Determination of extraction conditions of *Ginkgo biloba* L. leaves by supercritical CO_2 using response surface methodology

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Abstract

The effects of process parameters on the extraction of *Ginkgo biloba* L. leaves with supercritical carbon dioxide were investigated. The investigated parameters include particle size (mean particle diameter 0.19, 0.467 and 1.009 mm), solvent flow rate (1.58×10^{-3}, 3.22×10^{-3} and 4.16×10^{-3} kg CO_2/min) and pressure (100–300 bar), which were obtained by the response surface methodology (RSM) under the following condition ranges: temperature 40––50–60 °C, pressure 100–140–180 bar and extraction time of 2–3–4 h at the flow rate of 3.22×10^{-3} kg/min. Based on the experimental results of kinetics of *Ginkgo biloba* leaves extraction with supercritical carbon dioxide, modeling of the extraction system of *Ginkgo biloba*–supercritical CO_2 was done. Two mathematical models (Reverchon–Sesti Osseo and Sovová) were applied to correlate the experimental data. RSM was applied to optimize the process parameters of supercritical carbon dioxide extraction of *Ginkgo biloba* L. leaves. A second-order polynomial response surface equation was developed indicating the effect of variables on *Ginkgo biloba* extraction yield. The statistical analysis of the experiment indicated that pressure (X_1), extraction time (X_3), the quadratic of temperature (X_2^2), and the interaction between pressure and extraction time (X_1X_3), show significant effect on the extraction yield. The results showed that the data were adequately fitted into the second-order polynomial model. It was predicted that the optimum extraction process parameters within the experimental ranges would be the extraction temperature of 52.7 °C, the pressure of 184.4 bar, and the extraction time of 3.86 h. Under these conditions, the predicted extraction yield is 2.39% (g/100 g drug).

Keywords: *Ginkgo biloba* L.• supercritical extraction • supercritical CO_2 • Response surface methodology

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The dried leaves of *Ginkgo biloba* L. have been used as herbal remedies for centuries in China, and nowadays their extracts are one of the most widely used herbal products and/or dietary supplements in the world. *Ginkgo biloba* L. extract can reduce blood pressure, dilate peripheral blood vessels, increase capillary and venous blood flow to the head and may be effective in treating peripheral arterial diseases, tinnitus, hearing loss, inner ear neurological disorders, sclerosis of cerebral arteries and intermittent claudication (poor circulation in the legs). It is helpful in inhibiting the onset of vascular dementia (including Alzheimer's disease) and functional disability, and reducing the incidence of cardiovascular disease due to its ability to prevent free radical damage, improve brain function, and support microcirculation [1–5].

G*inkgo biloba* leaves contain different active ingredients [6], such as ginkgolides (A, B, C and J), bilobalides, flavonoids, flavonol aglycones, terpene trilaktones, proanthocyanidins, alkylphenols, simple phenolic acids, etc. Ginkgo flavonoids are an important class of compounds in *Ginkgo biloba* leaves and usually act as scavengers of different oxidizing species, *i.e.*, superoxide anions, hydroxyl, or peroxy radicals [7].

Ginkgo biloba extracts can be obtained using supercritical carbon dioxide extraction technology. In comparison to conventional extraction processes using liquid solvents, supercritical fluid extraction (SFE) is a good choice for the extraction of heat sensitive or easily oxidizable material. The application of SFE in the isolation and separation of natural products is becoming very popular due to the fact that the extraction is done without the addition of any toxic organic solvents.

At temperatures and pressures beyond the critical point, only the supercritical phase exists, whose properties are close to those of liquid, but with greater diffusivity and lower viscosity. High diffusivity and low viscosity improve mass transfer and therefore help decrease extraction time. Carbon dioxide is cheap, available in a pure state, non-toxic and has an accessible critical point (31.1 °C and 7.38 MPa (72.9 atm; 73.8 bar; 1071 psi)).

Carbon dioxide is the most preferable supercritical fluid for extraction. Other substances, with easily acces-

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sible critical points, are too expensive (xenon), toxic (ammonia, nitrous oxide), flammable (ethane, pentane) or corrosive (ammonia) [8].

Supercritical carbon dioxide has low viscosity, high diffusivity and low surface tension which allow it to penetrate easily throughout macro- and micro-porous materials. Also, it is a clean technology due to its non-toxic and environmental friendly nature. Furthermore, its critical point is sufficiently low for the processing of thermolabile materials, and most importantly it is simple to separate it from the reaction medium without extensive separation processes thus offering Industrial advantages [9–15].

The efficiency of supercritical carbon dioxide extraction can be affected by many factors including pressure, temperature and extraction time. In most of the previous studies, the process conditions have been merely optimized by conducting one factor-at-a-time experiments. The results of one-factor-at-time experiments do not reflect actual changes in the environment as they ignore interactions between factors that are present simultaneously. Therefore, these factors may be collectively studied to validate the optimal extraction conditions. The response surface methodology (RSM) has demonstrated to be a powerful tool for determining the factors effects and their interactions, which allow process optimization to be conducted effectively [16–22].

In this paper, the experimental design and response surface methodology (RSM) were applied to investigate the effect of operating pressure (100–140–180 bar), temperature (40–50–60 °C), and extraction time (2–3–4 h), on the *Ginkgo biloba* extraction yield. Also, two mathematical models, modified Reverchon–Sesti Osseo and Sovová model, were used to correlate the experimental data.

EXPERIMENTAL PROCEDURES

Plant material

Ginko (*Ginkgo biloba* L.) leaves were collected in the autumn (year 2007) from a female tree, on the location of University campus (Novi Sad), preserved by drying in the oven chamber (own making, Institute of Field and Vegetable Crops, Bački Petrovac, Serbia) at the temperature of 40–50 °C. Fresh *Ginkgo biloba* leaves contain 70–80% moisture have to be dried to prevent mold growth. Before the extraction, leaves were grinded in the mill and particle size diameter was determined by sieving (d_1 = 0.190 mm, d_2 = 0.467 mm i d_3 = 1.009 mm).

Reagents

Commercial grade carbon dioxide was used as the extraction agent (Tehnogas, Novi Sad, Serbia).

Supercritical carbon dioxide extraction

Supercritical fluid extraction (SFE) was carried out with the laboratory-scale high-pressure extraction plant (HPEP, NOVA–Swiss, Effretikon, Switzerland). The schematic diagram of the apparatus used for supercritical fluid extraction was described in detail previously [23,24]. The main part and characteristics (manufacturer specification) of the plant were as follows: diaphragm-type compressor (up to 1000 bar), extractor with an internal volume of 200 ml (p_{max} = 700 bar), separator with internal volume of 200 ml (p_{max} = 250 bar) and maximum CO_2 mass flow rate of approximately 5.7 kg/h. The mass of the sample in the extractor was 60.0 g. During the first four hours samples were taken every hour. The extract was collected into previously weighed glass vial and placed in the separator. The flow rate of carbon dioxide, expressed under normal conditions, was 3.22×10^{-3} kg/min, low enough to ensure the saturation of the supercritical CO_2 with solute. The separator conditions were 15 bar and 25 °C.

Experimental design

Response surface methodology (RSM) is a mathematical tool, which can help in reaching the optimum conditions for a reaction with minimum number of experiments with the goal of obtaining the statistically acceptable results. RSM enables evaluation of the effects of multiple parameters, alone or in combination, in response to variables and also predicts their behavior under given sets of conditions.

Response surface methodology (RSM) and Box–Behnken design (BBD) were applied for determining optimal extraction temperature, pressure and extraction time for supercritical carbon dioxide extraction of *Ginkgo biloba* leaves. The pressure (X_1), temperature (X_2) and extraction time (X_3) were independent variables studied to optimize the *Ginkgo biloba* extraction yield (y). The independent variables were transformed to range between –1 and 1 for the related factors. There are three levels of design (−1, 0, +1) with equally spaced intervals between these levels [17]. The carbon dioxide flow rate value was constant.

The experimental data were fitted with second order response surface model using the following form:

$$y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum\sum_{i<j} \beta_{ij} X_i X_j \quad (1)$$

where y is the response (extraction yield in %), β_0, β_j, β_{jj} and β_{ij} are the constant coefficients of intercept, linear, quadratic, and interaction terms, respectively, and X_i and X_j are coded independent variables (temperature, pressure, time). Analysis was performed using the commercial software Statistica 8.0, Series 0608c (StatSoft Europe, GmbH, Hamburg).

Response surface and contour plots were developed using the fitted quadratic polynomial equation, holding the independent variables with the least effect on the response at the central value and varying the levels of other two variables within the experimental range. The test of statistical significance was based on the total error criteria with a confidence level of 95.0%.

The linear, quadratic and cross product terms in the second order polynomial model can used to generate a three-dimensional response surface graph (with maximum, minimum or saddle point) and a two-dimensional contour plot (curves of equal response values are drawn on a plane whose coordinates represent the levels of the independent variables).

RESULTS AND DISCUSSION

The effect of single process parameter variable on the extraction process

The effect of solvent flow rate on the extraction yield

The flow rate of CO_2 showed an influence on the extraction yield. To determine the influence of the flow rate, *Ginkgo biloba* leaves were extracted at 100 bar and 40 °C for certain extraction time with different flow rates. Figure 1 shows the effect of the CO_2 flow rate on the extraction yield as function of time and positive correlation between extraction yield and the flow rate of CO_2. The yield of extract increases rapidly to somewhere around 10 h. For the two highest flow rates w_2 and w_3, after that extraction time, the curves lie on the same line indicating that the exit yield is independent from the flow rate and therefore equilibrium is considered to exist. For these reasons, as well as for further studies, the middle value of 3.22×10^{-3} kg/min for the flow rate was selected.

The effect of variation in the particle size on the total extraction yield

The extractions were performed for various particle sizes (0.190, 0.467 and 1.009 mm) at 100 bar, 40 °C and 3.22×10^{-3} kg/min flow rate. The influence of different grinding degree on extraction yield, y (g/100 g drug), is presented in Figure 2. The highest values of extraction yield were obtained with the smallest particle size (0.190 mm mean diameter of particle) which confirms that the convective mass transfer has the dominant influence. A properly reduced particle size increased the surface area and accordingly enhanced the contact of extract with supercritical fluid. Generally the extraction rate increases with the decrease in particle size.

The effect of pressure

It is well-known that the extraction pressure is one of the most important parameters in the SFE process because it is the major determinant of solvent power of supercritical fluids that may have strong influence on extraction efficiency. As shown in Figure 3, total extraction yield of *Ginkgo biloba* increased as the function of pressure.

Figures 1–3 clearly show that extraction yield increases more or less linearly as the function of extraction time during the initial phase of the process. Thereafter, the slope of the curves progressively decreases to reach approximately constant values.

The increase in pressure results in the increase of density of the supercritical fluid, and subsequent increase in its solvating power. However, as the pressure is increased the diffusivity decreases and the molecules have more difficulties diffusing into the pores to dissolve solute. The increase in pressure causes the solid matrix to become more packed while the void fraction decreases which also results in the reduction of the

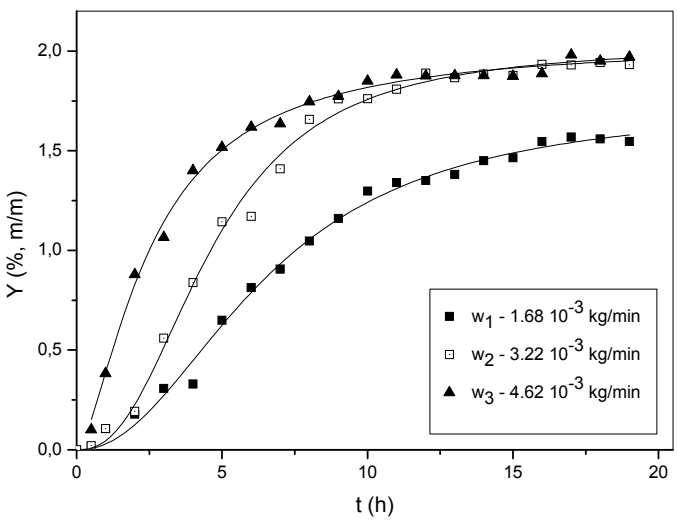

Figure 1. Effect of CO_2 flow rate, w, on the total extraction yield of Ginkg biloba (pressure 100 bar, temperature 40 °C, particle average diameter of ground material, d_{av}, 0.190 mm).

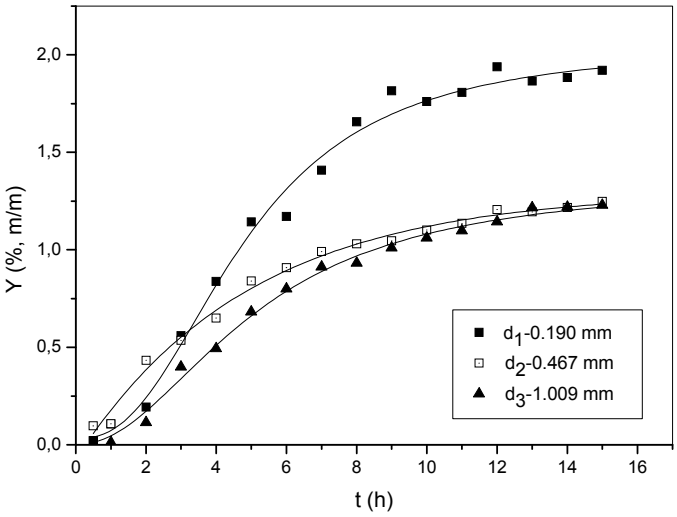

Figure 2. Effect of grinding degree, d, on the extraction yield of Ginkgo biloba (pressure 100 bar, temperature 40 °C, flow rate, w, 3.22×10^{-3} kg CO_2/min).

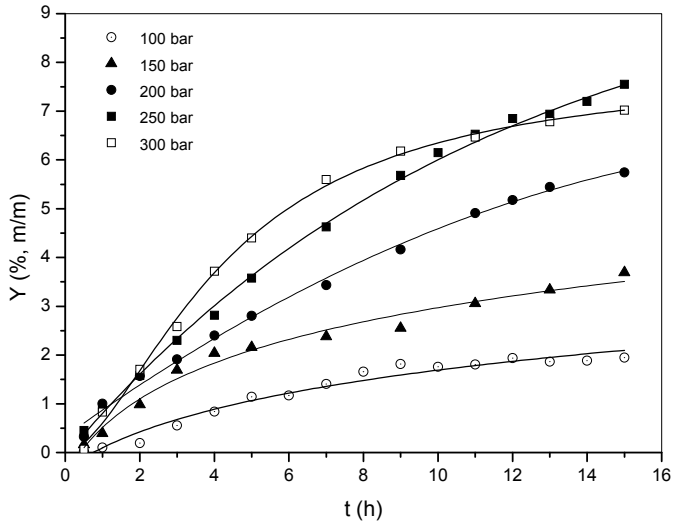

Figure 3. The extraction yield of Ginkgo biloba *in supercritical carbon dioxide as a function of time at different pressure (extraction temperature 40 °C, particle average diameter of ground material, $d_{av,}$ 0.190 mm, flow rate, w, 3.22×10^{-3} kg CO_2/min).*

extraction. The solubility of solute in CO_2 is a non-uniform function of temperature and pressure. The selectivity of solute extraction is a function of pressure, *i.e.*, as the pressure is increased different solutes are extracted [25].

An increase in pressure from 100 to 180 bar at constant temperature (40 °C), *i.e.*, increase in CO_2 density (630 to 820 kg/m^3) caused a 4.5 times greater extraction yield, but at 60 °C CO_2 density increased from 290 to 688 kg/m^3 and the extraction yield was 6.3 times greater.

Figure 4 is a plot showing the effect of pressure and temperature on the extraction yield in SC-CO_2 at a fixed extraction time of 4 h. Higher temperatures increased extraction yield, probably because vapor pressure of the extracted materials dominates the extraction. A contradictory effect was observed at lower pressure (100 and 140 bar), indicating that solvent density is a major factor enhancing the quantity of total extract.

Consequently, supercritical carbon dioxide at 60 °C and 180 bar is considered the most efficient operating condition for the investigated range of operating conditions.

The effect of temperature

Pressure has a positive effect on the extraction yield at lower temperature, however, the influence of temperature is dependent on the pressure. At 100 and 140 bar, *Ginkgo biloba* extraction yield decreases with the increase of temperature, while at 180 bar it increases with the increase of temperature.

The influence of temperature on extraction was more difficult to predict than that of pressure because of its two counter effects on the extraction yield. For

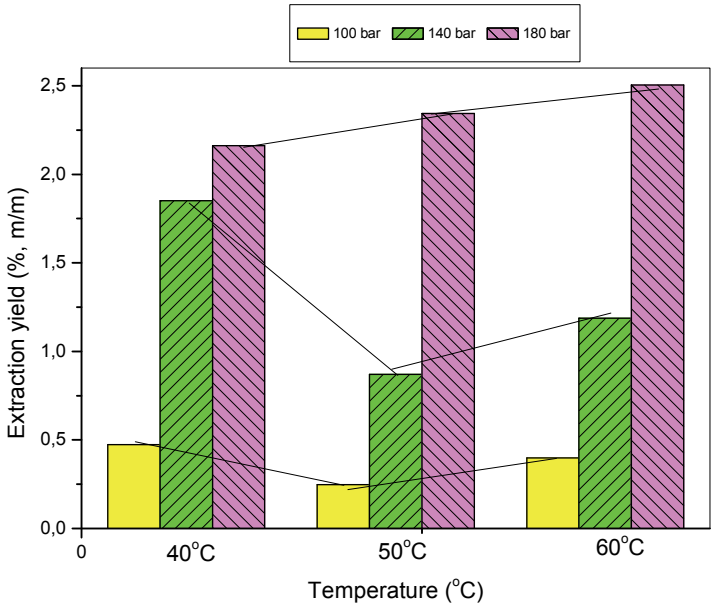

Figure 4. The Ginkgo biloba *extraction yield in supercritical carbon dioxide as a function pressure and temperature (extraction time 4 h).*

supercritical fluid extraction, the solubility of organic compounds depends largely on the balance between fluid density and solute vapor pressure, which are both controlled by fluid temperature and pressure. When the pressure increases the fluid density increases, thus the solubility increases too. On the other hand, when the temperature increases the fluid density decreases while the solute vapor pressure increases. The solubility of the solute may increase, become constant or decrease with the temperature rise at constant pressure, depending on whether the solute vapor pressure or the solvent density is predominant.

Increasing the temperature from 40 to 60 °C, *i.e.*, decreasing density of supercritical CO_2 from 764 to 563 kg/m^3, leads to the reduced extraction yield of 35%. However, the same temperature changes at 180 bar will lead to the increased extraction yield of 15%.

The effect of extraction time

As shown in the experimental results, the extraction yield increased gradually with the increase of the extraction time. According to the predicted model, the main effect of extraction time (X_3) was of great significance while its interactions with pressure (X_1X_3) were significant. At low-pressure levels extraction time has a positive linear effect on the extraction yield. However, at higher-pressure levels further increase in the extraction time resulted in significant positive changes in the extraction yield.

The result of a two hours longer supercritical extraction is a 4–5 times increased extraction yield at temperature of 40 or 60 °C, at the same pressure (140 bar).

Based on these results, it was deduced that pressure and extraction time play a significant role in extraction.

The modified Reverchon–Sesti Osseo mathematical model of extraction process

Together with supercritical fluid extraction investigations, mathematical models of this process were developed. Mathematical modeling allows a rational approach to the extraction problem, giving the opportunity to generalize the experimental results, and if successful, it can obtain indications about systems different from those studied.

To model the extraction of the *Ginkgo biloba* leaves–supercritical carbon dioxide system we used the form of the Reverchon–Sesti Osseo equation [26]:

$$Y = [1 - \exp(-t/t_i)] \quad (2)$$

that was transformed [27–29] where Y is the normalized extraction yield (%), t is the extraction time and t_i is the internal diffusion time. To avoid the evaluation of internal diffusion time, t_i, Eq. (2) was modified based on the assumption that for certain extraction system, t_i could be approximated as constant. This assumption allows one to assert that:

$$-t/t_i = at + b \quad (3)$$

Note that for $-t/t_i = Z$, a is constant; t is the extraction time, and b is the correction term, Eq. (3) can be written as:

$$Y = 100[1-\exp(at + b)] \quad (4)$$

Z is defined as:

$$Z = \ln(1 - Y/100) \quad (5)$$

where Y is the normalized extraction yield:

$$Y = 100 Y_{exp}/Y_{max} \quad (6)$$

where Y_{exp} is the extraction yield (g /100 g of drug) and Y_{max} is the maximum of the extraction yield (g/100 g).

Based on the experimental results, Z values were calculated and they are given in Table 1.

Table 1. The values of the parameters a and b in the modified equation Y = 100[1-exp(at + b)] for calculating normalized extraction yield of Ginkgo biloba for different extraction pressure

Pressure bar	Z = at + b			AARD, %
	a	b	|r|	
100	0.29677	–0.27108	0.96655	9.132
150	–0.1234	0.04084	0.9821	30.118
200	–0.1519	0.19643	0.9765	11.823
250	–0.1745	0.23929	0.9883	46.831
300	–0.1867	0.03196	0.9932	18.383

The yield of total extract of *Ginkgo biloba* (y_{RSO}) was calculated on the basis of Eq. (4) obtained for modified Reverchon–Sesti Osseo model and for normalized yield of total extract (Y), and graphically presented in Figure 5.

The modified equation Reverchon–Sesti Osseo well fitted the experimental results of extraction of *Ginkgo biloba* leaves obtained at 100, 150 and 200 bar (Figure 5).

There was an attempt to apply the Naik model [30] for the modeling of our extraction system, even though it was already used for extraction with liquid carbon dioxide. Applying this model to the extraction of *Ginkgo biloba* leaves by supercritical carbo dioxide did not give a satisfactory result.

The modeling of extraction procrss using Sovová model

The model of Sovová was also used to describe the extraction process. The pre-treatment of raw material, which includes milling, enables contact between solute and solvent easier. After milling, a great fraction of the solute becomes free for contact with solvent, however some of the cell walls may remain intact with solute inside the cells. Based on the broken and intact cell walls idea, Sovová proposed a model in which the solute is divided into two fractions [31]. The q fraction of the soluble components becomes free on the surface and can be extracted by simple dissolution which is characterized by the first stage of the yield curve. The other part of the soluble components, (1–q) fraction, remains inside the particles and can only reach the surface by diffusion which is characterized by the second stage of the yield curve. The Sovová model divides the extraction process into the following steps:

– diffusion of solvent through the film around the solid particle,

– streaming of solvent into the pores of the solid particle,

– solution of the soluble components,

– diffusion of the solute to the surface of the solid particle and

– mass transfer from the surface to the fluid bulk phase.

The mass transfer from solid matrix to surface is determined by the inner mass transfer coefficient k_s,

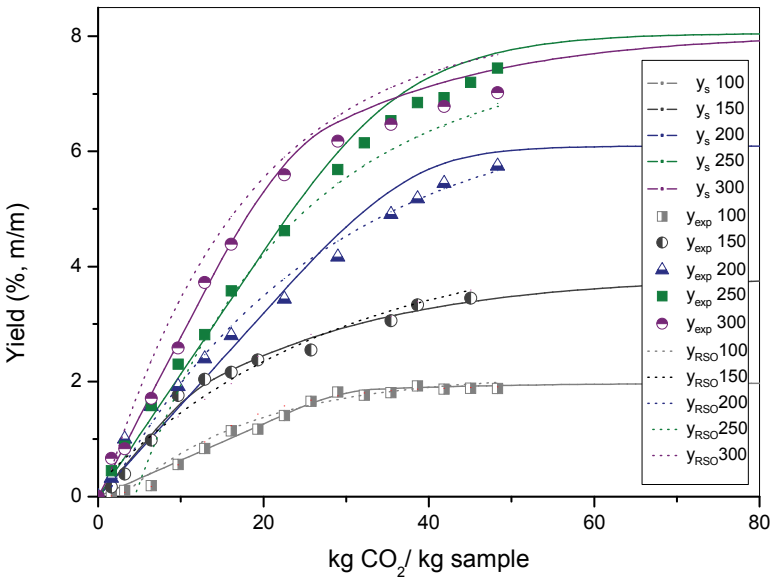

Figure 5. Yield of total Ginkgo biloba extract as a function of the specific amount of super-critical carbo dioxide (kg CO_2/kg sample). Symbols represents the experimental values of extraction yield, y_{exp}, solid lines correspond to data of extraction yield, y_s, calculated by Sovová model and dot lines correspond to data for extraction yield, y_{RSO}, calculated by modified Reverchon–Sesti Osseo model.

while k_f is surface to fluid phase by fluid mass transfer coefficient. The Sovová model assumes that the charge is homogenous and isotropic, and the soluble material is evenly dissolved in the raw material. Along the column, the particle size distribution, temperature, and pressure are constant, and pressure drop is negligible. The inlet solvent is solute free [31].

The experimental results of the extraction curves were fitted by using the Sovová model. For fitting yield curves a computer program was developed.

The Sovová model is represented by the following equations for the extraction curve:

$$qy^*[1-\exp(-Z)],\ q < q_m$$

$$e = \{[y^* - q_m \exp(z_w - Z)],\ q_m \le q < q_n$$

$$x_0 - \frac{y^*}{W}\ln[1+(\exp(\frac{Wx_0}{y^*})-1)\exp(\frac{Wx_k(q_m-q)}{x_0})],\ q > q_n \quad (7)$$

where:

$$q_m = \frac{(x_0 - x_k)}{y^* Z} \quad (8)$$

$$q_n = q_m + \frac{1}{W}\ln\left(\frac{x_k + (x_0 - x_k)\exp(\frac{Wx_0}{y^*})}{x_0}\right) \quad (9)$$

$$\frac{Z_W}{Z} = \frac{y^*}{Wx_0}\ln\left(\frac{x_0 \exp[W(q-q_m)-x_k)}{x_0 - x_k}\right) \quad (10)$$

Parameters Z and W are directly proportional to the fluid phase and to the solid-phase mass transfer coefficients, respectively, and are given below:

$$Z = \frac{k_f a \rho}{q(1-\varepsilon)\rho_s} \quad (11)$$

$$W = \frac{k_s a}{q(1-\varepsilon)} \quad (12)$$

The initial mass of the extract available in the solid is supposed to be equal to the asymptotical value of the mass of extract, $m_{e\infty}$. Given y_∞ the asymptotic yield, then:

$$x_0 = \frac{m_{e\infty}}{m_0 - m_{e\infty}} = \frac{y_\infty}{1-y_\infty} \quad (13)$$

Parameters Z, W and x_k were adjusted and determined by minimizing the errors between the experimental data and calculated yield values. The errors were quantified by defining average absolute relative deviations (*AARD*):

$$AARD\ (\%) = \frac{100}{n}\sum_{i=1}^{n}\left|\frac{y_i - y_{mod,i}}{y_i}\right| \quad (14)$$

$$SD\ (\%) = 100\sqrt{\sum_{i=1}^{n}\frac{(y_i - y_{mod,i})^2}{n-1}} \quad (15)$$

where n is the number of experimental points, y_i is the yield determined by the experimental point i, and $y_{mod,i}$ is the yield obtained by the model in point i. The model parameters were used to determine the hardly accessible solute x_k, volume mass transfer coefficients in the fluid phase $k_f a$, and the solid phase $k_s a$.

The mass transfer coefficient in the fluid phase $k_f a$ *and* mass transfer coefficient in the solid phase $k_s a$ are presented in Table 2.

Table 2. Parameters of the mass transfer model at different pressures (extraction conditions: temperature 40 °C, flow rate: 3.22×10^{-3} kg/min, d_{av}: 0.190 mm)

Parameter	Pressure, bar				
	100	150	200	250	300
x_k	1.453·10^{-3}	0.021	0.013	0.028	0.023
F / s^{-1}	0.405	0.81	0.757	0.674	0.674
S / s^{-1}	0.059	0.048	0.175	0.105	0.050
Z	444.06	872.03	798.16	697.02	697.01
W_k	64.2	52.07	184.35	108.6	51.4
a_0	3.158×10^4	3.158×10^4	3.158×10^4	3.158×10^4	3.158×10^4
k_s / m s^{-1}	8.62×10^{-7}	7.12×10^{-7}	2.58×10^{-6}	1.55×10^{-6}	7.313×10^{-7}
$k_s a_0$ / s^{-1}	0.027	0.022	0.081	0.049	0.023
U / m s^{-1}	6.782×10^{-5}	5.467×10^{-5}	5.084×10^{-5}	4.852×10^{-5}	4.690×10^{-5}
k_f / s^{-1}	1.418×10^{-5}	2.285×10^{-5}	1.987×10^{-5}	1.687×10^{-5}	2.447×10^{-5}
$k_f a_0$ / s^{-1}	0.448	0,722	0.627	0.533	0.773
q_m	23.80	7.23	21.10	17.96	13.17
q_n	33.63	14.07	40.09	33.12	27.96
r	0.073	0.541	0.213	0.347	0.281
SD / %	0.094	0.223	0.403	0.191	0.097
$AARD$ / %	48.791	19.479	19.232	6.135	3.087

The Sovová model applied to the description of the extraction curve showed good agreement with experimental data at 40 °C and different pressures (100–250 bar). Figure 5 presents the yield of total *Ginkgo biloba* extract as a function of the specific amount of supercritical carbon dioxide (kg CO_2/ kg sample). From the results, the Sovová mathematical model shows better agreement with experimental yield than modified Reverchon–Sesti Osseo model.

Response surface methodology at extraction process

Determination of the most suitable conditions for extraction of Ginkgo biloba leaves using multifactorial experiment

The effect of three main variables on supercritical CO_2 extraction was studied using three-factor design, with three levels for each factor. Since various parameters potentially affect the extraction process, the optimization of the experimental conditions represents the critical step in the development of supercritical fluid extraction method. The experimental design was adopted on the basis of coded level from three variables, resulting in fifteen simplified experimental sets (Table 3) with three replicates for the central point in order to confirm the mathematical model. The selected factors were extraction temperature (°C), pressure (bar) and extraction time (h) taking into consideration the fact that those factors are important in the extraction process. The carbon dioxide flow rate value was a fixed value.

The effect of the linear, quadratic or interaction coefficients on the response was tested for significance by the analysis of variance (ANOVA). Regression coefficients of intercept, linear, quadratic, and interaction terms of the model were calculated using the least square method. The degree of significance of each factor is represented in the Table 4 by its *p*-value. When *p*-value of a factor is smaller then 0.05, the factor has a

Table 3. Box–Behnken design matrix, uncoded and coded independent variables and their levels used in the RSM design, density of CO_2, experimental and predicted value for Ginkgo biloba extraction yield (X_1 - pressure (bar), $X_1 = (P – 140)/40$; X_2 - temperature (°C), $X_2 = (T – 50)/10$; X_3 - extraction time (h), $X_3 = (\tau – 3)/4$)

SFE run No.	Coded levels			Independent variables			Density of CO_2, kg/m^3	Experimental yield, %	Predicted yield, %
	X_1	X_2	X_3	p / bar	T / °C	τ / h			
1	–1	–1	0	100	40	3	630	0.559	0.621
2	1	–1	0	180	40	3	820	1.414	1.545
3	–1	1	0	100	60	3	290	0.261	0.139
4	1	1	0	180	60	3	688	1.680	1.618
5	–1	0	–1	100	50	2	385	0.053	0.044
6	1	0	–1	180	50	2	758	0.506	0.428
7	–1	0	1	100	50	4	385	0.247	0.325
8	1	0	1	180	50	4	758	2.344	2.353
9	0	–1	–1	140	40	2	764	0.401	0.347
10	0	1	–1	140	60	2	563	0.257	0.397
11	0	–1	1	140	40	4	764	1.850	1.709
12	0	1	1	140	60	4	563	1.188	1.241
13	0	0	0	140	50	3	674	0.570	0.580
14	0	0	0	140	50	3	674	0.560	0.580
15	0	0	0	140	50	3	674	0.566	0.580

Table 4. Regression coefficients of predicted second-order polynomial model for the response variable

Term	Coefficient	Value	*t*-Value	*p*-Value
Constant	β_0	0.580	7.11840	0.000849[a]
X_1	β_1	0.603	12.08413	0.000069[a]
X_2	β_2	–0.105	–2.09801	0.089980
X_3	β_3	0.552	11.05561	0.000105[a]
X_1^2	β_{11}	0.131	1.78614	0.134131
X_2^2	β_{22}	0.267	3.64129	0.014883[b]
X_3^2	β_{33}	0.076	1.03914	0.346356
X_1X_2	β_{12}	0.141	1.99981	0.101964
X_1X_3	β_{13}	0.411	5.82459	0.002107[a]
X_2X_3	β_{23}	–0.129	–1.83879	0.125343

[a]$P < 0.01$, highly significant; [b]$0.01 \le P < 0.05$, significant and $P \ge 0.05$, not significant

significant influence on the process (for a confidence level of 0.95).

The second order polynomial model used to express the total extract yield y, as a function of independent variables (in terms of coded values) is shown below:

$$y = 0.580 + 0.603X_1 - 1.105X_2 + 0.552X_3 + 0.131X_1^2 + 0.267X_2^2 + 0.076X_3^2 + 0.141X_1X_2 + 0.411X_1X_3 - 0.129X_2X_3 \quad (16)$$

where y is the extraction yield of Ginkgo biloba, X_1 is the temperature, X_2 is pressure and X_3 is the extraction time.

By computation, the optimal conditions to obtain the optimal extraction yield of *Ginkgo biloba* were determined at 184.4 bar, 52.7 °C and 3.86 h of extraction, and the predicted extraction yield was 2.39%.

Figure 6 shows predicted *versus* experimental values. The coefficient of determination for this model was 0.9924, which indicated that the model adequately represented the real relationship among the selected extraction parameters.

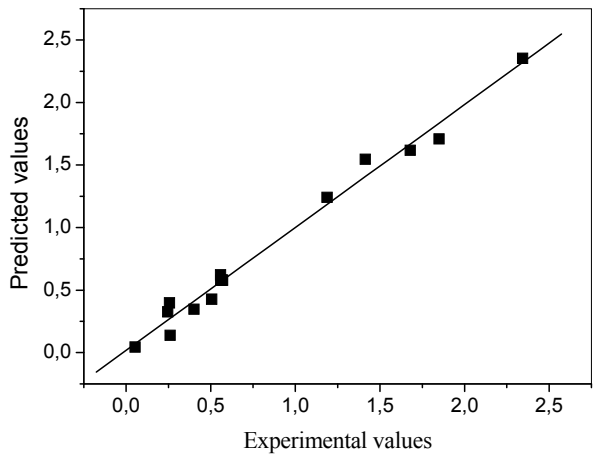

Figure 6. Predicted versus experimental values in the supercritical extraction of Ginkgo biloba leaves.

CONCLUSION

Extraction of *Ginkgo biloba* leaves using supercritical CO_2 as solvent under various conditions was investigated. The influence of solvent flow on the extraction yield of *Ginkgo biloba* leaves was investigated with three different solvent flow rates: 1.58×10^{-3}, 3.22×10^{-3} and 4.16×10^{-3} kg CO_2/min at 100 bar and 40 °C, where the flow rate $w_2 = 3.22\times10^{-3}$ kg CO_2/min was selected as the most convenient. Two extraction periods are observed during the fast and slow extraction. Rapid extraction is finished within about 10 hours, and slow extraction is a long term process. The grinding degree of the drug has a significant role in the extraction. The highest yield was achieved using the highest degree of grinding that is understandable because it is the highest level of destroyed cells, so the mass transfer is done mainly through mass convection.

Mathematical modeling of the extraction system of *Ginkgo biloba* – supercritical carbon dioxide was based on the model equation Reverchon–Sesti Osseo using the modified model equation $Y = 100[1 - \exp(at + b)]$. The modified equation fitted well the experimental results of extraction of *Ginkgo biloba*-supercritical carbon dioxide at pressures in range from 100 to 200 bar. Regarding the kinetics of supercritical fluid extraction, the Sovová model was selected and successfully applied to the description of the extraction curve. The values of *SD* (%) were in the range from 0.094 to 0.403 indicating a relatively good agreement with experimental results in the range of studied conditions. These models can be applied to design the supercritical extraction process of *Ginkgo biloba*, considering that the process of supercritical fluid extraction is in progress, especially in the field of nutraceuticals and pharmaceutical products.

The current results also showed that the second-order polynomial model was sufficient to describe and predict the response variable of the *Ginkgo biloba* extraction yield to the change in process parameters for supercritical CO_2 extraction of *Ginkgo biloba* leaves within the experimental ranges. For the extraction yield, examination of these coefficients using the *p*-test indicated that the linear terms of pressure and extraction time, and the interaction between pressure and the extraction time were highly significant ($p < 0.01$), as well as the quadratic term of temperature ($0.01 < p < 0.05$) for the extraction yield. There were no significant linear term of temperature, interaction between temperature and pressure, and extraction time and temperature within the experimental range ($p > 0.05$). Therefore, these results suggest that linear, quadratic and (or) interaction effects of the independent variables may be the primary determining factors affecting the extraction yield.

Based on the proposed model, the most acceptable conditions for *Ginkgo biloba* extraction were found to be at 184.4 bar, 52.7 °C and 3.86 h. Thus, this methodology could provide a basis for a model to examine the non-linear nature between the independent variables and the response in a short-term experiment.

Nomenclature

a	Specific interfacial area (m^{-1})
e	Mass of extract
$k_f a$	Fluid-phase mass transfer coefficient (s^{-1})
$k_s a$	Solid phase mass transfer coefficient (s^{-1})
q	Mass of solvent (kg)
Q	Solvent flow rate (kg/s)
t	Time (s)
W	Model parameter related to diffusion in the solid phase of the extraction bed

x_k Initial mass of extractable material in intact cells, relative to mass of non-extractable material (kg/kg)
x_p Initial mass of extractable material in broken cells, relative to mass of non-extractable material (kg/kg)
x_0 Initial mass of extractable material, relative to mass of non-extractable material (kg/kg)
y Yield, expressed as mass of extract per mass of sample
y^* Pseudo-solubility, expressed as mass of extract per mass of solvent (kg/kg)
y^* Extract solubility in the solvent at given pressure and temperature (kg/kg)
Z Model parameter related to convection in the fluid phase of the extraction bed
Z_W Parameter of the second extraction period
ε Extraction bed porosity
ρ Solvent density (kg/m^3)
ρ_S Solid density (kg/m^3)

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IZVOD

ISPITIVANJE USLOVA EKSTRAKCIJE LIŠĆA *Ginkgo biloba* L. SUPERKRITIČNIM UGLJEN-DIOKSIDOM PRIMENOM METODE ODZIVNE POVRŠINE

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(Naučni rad)

U radu je ispitana ekstrakcija lišća Ginkgo biloba superkritičnim ugljen-dioksidom određivanjem prinosa ekstrakcije ukupnih ekstraktivnih materija u funkciji parametara ekstrakcije kao što su protok rastvarača, *w*, stepen usitnjenosti, *d*, pritisak, *p*. Odabran je protok rastvarača w_1 = 3,22×10^{-3} kg/min kao najpovoljniji za ekstrakciju lišća *Ginkgo biloba*. Ispitivanjem uticaja stepena usitnjenosti droge na količinu ekstraktivne materije za vreme brze ekstrakcije, nađeno je da se najveće vrednosti dobijaju za drogu sa najvećim stepenom usitnjenosti, što je i razumljivo jer se radi o drogi sa najvećim stepenom razrušenosti ćelija. Na osnovu rezultata ispitivanja kinetike ekstrakcije izvršeno je modelovanje ekstrakcionog sistema polazeći od model jednačine Reverchon–Sesti Osseo, korišćenjem modifikovane model jednačine $Y = 100[1- \exp(at + b)]$ i pokazalo se da modifikovana jednačina dobro fituje eksperimentalne rezultate ekstrakcije *Ginkgo bilobe* superkritičnim ugljen-dioksidom dobijene na pritisku do 200 bar. Izračunate vrednosti prinosa na osnovu ispitanog modela ne odgovaraju u potpunosti eksperimentalno utvrđenim vrednostima prinosa na ostalim pritiscima. Sovová model pokazao je daleko veću saglasnost sa eksperimentalnim podacima za prinos ukupnog ekstrakta skoro na svim ispitivanim pritiscima. Radi određivanja najpovoljnijih parametara ekstrakcije primenjen je metod odzivne površine variranjem parametara ekstrakcije kao što su pritisak (100–140–180 bar), temperatura (40–50–60 °C), i vreme ekstrakcije (2–3–4 h), pri protoku od 3,22×10^{-3} kg/min. Značajan uticaj pokazali su pritisak, vreme ekstrakcije, kvadrat temperature, i međusobna interakcija pritiska i vremena ekstrakcije. Određeni su najpovoljniji uslovi ekstrakcije: pritisak 184,4 bar, temperatura 52,7 °C i vreme ekstrakcije 3,86 h.

Ključne reči: *Ginkgo biloba* L.• Superkritični CO_2 • Superkritična ekstrakcija • Metod odzivne površine