-assisted (UAE) and microwave-assisted extraction (MAE) can provide higher yields and improve process, TP obtained with these techniques were 0.0418 and 0.0821 g GAE/100 g CSS [26], which once again confirmed higher yields obtained using SWE.

Total flavonoids content (TF) in vegetative parts of *C. sativum* was reported to be 0.5259 g/100 g CSS, and flavonoid fraction mainly consisted of quercetin and kaempferol glycosides [27,28]. Experimentally obtained values for TF varied from 0.2315 to 0.6280 g CE/100 g CSS (Table 2). Once again, temperature had the most significant influence since TF obtained at 200 °C was approximately twice higher comparing to TF obtained on 100 °C and 150 °C, regardless of pressure and extraction time. TF obtained with SWE at 200 °C in this work was, also, significantly higher comparing to previously reported results of TF obtained with extraction for 1 h using water, 70% ethanol, 70% methanol and 70% acetone, which varied from 0.19 g QE/100 g CSS obtained with 70% methanol to 0.35 g QE/100 g CSS obtained with 70% ethanol [29].

Experimentally obtained values for antioxidant activity, i.e. IC_{50}, varied from 0.01706 to 0.06336 mg/ml (Table 2). The lowest IC_{50}, value i.e. the highest antioxidant activity, was obtained at high levels of temperature and pressure and low level of extraction time, whilst on low levels of temperature and extraction time and middle level of pressure, the antioxidant activity was the lowest. It is expected that correlation exist between contents of phenolic and flavonoid compounds and antioxidant activity, however, some found no such relationship [29,30]. Significant linear correlation was observed between TP and IC_{50} value ($R^2 = 0.965$) suggesting that higher TP content provides higher antioxidant activity (Fig. 3a). In case of TF and IC_{50}, moderate correlation was observed ($R^2 = 0.709$). Most of the experimental points exhibit similar correlation like TP, however, TF obtained at 200 °C, 60 bar for 10 min, deviates from linear regression (Fig. 3b). Even though TF at this experimental point was rather low, this extract exhibited high antioxidant activity (Table 2).

Although, it has been reported that SWE on temperatures between 100 and 150 °C can be suitable for recovery of volatile compounds from essential oils [13,31], liquid extracts obtained in this work were subjected to hydrodistillation, but no essential oil was isolated. The reason for this could be terpene transformation and degradation on prolonged time during SWE [32]. Appearance of extracts could be indicator of degradation since with increase of temperature, color of extracts was from light brown to dark brown. Odor was still pleasant and recognizable for coriander in extracts obtained on 100 °C, while on higher temperatures, odor was rather unpleasant suggesting that complete degradation of essential oil components occurred.

3.1. Model fitting

Experimental values were fitted to a second-order polynomial model (Eq. (3)) and multiple regression coefficients were generated for all responses using statistical approach called the method of least square (MLS) which represents a multiple regression technique generating the lowest residual possible [24]. The regression coefficients of the model for each response are presented in Table 3, while results of the analysis of variance (ANOVA) are summarized in Table 4.

According to particularly high values of coefficients of multiple determination (R^2) for TP, TF and IC_{50} (0.994, 0.934 and 0.991, respectively), model equations provides good representation of experimental values. Moreover, for all three responses, mathematical models were statistically acceptable due to significant regression for the model ($p < 0.05$) (Table 4). Lack of fit testing confirmed adequacy of fitting experimental data to a second-order polynomial model in case of TP and IC_{50}, where p-value for lack of fit was insignificant ($p > 0.05$) (Table 4). However, for TF content, p-value for lack of fit was 0.0005, which suggested not a good fit to the mathematical model Eq. (3). Similar problem with fitting flavonoid extraction to this model was previously reported by Gan and Latiff [23]. Acquired experimental data was used for creation of response surface three-dimensional plots and regression equations which could predict response values at investigated experimental domain (Table 5). ANOVA suggests that these equations would be able to adequately describe behavior of TP and IC_{50}, however, due to significant lack of fit in case of TF, regression equation for this response could provide suspicious results [18].

Table 3
Estimated coefficients of the fitted second-order polynomial model for TP, TF and IC_{50} value.

Regression coefficient	Response		
	TP	TF	IC_{50}
β_0	0.94808*	0.30578*	0.05447*
Linear			
β_1	0.99587*	0.14326*	−0.02078*
β_2	−0.01558	0.01043	−0.00024
β_3	0.04378	0.06337*	−0.00126
Cross product			
β_{12}	−0.03846	0.00708	−0.00036
β_{13}	−0.05727	0.07103**	−0.00038
β_{23}	0.03032	0.01138	0.00128
Quadratic			
β_{11}	0.57929*	0.08061*	−0.01347*
β_{22}	0.00889	0.03632	−0.00449*
β_{33}	−0.00710	−0.02763	−0.00029

* Significant at 0.05 level.
** Significant at 0.10 level.

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

	Sum of squares	DF	Mean square	*F*-value	*p*-Value
Total phenolics content[a]					
Model	9.226781	9	1.025198	91.57549	<0.0001
Residual	0.055976	5	0.011195		
Lack of fit	0.053999	3	0.018000	18.21139	0.0525
Pure error	0.001977	2	0.000988		
Total	9.282757	14			
Total flavonoids content[b]					
Model	0.250281	9	0.027809	7.91	0.0174
Residual	0.017577	5	0.003515		
Lack of fit	0.017572	3	0.005857	2173.01	0.0005
Pure error	0.000005	2	0.000002		
Total	0.267858	14			
IC_{50} value[b]					
Model	0.004192	9	0.000466	64.70781	0.0001
Residual	0.000036	5	0.000007		
Lack of fit	0.000030	3	0.000010	3.56171	0.2269
Pure error	0.000006	2	0.000002		
Total	0.004228	14			

[a] The coefficient of determination (R^2) of the model was 0.994.
[b] The coefficient of determination (R^2) of the model was 0.934.
[c] The coefficient of determination (R^2) of the model was 0.991.

3.2. Influence of independent variables on investigated responses

The influence of extraction conditions toward investigated responses was reported through significant ($p < 0.05$) and moderately significant ($p < 0.10$) regression coefficients of the second-order polynomial regression equation. In case of total phenolics content, only linear and quadratic terms of temperature had significant influence ($p < 0.05$), while all other effects were insignificant. As the extraction and separation of phenolic compounds depend largely on the polarity of solvents and extractable compounds [33], extractive power of the water could be limited toward phenolics due to relatively high dielectric constant at room temperature. Temperature has influence both on solvent by changing its physical properties such as viscosity, surface tension and dielectric constant, and herbal matrix by causing its disruption and increasing mass transfer through solid phase [33]. Positive effects of linear and quadratic terms of temperature can be observed on Fig. 4a and b from which could be seen that pressure and extraction time have limited influence. Therefore, the highest TP was observed on high level of temperature (200 °C) regardless of pressure and extraction time, and it was more than twice higher comparing to TP obtained on 100 °C and 150 °C. Significant increase in TP yield on 200 °C could be explained by decreased dielectric constant of water at higher temperature, resulting in a better extraction of phenolics [34]. By setting temperature at the fixed high level (200 °C) and observing effects of pressure and extraction time, it could be seen that highest TP would be obtained on low levels of pressure and extraction time, i.e. 30 bar and 10 min (Fig. 4c). Decrease of TP was observed on prolonged extraction time and high pressure. While effects of extraction time could be explained by temperature degradation and/or possible reaction of phenolics with other plant constituents [35], pressure effects on this phenomenon is not yet defined.

According to *p*-values of regression coefficients, linear terms of temperature and extraction time and quadratic terms of temperature had significant influence, while interaction between temperature and extraction time had moderate influence (Table 3). Once again, the influence of extraction pressure on TF yield was limited (Fig. 4d and f). From Fig. 4e, it could be seen that temperature influence was the most dominant, but interaction with extraction time also had influence and on prolonged extractions, higher TF yields would be obtained in high levels of temperature and extraction time. This indicates that flavonoids fraction from *C. sativum* seeds is rather stable on elevated temperatures. Therefore, temperature exhibited its positive influence by moderating water physical properties and causing softening of the plant tissue which lead to disruption of interactions between flavonoids and proteins or polysaccharides and increase of the solubility of flavonoids in hot water [36]. Since extended extraction time is needed for complete extraction of flavonoids from coriander seeds, it could be concluded that more time is needed for diffusion of the solute from solid matrix to crude extract. By setting temperature at the fixed high level (200 °C) and observing effects of pressure and extraction time, it could be seen that extraction time has positive linear effect on TF yield at all levels of pressure.

Comparing to pressure effects on TP and TF, in case of IC_{50} value, quadratic term of pressure exhibited significant negative influence (Table 3). Besides that, linear and quadratic terms of temperature had significant influence while all other terms were insignificant (Table 3). From Fig. 4g and h, it could be seen that temperature influence was dominant comparing to other independent variables. Since good linear correlation was observed between antioxidant activity and polyphenolics content (TP and TF), it is expected that independent variables will exhibit similar effects on these responses. Therefore, the lowest IC_{50} values, i.e. the highest antioxidant activities, were observed on high level of temperature (Fig. 4g and h). At the fixed high level of temperature (200 °C), negative quadratic influence of pressure could been observed (Fig. 4i). The lowest IC_{50} values were obtained on low (30 bar) or high (90 bar) level of pressure, regardless of extraction time. Since, low pressures are more suitable for manipulation due to safety reasons, extractions on 30 bar could be used for obtaining extracts with higher antioxidant activity.

Table 5
Second-order polynomial equations for investigated response variables.

Response	Second-order polynomial model equation
Total phenolics content	$TP = 0.9481 + 0.9959X_1 - 0.0156X_2 + 0.0438X_3 - 0.0385X_1X_2 - 0.0573X_1X_3 + 0.0303X_2X_3 + 0.5793X_1^2 + 0.0089X_2^2 - 0.0071X_3^2$
Total flavonoids content	$TF = 0.3058 + 0.1433X_1 + 0.0104X_2 + 0.0634X_3 + 0.0071X_1X_2 + 0.0710X_1X_3 + 0.0114X_2X_3 + 0.0806X_1^2 + 0.0363X_2^2 - 0.0276X_3^2$
IC_{50} value	$IC_{50} = 0.05447 - 0.02078X_1 - 0.00024X_2 - 0.00126X_3 - 0.00036X_1X_2 - 0.00038X_1X_3 + 0.00128X_2X_3 - 0.01347X_1^2 - 0.00449X_2^2 - 0.00029X_3^2$

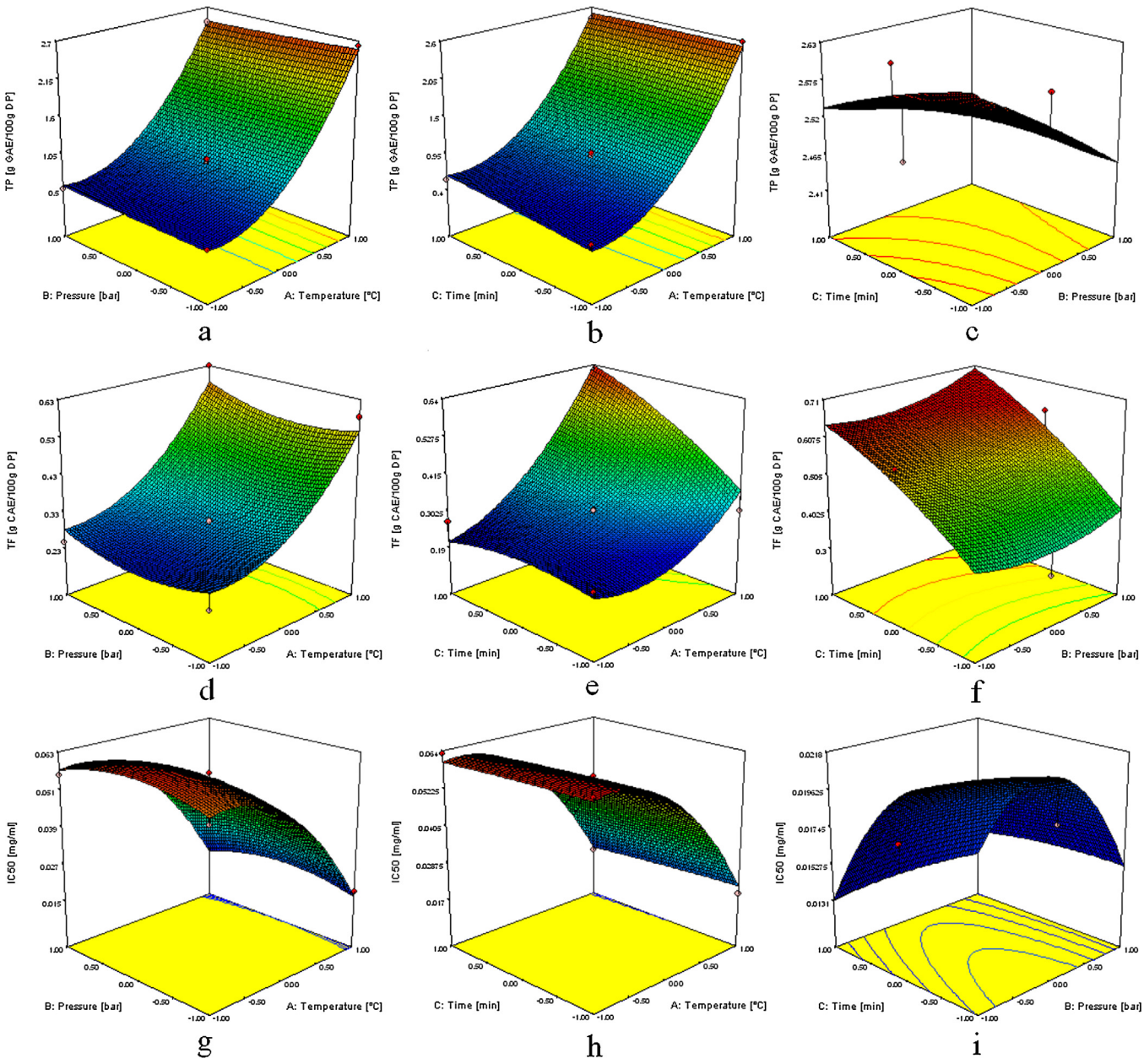

Fig. 4. Response surface plots showing combined effects of temperature, pressure and extraction time on total phenolics content ((a)–(c)), total flavonoids content ((d)–(f)) and IC_{50} value ((g)–(i)).

3.3. Optimization of SWE process

Optimization of industrial process is crucial for its efficiency and profitability. Therefore, in subcritical water extraction, several variables must be optimized. SWE has certain advantages comparing to conventional extraction process, but, temperature, as the most influential variable has to be particularly high in order to obtain satisfying yield. Since pressure effects are rather insignificant, it is desirable to carry out process on lower pressures due to lower exhausting of the equipment and safety reasons. Reduction of extraction time can significantly reduce operational costs and using SWE, higher yields could be obtained for 10 min, comparing to conventional solvent extraction for 24 h [11]. Optimized extraction conditions for maximized yields of TP and TF and minimized IC_{50} value, i.e. maximized antioxidant activity, are presented in Table 6. In order to optimize all three responses at the same time, desirability function was employed and optimized condition were

Table 6
Optimized SWE conditions for total phenolics content, total flavonoids content and IC_{50} value.

Optimized conditions			
Temperature [°C]	200	200	200
Pressure [bar]	30	90	35.7
Extraction time [min]	10	29.4	29.4
Predicted values	TP [g GAE/100 g CSS]	TF [g CE/100 g CSS]	IC_{50} [mg/ml]
	2.6228	0.6950	0.01519

temperature of 200 °C, pressure of 30 bar and extraction time of 28.3 min. Obtained values of TP, TF and IC_{50} value at this experimental point would be 2.5452 g GAE/100 g CSS, 0.6311 g CE/100 g CSS and 0.01372 mg/ml, respectively, while desirability was 0.987. Since pressure has rather insignificant effects, it would be much

easier to operate on lower pressures which should not affect TF yield significantly.

4. Conclusions

Subcritical water extraction was employed in order to obtain antioxidant-rich extracts from coriander seeds. Significant improvement was observed comparing yields of phenolics and flavonoids obtained with this technique, with conventional solid–liquid extraction and modern extraction techniques, such as ultrasound-assisted and microwave-assisted extraction. Response surface methodology was used for optimization of SWE conditions (temperature, pressure and extraction time). The highest yields of investigated responses and lowest IC_{50} were observed on high level of temperature (200 °C), while influence of other factors was limited. Total phenolics and total flavonoids content were maximized, while IC_{50} value was minimized, and optimum conditions were determined using desirability function. The most efficient extraction conditions for all three responses were temperature of 200 °C, pressure of 30 bar and extraction time of 28.3 min, while obtained values of TP, TF and IC_{50} value would be 2.5452 g GAE/100 g CSS, 0.6311 g CE/100 g CSS, and 0.01372 mg/ml, respectively. Moreover, good and moderate linear correlation was observed between antioxidant activity (IC_{50} value) and total phenolics content ($R^2 = 0.965$) and total flavonoids content ($R^2 = 0.709$), which indicated that these groups of compounds are responsible for antioxidant activity of *C. sativum* extracts.

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