DETERMINATION OF OPTIMAL PARAMETERS OF BASIL SUPERCRITICAL FLUID EXTRACTION BY RESPONSE SURFACE METHODOLOGY

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The supercritical fluid extraction of aroma compounds from basil (Ocimum basilicum L.) was studied. Response surface methodology was used to optimize the parameters of the process. Full factorial design was applied to evaluate the effects of two independent variables (pressure and temperature) on the extraction yield and linalool yield. From the response surface plots, pressure and temperature exhibited independent and interactive effect on the extraction yield. The optimal conditions to obtain the highest extraction yield (1.91%) of O. basilicum were the pressure of 29.7 MPa and temperature of 59.2°C, whereas the highest yield of linalool (1.998 g·kg⁻¹) was obtained at the pressure of 20 MPa and temperature of 40°C. The experimental values agreed with the predicted ones, indicating suitability of the response surface methodology for optimizing the extraction process.

KEY WORDS: basil, supercritical carbon dioxide extraction, linalool, response surface methodology

INTRODUCTION

In the recent years, there has been an increasing interest in essential oils and natural antioxidants extracted from different herbs and aromatic vegetable crops (1-3). Polyphenols, as biologically active compounds are used for preparation of dietary supplements, nutraceuticals, functional food ingredients or cosmeceuticals (4).

Basil (Ocimum basilicum L.) is one of the most popular plants grown extensively in many countries around the world (especially in Mediterranean countries). It is mostly used as culinary herb for its characteristic aroma. Besides its use in traditional medicine, it is a well-known source of food packaging and flavoring (5,6). Essential oil isolated from O. basilicum has antimicrobial, antiviral, anti-inflammatory and antioxidant activity (7-9). Also, basil extracts exhibit a wide spectrum of properties, including bactericidal, anti-inflammatory, antioxidant, chemopreventive, and anti-diabetic activities, and act as nervous system stimulatory (1, 10-12).

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Basil extracts are mostly obtained by different solvent extractions, using solvents with different polarity (such as methanol, ethanol and water, pure or mixed) and by supercritical fluid extraction (SFE) (13). Solvent extraction is limited by economic, environmental and safety problems. It is also non-selective, with large volumes of organic solvents used and long extraction times. The final products are with toxic organic residues and, most often, accompanied with degradation or loss of target compounds (14).

Among innovative process technologies, SFE has shown increasing importance. SFE can be a fast, clean and efficient method for the extraction of valuable natural compounds from plant materials (15). Carbon dioxide has been proved to be effective solvent for the application in the pharmaceutical, chemical and food industry. The solvating power of supercritical carbon dioxide can be summarized by a few rules (16): 1) it dissolves non-polar or slightly polar compounds; 2) the solvent power for low molecular weight compounds is high and decreases with increasing molecular weight; 3) free fatty acids and their glycerides exhibit low solubility; 4) pigments are even less soluble; 5) proteins and polysaccharides are insoluble and 6) supercritical CO$_2$ is capable of extracting compounds that are less volatile, having a higher molecular weight and/or a higher polarity as pressure increases.

In order to optimize the process, one-factor at-a-time approach is generally applied. Response surface methodology (RSM) is a collection of mathematical and statistical techniques used for developing, improving and optimizing process. That experimental methodology is based on the analysis of the effects of the independent variables, where influences of independent variables on responses are investigated one by one, while all the others are kept constant. For the possible industrial application, the optimization of the extraction process is essential. In the literature, the effects of supercritical extraction parameters on the basil extraction yields were poorly investigated.

In this study, the effect of pressure and temperature on the extraction yield and linalool yield were investigated. Response surface methodology was used to build a model between extraction yield and these independent factors, and to optimize the extraction conditions.

**EXPERIMENTAL**

**Chemicals**

Commercial carbon dioxide (Messer, Novi Sad, Serbia) purity >99.98% (w/w) was used for the laboratory SFE. The standard compound linalool, used as external standard, was purchased from Carl Roth GmbH (Germany). All other chemicals were of analytical reagent grade.

**Plant material**

*Ocimum basilicum* was cultivated at the Department for Organic Production and Biodiversity, Bački Petrovac, Serbia, in 2011. The collected plant material (leaves and
flowering tops) were air dried and stored at room temperature. Moisture content of the dried plant was determined using standard procedure, i.e., after drying the plant sample at 105°C as 11.44% (w/w). The dried basil was ground and the particle size of ground material was determined using sieve sets (Erweka, Germany).

**Supercritical carbon dioxide extraction**

The extraction was carried out on a laboratory scale high pressure extraction plant (HPEP, NOVA-Swiss, Effertikon, Switzerland; Figure 1). The main plant parts and properties, by manufacturer specification were: gas cylinder with CO₂, the diaphragm type compressor with pressure range up to 100 MPa, extractor with heating jacket for heating medium with internal volume of 200 mL and maximum operating pressure of 70 MPa, separator with heating jacket for heating medium with internal volume of 200 mL and maximum operating pressure of 25 MPa, pressure control valve, temperature regulation system and regulation valves. Maximum carbon dioxide mass flow rate is 5.7 kg·h⁻¹.

The ground sample of basil (50.0 g) mean particle size of 0.65 mm was placed in the extractor vessel. The extraction process was carried out and extraction yield was measured after 4 h of extraction. A flow rate of carbon dioxide, expressed under normal conditions, was 97.725 dm³·h⁻¹. The investigated values of pressure were 10, 15, 20 and 30 MPa, and of temperature 40°C, 50°C and 60°C. The separator conditions were 1.5 MPa and 23°C. After each extraction, the obtained extract was placed in a glass bottle, sealed and stored at 4°C to prevent any possible degradation.

**Figure 1.** Schematic diagram of the apparatus used for supercritical fluid extraction

Chromatographic procedure

The GC/MS analysis was run on an Agilent GC 6890N system coupled to an Agilent MS 5795 mass spectrometer. An HP-5MS column (30 m length, 0.25 mm inner diameter and 0.25 μm film thickness) was used. The injected volume of sample solution in methanol was 5 μl with split ratio 30:1. The compounds were identified using the NIST 05 and Wiley 7n mass data base and by comparing their retention times to those in mass spectral libraries. Quantifications of the aromatic compound linalool was performed by FID detector and calibration curve for the compound. The percentage composition (relative amount) was calculated from the peak area. The GC/MS operating conditions were as follows: injector temperature 250°C, temperature program was: from 60°C to 150°C (4°C·min⁻¹), carrier gas He with flow rate 2 ml·min⁻¹. The GC/FID operating conditions were: injector temperature 250°C, temperature program from 60°C to 150°C (4°C·min⁻¹), detector temperature 300°C.

All experiments were performed in triplicate and results are given as mean values.

Experimental design and statistical analysis

In this study, the RSM and 3² factorial design were applied to determine the optimal extraction temperature and pressure for SFE. RSM is an optimization procedure based on physical experiments or computer experiments (simulations) and experimented observations (17, 18).

The experimental data were fitted to a second order response surface model (Eq. [1]) of the following form:

$$ Y = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_i^2 + \sum_{i<j=1}^{4} \beta_{ij} x_i x_j $$  

[1]

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

If the response is defined by a linear function of independent parameters, then it is a first-order function that can be expressed as Eq. [2]:

$$ Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 $$  

[2]

Generally, first-order equations cannot give the desired approximation to real experiments, so second-order models or models with more parameters are used.

In this study, two independent variables, pressure ($X_1$) and temperature ($X_2$) were evaluated to optimize the extraction yield (in percent) and linalool yield (in grams per kilogram). The carbon dioxide flow rate and extraction time were fixed values. The
coded and uncoded independent variables used in the RSM design are shown in Table 1. Experiments were randomized, to maximize the effects of unexplained variability in the observed responses.

**Table 1. The uncoded and coded independent variables used in RSM design**

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Symbol</th>
<th>Coded level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-1</td>
</tr>
<tr>
<td>Pressure (bar)</td>
<td>X₁</td>
<td>100</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>X₂</td>
<td>40</td>
</tr>
</tbody>
</table>

The analysis was performed using commercial software Design-Expert v.7 Trial (Stat-Ease, Minneapolis, Minnesota, USA). The analysis of variance (ANOVA) was also used to evaluate the quality of the fitted model. The test of statistical difference was based on the total error criteria with confidence level of 95.0%.

**RESULTS AND DISCUSSION**

**Effect of SFE parameters on extraction yield**

The effects of pressure (10-30 MPa) and temperature (40-60°C) on the basil supercritical CO₂ extraction yield and linalool yield were investigated. The 3² factorial design was used to optimize important operating variable (pressure and temperature) in order to achieve the optimal yield of basil extract. The optimization of the experimental conditions represents the critical and most important step in the development of supercritical extraction method. The obtained experimental results of 9 runs are summarized in Table 2.

**Table 2. Full factorial design (3²) with independent variables and experimentally obtained results of the extraction yield (Y) and linalool content**

<table>
<thead>
<tr>
<th>Run order</th>
<th>Pressure (MPa)</th>
<th>Temperature (°C)</th>
<th>Y* (%</th>
<th>Linalool (g·kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 (-1)</td>
<td>40 (-1)</td>
<td>0.719</td>
<td>1.194</td>
</tr>
<tr>
<td>2</td>
<td>20 (0)</td>
<td>40 (-1)</td>
<td>1.322</td>
<td>1.998**</td>
</tr>
<tr>
<td>3</td>
<td>30 (1)</td>
<td>40 (-1)</td>
<td>1.287</td>
<td>1.379**</td>
</tr>
<tr>
<td>4</td>
<td>10 (-1)</td>
<td>50 (0)</td>
<td>0.894</td>
<td>1.054</td>
</tr>
<tr>
<td>5</td>
<td>20 (0)</td>
<td>50 (0)</td>
<td>1.447</td>
<td>1.386**</td>
</tr>
<tr>
<td>6</td>
<td>30 (1)</td>
<td>50 (0)</td>
<td>1.715</td>
<td>1.739**</td>
</tr>
<tr>
<td>7</td>
<td>10 (-1)</td>
<td>60 (1)</td>
<td>0.382</td>
<td>0.382</td>
</tr>
<tr>
<td>8</td>
<td>20 (0)</td>
<td>60 (1)</td>
<td>1.666</td>
<td>1.609</td>
</tr>
<tr>
<td>9</td>
<td>30 (1)</td>
<td>60 (1)</td>
<td>1.879</td>
<td>1.313</td>
</tr>
</tbody>
</table>

Published data * (19) ** (20)
The extraction yield after the supercritical extraction was in a range from 0.382 to 1.879 %. The highest extraction yield of 1.879 %, in the investigated conditions was achieved at a pressure of 30 MPa and temperature of 60ºC. Furthermore, it was observed that the extraction yield increased by increasing pressure from 10 to 30 MPa, at constant temperature. This can be explained by the increase in the solvent power of the supercritical CO₂, i.e. solvent density, with the increase in the pressure.

The analysis of variance (ANOVA) proved the suitability of the fitted model (Table 3). The coefficient of determination (R²) of the model was 0.966, indicating that the model adequately represents the real relationship between parameters. This claim is supported by the relatively low value of the coefficient of variation, and also the good correlation between the predicted values by the mathematical model and experimental results obtained shown in Figure 2. Based on the p-value of the model (p<0.05), it can be concluded that the assumed model fits well to the experimental data.

Table 3. Analysis of variance (ANOVA) of the fitted second-order polynomial model

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>Df</th>
<th>MS</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>1.9254</td>
<td>5</td>
<td>0.3850</td>
<td>17.3535</td>
<td>0.0201</td>
</tr>
<tr>
<td>Residual</td>
<td>0.0665</td>
<td>3</td>
<td>0.0221</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.9919</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SS - Sum of squares, Df - Degrees of freedom, MS - Mean square

Figure 2. Distribution of the predicted and experimental values of extraction yield (Y)

The regression coefficients of the intercept, linear, quadratic and interaction terms of the model were calculated using the least squares technique. The degree of significance of each factor is presented in Table 4. It is evident that the linear parameter of extraction
pressure has very significant (p<0.05) effect on the extraction yield, while the effect of the quadratic and interaction parameters on the extraction yield is significant (p<0.10).

Table 4. Regression coefficients of the fitted second-order polynomial model

<table>
<thead>
<tr>
<th>Regression coefficient</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_0$</td>
<td>1.573*</td>
</tr>
<tr>
<td>Linear</td>
<td></td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>0.481*</td>
</tr>
<tr>
<td>$\beta_2$</td>
<td>0.099</td>
</tr>
<tr>
<td>Cross product</td>
<td></td>
</tr>
<tr>
<td>$\beta_{12}$</td>
<td>0.232**</td>
</tr>
<tr>
<td>Quadratic</td>
<td></td>
</tr>
<tr>
<td>$\beta_{11}$</td>
<td>-0.332**</td>
</tr>
<tr>
<td>$\beta_{22}$</td>
<td>-0.142</td>
</tr>
<tr>
<td>Coefficient of multiple determination $R^2$</td>
<td>0.966</td>
</tr>
<tr>
<td>Coefficient of variance [%]</td>
<td>11.9</td>
</tr>
</tbody>
</table>

* Significant at 5%; ** Significant at 10%

Figure 3 shows a three-dimensional plot of the response surface for the extraction yields of basil obtained by extraction with supercritical carbon dioxide. From the surface plot, it is evident that the extraction yield significantly increases with increased pressure and increased temperature, at the constant extraction time. The pressure had a positive linear effect on the extraction yield, however, the negative quadratic effect also became significant because the further increase in pressure resulted in a slight decline of the extraction yield. Also, it can be seen that the pressure influence was dominant comparing to the second independent variable. This can be explained by the fact that the increase in pressure can result in an increase in fluid density, which accelerates the mass transfer and improves extraction yield (15).

Figure 3. Response surface plot showing effects of the investigated parameters on the extraction yield (Y)
The mathematical model representing the extraction yield of basil as a function of the independent variables within the region under investigation was expressed by the following equation:

\[ Y = 1.573556 + 0.481 \ p + 0.099833 \ T + 0.23225 \ pT - 0.33233 \ p^2 - 0.14283 \ T^2 \]  \[3\]

where \( p \) is the pressure and \( T \) is the temperature.

Using the above equation, the optimal conditions to obtain the highest extraction yield of basil were the pressure of 29.7 MPa and temperature of 59.2°C. Under these conditions, the calculated total extraction yield was 1.91%. As the experimentally obtained yield (1.879%) was not significantly different from the predicted value, at the 95% confidence level, it can be concluded that the optimization has been performed successfully.

These findings are in agreement with the results of other studies. In the supercritical extraction of basil reported by Mazutti et al. (21) the highest extraction yield of 1.95% was obtained at a pressure of 25 MPa and temperature of 50°C during 3 h of extraction. Lachowicz et al. (22) reported that during the supercritical carbon dioxide extraction at 10.33 MPa and 40°C extraction yield was 0.51% and at 31 MPa and 40°C it was 0.97%. The extraction times in both processes were 2 h.

**Effect of SFE parameters on linalool yield**

In order to optimize the extraction parameters (temperature and pressure) to obtain the highest yield of linalool, the optimization could not be performed in the same manner, because the quadratic model (Eq. [1]) did not give the desired approximation to real experiments (\( p > 0.05 \)). It was found that the linear model (Eq. [2]) describes better the extraction system (\( p < 0.05 \)). The coefficient of determination (\( R^2 \)) of the model was 0.85, indicating that the model had a certain deficiency. The predicted linear model used to express the yield of linalool (\( Y \)) obtained by supercritical carbon dioxide as a function of two independent variables is as follows:

\[ Y = 1.79417 + 0.03003 \ p - 0.021108 \ T \]  \[4\]

The influence of the pressure and temperature on the linalool yield is presented in Figure 4. It can be observed that pressure had a positive effect on the yield of linalool, while temperature had a negative effect. The increase in pressure from 10 to 30 MPa at 40 and 60°C, led to the increase and then a slight decrease in the linalool yield. At the temperature of 50°C, the increase in pressure from 10 to 30 MPa (increasing in solvent density from 0.378 to 0.881 kg·m\(^{-3}\)) resulted in the enhanced linalool yields. From the experimental results (Table 2), it can be seen that the highest yield of linalool (1.998 g·kg\(^{-1}\)) was obtained at a pressure of 20 MPa and temperature of 40°C (fluid density 0.831 kg·m\(^{-3}\)). So, the supercritical optimum operating conditions to achieve maximum yield of linalool were close to the fluid density of 0.8 kg·m\(^{-3}\).
Response surface methodology was successfully applied for optimization the parameters in SFE from *O. basilicum*. The results were very useful for the selection of pressure and temperature in order to obtain the highest extraction yield by supercritical carbon dioxide. The high correlation of the mathematical model indicated that a quadratic polynomial model could be employed to optimize supercritical carbon dioxide extraction of basil. From the response surface plots it can be concluded that pressure and temperature significantly influenced extraction yield, independently and interactively. The optimal conditions to obtain the highest yield were determined to be 29.7 MPa and 59.2°C. Under the optimal conditions, the experimental values agreed with the predicted.

For optimization of the parameters leading to the highest yield of linalool, linear model was employed. The low correlation between experimental and predicted values indicated that further research must involve more independent variables in the non-linear model with the aim to better optimize this response.

This study can be useful in the development of industrial extraction process concerning the optimal sequential steps to enhance the efficacy of a large-scale extraction system.

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**ОДРЕЂИВАЊЕ ОПТИМАЛНИХ ПАРАМЕТАРА ЗА СУПЕРКРИТИЧНУ ЕКСТРАКЦИЈУ БОСИЉКА МЕТОДОМ ОДЗИВНЕ ПОВРШИНЕ**

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У овом раду суперкритична екстракција испарљивих компонената босилјка (*Ocimum basilicum* L.) је испитивана. У циљу оптимизације параметара суперкритичне екстракције примењена је метода одзивне површине. Факторијални дизајн је употребљен за евалуацију ефеката две независно променљиве (притиска и температуре) на принос екстракције и принос линалоола. Утицај притиска на процес суперкритичне екстракције испитан је у опсегу притиска од 10 до 30 МPa, док је утицај температуре испитан у опсегу од 40 до 60°C. На основу одзивних површина, притисак и температура испољавају независни и међусобни утицај на принос екстракције. Оптимални услови при којима се добија највећи принос екстракције (1,91%) је притисак од 29,7 МPa и температура од 59,2°C, док је највећи принос линалоола (1.998 g·kg⁻¹) добијен при притиску од 20 МPa и температуре од 40°C. Експерименталне вредности које се одлично слажу са предвиђеним квадратним моделом, указују да је полиномска зависност посматраног одзива погодна за оптимизацију суперкритичне екстракције босилјка.

**Кључне речи:** босилјак, суперкритична екстракција, линалоол, метода одзивне површине

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