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Optimization of the Supercritical CO_2 Extraction of Oil from Rapeseed Using Response Surface Methodology

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Summary

Three operating parameters (pressure, temperature and extraction time) of the supercritical CO_2 extraction of oil from rapeseed have been optimised by response surface methodology to obtain high yield of oil. Results showed that data were adequately fitted into the second-order polynomial model. The linear and quadratic terms of independent variables of temperature, pressure and extraction time had a significant effect on the oil yield. Optimal conditions for oil yield within the experimental range of the studied variables were 29.7 MPa, 52.14 °C and 3.36 h, and oil yield was predicted to be 28.27 %. In addition, the mass transfer model was used to describe the extraction kinetic curves of rapeseed oil and very good agreement was found between the observed and predicted values. The fatty acid composition of rapeseed oil extracted by supercritical CO_2 at optimal operating parameters and that by *n*-hexane were analysed by gas chromatography. The obtained oil showed similar fatty acid composition with an unsaturated fatty acid content up to 92.9 %.

Key words: fatty acids, kinetics, rapeseed oil, response surface methodology, supercritical CO_2 extraction

Introduction

Rapeseed oil is one of the most popular vegetable oils on the global market of edible fats used for cooking, consumption and food production. Due to high level of total antioxidant compounds, rapeseed oil is regarded as the most useful of all cooking fats. Namely, rapeseed oil is rich in monounsaturated fat and in ω-3 fatty acids, while antioxidant compounds present in this oil are polyphenols, sterols, tocopherols, flavonoids *etc.*, which exhibit antiradical activity (*1*). To maintain rapeseed oil quality and yield, choosing the right method for oil extraction is a crucial factor. Traditionally, oil from rapeseed is produced by a combined process using mechanical pressing of the seeds, followed by organic solvent extraction of residual oil from the press cake. The use of organic solvents in rapeseed oil processing involves problems regarding safety concerns, emission of volatile organic compounds into the atmosphere, high operation costs, and poor product quality caused by high processing temperatures. If the oil is used in food processing and pharmaceutical industry, relatively high number of processing steps is mandatory, such as the removal of phospholipids (degumming) and the removal of residual organic solvents after extraction and oil refining (*2,3*).

Due to the drawbacks associated with the time-consuming conventional two-step procedure for oilseed processing, much effort has been put into the development of alternative single-step extraction method by

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using a solvent that would have high solvent power and selectivity, be easily removed from meal and oil, have low flammability, be stable, nonreactive and nontoxic (*4*). Among alternative solvents, CO_2 under supercritical conditions of temperature (over 31 °C) and pressure (over 7.28 MPa) has been of great interest. This inexpensive gas, available on an unlimited scale both from renewable organic resources and from inorganic material, has low toxicity, is nonflammable, and it is easily recovered from meal leaving no trace in the processed matrix (*2*). Supercritical CO_2 extraction produces superior oil in terms of oil acidity, but also undesirable compounds such as phospholipids, pigments and sterols, therefore simpler refining processes are required (*5,6*). Processing of seeds in the supercritical CO_2 at low temperatures is especially advantageous for the production of oil used in food industry because it minimizes undesirable oxidation reactions, which is especially beneficial for the sensitive bioactive components of oil such as sterols, tocopherols, and unsaturated fatty acids (*7,8*). The disadvantages of supercritical fluid extraction are high investment costs for equipment acquisition and high energy demand of the CO_2 extraction unit.

Some authors have studied the supercritical fluid extraction of rapeseed oil (*2,9-12*), but according to our knowledge there is no report regarding the optimization of the supercritical CO_2 extraction of oil from rapeseed using response surface methodology (RSM). RSM, originally described by Box and Wilson (*13*), is a collection of mathematical and statistical techniques useful for the modelling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response (*14*). In this study, the Box–Behnken design (BBD) is applied to investigate the effects of pressure, temperature and extraction time on the yield of rapeseed oil extracted by supercritical CO_2 under the applied experimental range. The extraction curves of rapeseed oil were correlated using the mass transfer model described by Martínez *et al.* (*15*). Furthermore, fatty acid composition of the extracted oil obtained at optimal extraction point was analysed by gas chromatography and compared with the rapeseed oil extracted by organic solvent *n*-hexane.

Materials and Methods

Chemicals

Commercial CO_2 (Messer, Novi Sad, Serbia), *n*-hexane (J.T. Baker, Milan, Italy), kieselguhr (Merck, Darmstadt, Germany) and F.A.M.E. Mix C4-C24 (18919 Supelco, Sigma-Aldrich, St. Louis, MO, USA) were used. All other chemicals were of analytical reagent grade.

Plant material preparation

Rapeseed cultivar Triangle from the Croatian National list of varieties was obtained from the rapeseed producers and supplied by the Institute for seed and seedlings, Osijek, Croatia, in 2008. The water content was determined by drying the seeds to constant mass in an oven at 105 °C and expressed in percentage ((15.3±0.04) %). Prior to the extraction process, the seeds were ground in a blender and sieved using a sieve sets (Erweka, Munich, Germany) and the average particle size was measured ((0.3823±0.14) mm). Ground seeds were stored at 4 °C and used without dehulling or any other pretreatment such as predrying.

Soxhlet extraction

About 30 g of ground rapeseed sample was extracted with 120 mL of *n*-hexane, until totally depleted. The whole process took 14 h. The measurements were done in triplicate. The average initial oil content for three replicates was 34.17 %.

Supercritical CO_2 extraction

The extraction process was carried out on laboratory-scale high-pressure extraction plant (HPEP, NOVA-Swiss, Effretikon, Switzerland). The schematic diagram of the apparatus used for supercritical fluid extraction is given in detail elsewhere (*16,17*). The ground sample of 30 g was placed into extractor vessel. To fill up the extraction vessel, diatomaceous earth (kieselguhr) was used as inert material. The extracts were collected into previously weighed glass vials and placed in the separator at ambient temperature and pressure. A balance (precision of ±0.0001 g) was used to weigh the extract at regular time intervals. A mass flow rate of CO_2, expressed under normal conditions, was 0.194 kg/h, low enough to ensure the saturation of supercritical CO_2 with the solute. The investigated values of pressure varied from 20 to 30 MPa, temperature varied from 40 and 60 °C during the extraction time up to 4 h. Separator conditions were 1.5 MPa and 25 °C. After each extraction, the obtained extract was placed into glass vials, sealed and stored at 4 °C to prevent any possible degradation.

Experimental design

Box–Behnken design (BBD) was applied for determining optimal extraction temperature, pressure and time for supercritical CO_2 extraction of rapeseed oil. The temperature (X_1), pressure (X_2) and extraction time (X_3) were independent variables studied to optimize the oil yield (Y) from rapeseed. The CO_2 mass flow rate value was constant.

Box–Behnken design requires an experiment number (*N*) according to the following equation:

$$N=2k(k-1)+c_p \qquad /1/$$

where *k* is the factor number and c_p is the replicate number of the central point. There are three levels of design (-1, 0, +1) with equally spaced intervals between these levels (*18*).

The variables were coded according to the following equation (*19*):

$$X=\frac{x-[x_{max}+x_{min}]/2}{[x_{max}-x_{min}]/2} \qquad /2/$$

where x is the natural variable, X is the coded variable and x_{max} and x_{min} are the maximum and minimum values of the natural variable, respectively. The investigated factors and tested levels are reported in Table 1.

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

Independent variable	Symbol	Level		
		low (–1)	middle (0)	high (+1)
Temperature/°C	X_1	40	50	60
Pressure/MPa	X_2	20	25	30
Time/h	X_3	1	2.5	4

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

$$Y=\beta_0+\sum_{j=1}^{k}\beta_j X_j+\sum_{j=1}^{k}\beta_{jj}X_j^2+\sum\sum_{i<j}\beta_{ij}X_i X_j \qquad /3/$$

where Y is the response (extraction yield in %), β_0, β_j, β_{jj} and β_{ij} are constant coefficients of intercept, linear, quadratic, and interaction terms, respectively. X_i and X_j are coded independent variables (temperature, pressure or time). Analysis was performed using commercial software Design-Expert® v. 7.1.5 (*20*).

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 a confidence level of 95 %.

Modelling the extraction curves

The supercritical fluid extraction curve modelling is important for process optimization and economic evaluation of the process and for the ability to predict the extraction process, which is useful for scale-up as well as for the design and optimization of an industrial plant.

The extraction curves of rapeseed oil were adjusted with the model of Martínez *et al.* (*15*) represented by the following equation:

$$m_{ext}(t)=\frac{m_t}{\exp(b\cdot t_m)}\left\{\frac{1+\exp(b\cdot t_m)}{1+\exp[(b(t_m-t)]}-1\right\} \qquad /4/$$

where m_{ext} is the mass of extract (in kg), m_t is the total initial mass of solid in the extraction bed (in kg), t is the extraction time (in min), while b (in min^{-1}) and t_m (in min) are the adjustable parameters.

The concordance between the experimental data and the calculated value was established by the average absolute relative deviation (AARD) as follows:

$$AARD=\frac{1}{n}\cdot\sum_{i=1}^{n}\left|\frac{m_{exp}-m_{cal}}{m_{exp}}\right| \qquad /5/$$

where n is the number of data used for obtaining the parameters, and m_{exp} and m_{cal} are the experimental and calculated mass, respectively.

The parameters of all models were calculated by non-linear regression method using Mathcad software v. 14.0 (*21*).

Determination of fatty acid composition of rapeseed oil

The fatty acid composition of rapeseed oil was determined by gas chromatography using an GC Agilent 7890 A (Agilent Technologies, Santa Clara, CA, USA), equipped with a DB-23 (J&W 122-2361) capillary column (60 m×0.25 mm i.d.; film thickness 0.25 μm). Agilent 5975 MSD mass detector and Agilent 5975 MSD autoinjector were used. The prepared sample for analysis (1 μL) was injected with a split ratio of 1:15. The inlet temperature was set at 250 °C. The initial oven temperature was 50 °C (held for 1 min), then increased for 25 °C/min to 175 °C and finally increased for 4 °C/min to 235 °C, and held at that temperature for 5 min. Total analysis time was 26 min. Helium was used as the carrier gas. The mass spectrometry (MS) conditions were as follows: scan 40 to 500 amu, threshold 100, MS temperature 150 °C (quad) and 230 °C (source). The constituents of the extract were determined qualitatively based on the retention time and mass spectra using matching with the NIST 2008 MS libraries. For qualitative determination, based on retention time, F.A.M.E. Mix C4-C24 was used. Quantitative determination was done by applying normalization using correction factors (*22*).

One-way analysis of variance (ANOVA) and multiple comparisons (Duncan's *post-hoc* test) were used to evaluate the significant differences of the data at $p<0.05$. Data were expressed as mean values±standard deviation.

Results and Discussion

Response surface analysis

Since various parameters potentially affect the extraction process, the optimization of the experimental conditions represents a critical step in the development of a supercritical fluid extraction method. The experimental design was adopted on the basis of coded level from three variables (Table 1), resulting in seventeen simplified experimental sets (Table 2) with five replicates for the central point. The selected factors were extraction temperature (in °C), pressure (in MPa) and extraction time (in h) with consideration that these factors are important factors in the extraction process. The CO_2 mass flow rate value was constant (0.194 kg/h).

The effect of linear, quadratic or interaction coefficients on the response was tested for significance by 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 Table 3 by its p-value. When the p-value of a factor is less than 0.05, the factor has a significant influence on the process (for a confidence level of 0.95). Table 3 shows that the linear term of pressure, extraction time and temperature had a significant effect on the oil extraction yield, followed by the quadratic term of all three investigated parameters. The interactions between the investigated process parameters were not statistically significant.

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

$$Y=23.71-1.20X_1+5.03X_2+6.52X_3-3.10X_1^2-1.36X_2^2-5.79X_3^2-0.62X_1X_2-0.58X_1X_3-0.40X_2X_3 \qquad /6/$$

where Y is the extraction yield of oil from rapeseed, X_1 is temperature, X_2 is pressure and X_3 is extraction time.

By computation, the optimal conditions to obtain the highest extraction yield of oil from rapeseed were de-

Table 2. Experimental matrix and values of the observed response

Run	Temperature °C	Pressure MPa	Time h	Coded temperature variable	Coded pressure variable	Coded time variable	Observed extraction yield %
1	40	20	2.5	–1	–1	0	15.3877
2	60	20	2.5	1	–1	0	14.0874
3	40	30	2.5	–1	1	0	25.6684
4	60	30	2.5	1	1	0	21.8815
5	40	25	1	–1	0	–1	8.8021
6	60	25	1	1	0	–1	7.7123
7	40	25	4	–1	0	1	23.0836
8	60	25	4	1	0	1	19.6732
9	50	20	1	0	–1	–1	4.1358
10	50	30	1	0	1	–1	16.0251
11	50	20	4	0	–1	1	17.8992
12	50	30	4	0	1	1	28.1936
13	50	25	2.5	0	0	0	23.2753
14	50	25	2.5	0	0	0	23.1421
15	50	25	2.5	0	0	0	24.2137
16	50	25	2.5	0	0	0	23.9554
17	50	25	2.5	0	0	0	23.9853

Table 3. Regression coefficient of polynomial function of response surface of oil yield obtained by supercritical CO_2 extraction

Variable	Coefficients	Standard error	F-value	p-value
Intercept	23.71	0.30		
X_1	–1.20	0.23	26.23	0.0014
X_2	5.03	0.23	462.45	<0.0001
X_3	6.52	0.23	776.72	<0.0001
X_1^2	–3.10	0.32	92.47	<0.0001
X_2^2	–1.36	0.32	17.68	0.0040
X_3^2	–5.79	0.32	322.73	<0.0001
X_1X_2	–0.62	0.33	3.53	0.1024
X_1X_3	–0.58	0.33	3.07	0.1230
X_2X_3	–0.40	0.33	1.45	0.2674

X_1=temperature, X_2=pressure, X_3=extraction time
$p<0.01$ highly significant, $0.01\leq p<0.05$ significant, $p\geq 0.05$ not significant

Table 4. Analysis of variance (ANOVA) for the response surface quadratic model for the oil yield from rapeseed obtained by supercritical CO_2 extraction

Source	Sum of squares	Degree of freedom	Mean square	F-value	p-value
Recovery					
model	762.77	9	84.75	193.46	<0.0001
residual	3.03	7	0.44		
lack-of-fit	2.17	3	0.72	3.20	0.1450
pure error	0.90	4	0.20		
total	765.84	16			

termined at 29.7 MPa, 52.14 °C and 3.36 h, and the predicted extraction oil yield was 28.27 %. Under these optimal conditions, the experimental value was 28.11 %, which is in agreement with those predicted by computation.

Analysis of variance (ANOVA) results of the model are shown in Table 4. The regression model for the oil yield was highly significant ($p<0.01$) with satisfactory coefficient of determination (R^2) of 0.9908. These results show that the model predicted for the oil yield was adequate, as indicated by error analysis that showed non-significant lack-of-fit. Low residual values indicate good agreement of the experimental data with the mathematical model. Additionally, an excellent agreement between the observed and predicted values for oil yield from rapeseed is obvious from parity plot shown in Fig. 1. The best way to visualize the effect of the independent variables on the dependent ones is to draw surface response plots of the model. Eq. 6 is represented graphically on three-dimensional surface of rapeseed oil obtained by supercritical CO_2 extraction as shown in Figs. 2–4. From these figures it can be seen that oil yield increased with increased extraction pressure and with longer

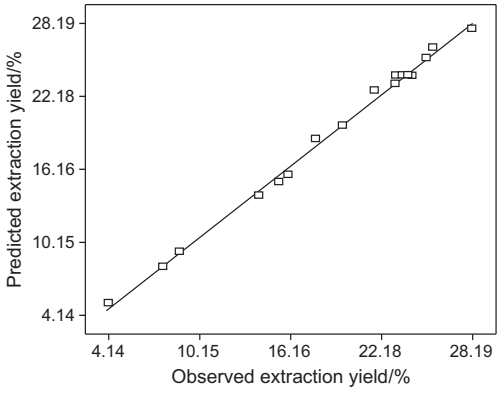

Fig. 1. Predicted *vs.* observed values for extraction yield of oil from rapeseed

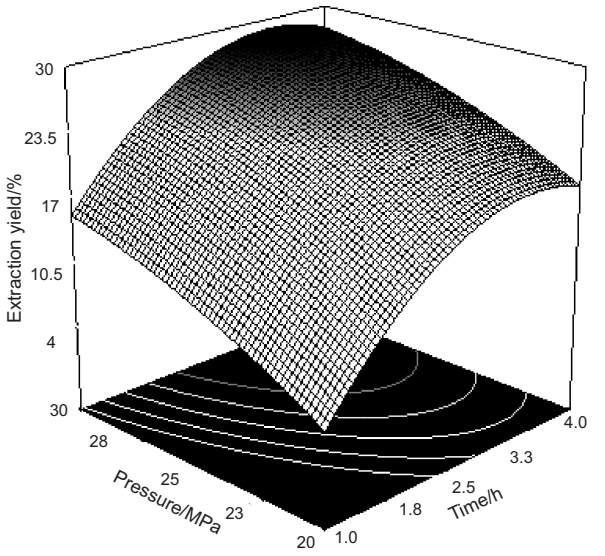

Fig. 2. Surface plot of oil yield from rapeseed as a function of pressure and extraction time at constant temperature of 50 °C

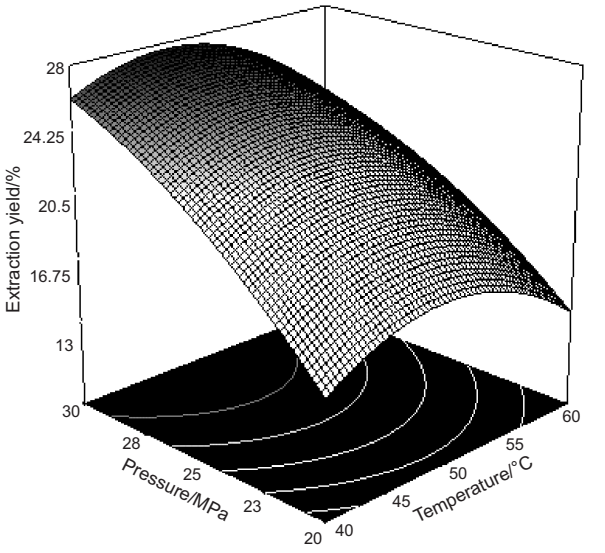

Fig. 3. Surface plot of oil yield from rapeseed as a function of pressure and extraction temperature at constant time of 2.5 h

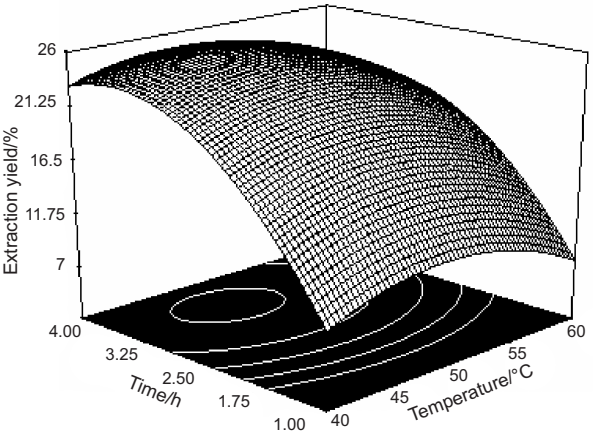

Fig. 4. Surface plot of oil yield from rapeseed as a function of temperature and extraction time at constant pressure of 25 MPa

extraction time. The oil yield increased with increased extraction temperature up to about 50 °C, while further increase of temperature led to the decrease in the oil yield.

Kinetic study

The kinetics of the supercritical CO_2 extraction of oil from rapeseed was investigated by modelling the extraction curves using the model described by Martínez *et al.* (*15*). The values for the adjustable model parameters and AARD values for all temperature and pressure ranges are presented in Table 5. It can be observed that the values of t_m were negative, which means that the extraction was constantly decreasing, having its maximum value at the very beginning (*23*). The physical meaning of adjustable parameter *b* is still not well defined, but from the results in Table 5, it can be seen that the value of parameter *b* increased with pressure at isothermal conditions. This indicates that the equilibrium is quickly established in the systems with higher pressure (*16*). The AARD values in Table 5 ranged from 3.08 to 15.32 %, which indicates that the model proposed by Martínez *et al.* (*15*) showed very good agreement between the experimental and modelled results. The best adjustments of the experimental results were obtained at 30 MPa and 60 °C (the lowest AARD value).

Table 5. Calculated parameters and deviations for mass transfer model

p	Temperature	*b*	t_m	AARD
MPa	°C	min^{-1}	min	%
20	40	0.006593	–1052.559	13.94
25	40	0.011898	–720.256	9.725
30	40	0.023646	–290.499	10.04
20	50	0.004539	–2247.733	11.14
25	50	0.013581	–709.443	15.32
30	50	0.026726	–525.211	11.48
30	60	0.015439	–379.231	3.08

AARD=average absolute relative deviation

Fig. 5 shows the effect of pressure on the supercritical CO_2 extraction of oil from rapeseed at 40 °C. At

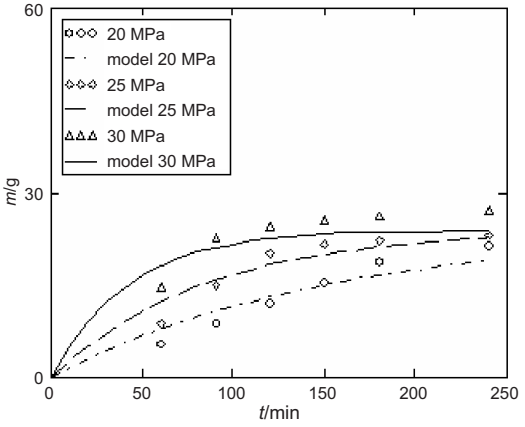

Fig. 5. Model and experimental extraction curves: effect of pressure (at 40 °C)

lower pressure, the yield of extracted oil was also lower. Furthermore, with the increase of pressure, the amount of extracted oil increased. This performance is caused by the enhancement in the solvent density with extraction pressure. Similar results were published by other researchers (*24–26*). From the experimental curve obtained at 30 MPa, it can be clearly seen that the extraction curve is divided into three periods. The first period is constant extraction rate period, where the external surface of the particles is covered with the solute (easily accessible solute) and the convection is the dominant mass transfer mechanism. The second is the falling extraction rate period, where flaws in the external surface oil layer appear and the diffusion mechanism starts, operating in combination with convection. In the third period the mass transfer occurs mainly by the diffusion in the bed and inside the solid substrate particles (*27,28*).

The temperature effect for the experimental curves obtained at the pressure of 30 MPa is shown in Fig. 6. The lowest AARD value between experimental and model results was obtained at higher temperature (60 °C). Furthermore, the highest extraction yield was obtained at 50 °C. Figs. 5 and 6 show a good agreement of experimental data with approximation data using the model of Martínez *et al*. (*15*) for the extraction yield of oil from rapeseed obtained by supercritical CO_2.

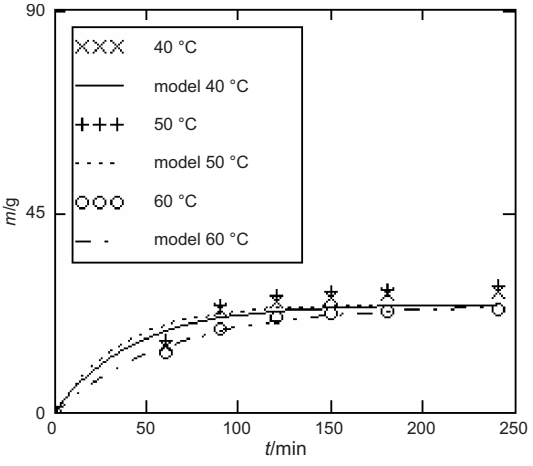

Fig. 6. Model and experimental extraction curves: effect of temperature (at 30 MPa)

The extraction yield was calculated as the mass of oil extracted and expressed as a percentage of the mass of raw material fed into the extractor. Soxhlet extraction using *n*-hexane as a solvent produced higher yield (34.17 %) compared to supercritical CO_2 extraction under optimal extraction conditions (28.27 %). The yield of oil obtained using this method from rapeseed was about 27 % lower in comparison with the Soxhlet method (83 % of the *n*-hexane-extracted oil). This difference has already been reported (*29-31*). The authors have suggested that *n*-hexane is much less selective than CO_2, thus the produced oil contains some undesirable compounds. Namely, in the work of Friedrich and List (*29*) soybean oil extracted with supercritical CO_2 showed significantly less phosphorus compared to *n*-hexane-extracted oil with the phosphatide content of 0.19 % in the oil obtained by supercritical CO_2 and 2 % in the oil obtained by *n*-hexane extraction.

Pederssetti *et al.* (*8*) also studied the extraction of rapeseed oil using compressed propane, supercritical CO_2 and *n*-hexane as solvents. The influence of pressure (20–25 MPa) and temperature (40–60 °C) on the extraction yield of rapeseed oil was investigated. Even though the extraction yield of *n*-hexane-extracted oil was similar to the yield obtained in our study, the values for extraction yield with supercritical CO_2 significantly differed. Namely, the authors exhibited the highest extraction yield among 5 different experiments of just 19.49 % at 40 °C and 25 MPa. This implicates that by the optimization of extraction operating parameters, such as in this study, the yield could be significantly improved.

Fatty acid composition of rapeseed oil

Fatty acid profiles of rapeseed oil extracted by *n*-hexane extraction and supercritical CO_2 are presented in Table 6. No significant differences were found in the oils extracted by two different extraction methods. This has already been reported by many authors (*2,29*), who reported that supercritical CO_2 and *n*-hexane-extracted oils have a similar content of unsaponifiable matter. *n*-Hexane and CO_2 are non-polar solvents so they exhibit similar behaviour for extracting chemical compounds from plant materials (*30*).

Table 6. Fatty acid composition of rapeseed oil obtained at optimal conditions by supercritical CO_2 extraction and by Soxhlet extraction

Fatty acid	SC-CO_2 extraction	Soxhlet extraction
	%	%
C 16:0	(4.72±0.07)[a]	(4.86±0.076)[a]
C 16:1	(0.21±0.01)[a]	(0.23±0.015)[a]
C 16: 1 trans	(0.13±0.015)[a]	(0.15±0.02)[a]
C 17:0	<0.05[a]	<0.05[a]
C 17:1	<0.05[a]	<0.05[a]
C 18:0	(1.64±0.04)[a]	(1.63±0.046)[a]
C 18:1 trans	<0.05[a]	<0.05[a]
C 18:1	(66.31±0.145)[a]	(64.30±0.136)[b]
C 18:2 trans	<0.05[a]	<0.05[a]
C 18:2	(18.08±0.060)[a]	(19.31±0.058)[b]
C 18:3	(6.96±0.060)[a]	(7.76±0.065)[b]
C 20:0	(0.71±0.043)[a]	(0.58±0.052)[a]
C 20:1	(0.92±0.045)[a]	(0.91±0.043)[a]
C 22:0	<0.05[a]	<0.05[a]
C 22:1	(0.31±0.025)[a]	(0.27±0.023)[a]
SFA	7.07	7.06
UFA	92.92	92.94
MUFA	67.88	65.87

Data are expressed as mean value of replication (N=3)±S.D. (standard deviation); the same letter in the same row indicates no significant differences (Duncan's test, $p<0.05$); SFA=saturated fatty acids, UFA=unsaturated fatty acids, MUFA=monounsaturated fatty acids

Fatty acid composition is a major determinant of oil quality. The good quality oil mainly contains high percentages of unsaturated fatty acids, usually oleic and linoleic. The dominant fatty acid obtained either by supercritical CO_2 or *n*-hexane extraction was oleic (66.3 and 64.3 %, respectively), followed by a significant amount of linoleic (18.1 and 19.3 %, respectively) and palmitic acid (4.7 and 4.9 %, respectively). Váradyová *et al.* (*32*) also found that oleic acid was the principal fatty acid in the rapeseed oil (>60 % of total fatty acids), followed by linoleic and palmitic acids at similar percentages (23 and 5 %, respectively). Furthermore, it is very important to detect the quantity of linolenic acid. In the case of the investigated rapeseed oil samples this content can be considered as very high. It is higher in comparison with most types of oil present on the market (pumpkin, olive, avocado), similar to soybean oil, but lower compared to canola oil, where according to Haiyan *et al*. (*33*) and Przybylski *et al*. (*34*) this fatty acid content was determined to be higher. α-Linolenic acid, found in green leafy vegetables, flaxseed, rapeseed, and walnuts, is significant as it desaturates and elongates in the human body to eicosapentaenoic and docosahexaenoic acids, and by itself may have beneficial effects on health and in the control of chronic diseases (*35*). Content of total unsaturated fatty acids in the investigated rapeseed oil was very high, higher than 90 %, and the same could also be claimed for the total content of monounsaturated fatty acids (MUFA), as it was higher than 65 %. Except for palmitic acid, present at about 4.7 %, and stearic acid, present at about 1.8 %, the quantity of other saturated fatty acids was much lower. In the obtained oil samples, there were no fatty acids with a chain shorter than 16 carbon atoms, and longer than 22 carbon atoms.

Conclusion

Supercritical CO_2 extraction was demonstrated to be a valuable alternative technology to the traditional techniques for oil processing. In terms of extraction time, supercritical CO_2 extraction was the quickest process as it is less time consuming than Soxhlet extraction. Since the tendency of modern industry is to minimize the environmental impact, decrease the toxic residues, and produce higher quality foods, the priority should also be given to the extraction with supercritical CO_2 compared to the extraction with organic solvents. The current results show that the second-order polynomial model was sufficient to describe and predict the response variable of the rapeseed oil yield obtained by supercritical CO_2 extraction within the experimental ranges. The linear and quadratic terms of temperature, pressure and extraction time highly significantly affected the oil yield. Based on the proposed model, the optimal conditions for rapeseed oil yield within the experimental range were found to be 29.7 MPa, 52.14 °C and 3.36 h, and the predicted oil yield was found to be 28.27 %. Under these optimal conditions, the experimental values were in agreement with the predicted values. 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. The model proposed by Martínez *et al.* (*15*) fitted well to all stages of the extraction curves for rapeseed oil in supercritical CO_2 meaning that the model based on the mass transfer equations could be successfully used to describe the extraction curves through the adjustable parameters (*b* and t_m). Both rapeseed oil samples, obtained by *n*-hexane and by supercritical CO_2 extraction, showed good qualitative and quantitative characteristics considering fatty acid composition. The fatty acid composition of the oil obtained by supercritical CO_2 was similar to the oil obtained by *n*-hexane extraction. In both cases the oil contained significantly high amount of unsaturated fatty acids (higher than 90 %), very high amount of monounsaturated oleic acid and, most importantly, in comparison with commercial oil on the market, its polyunsaturated linolenic fatty acid carries the health benefits.

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