Optimization of walnut oil production

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Summary

Walnut is recognized worldwide as a functional health food. In the walnut oil production it is very important to find an appropriate method to recover the oil from seeds. Walnut oil in this study was obtained by pressing the seeds followed by extraction with supercritical CO_2. The effects of pressing temperature (70, 85, 100 °C), frequency (20, 30, 40 Hz) and nozzle size (8, 10, 12 mm) in pressing experiments on oil recovery and oil temperature were monitored. The optimal calculated pressing condition within the experimental range of the variables studied was determined. In obtained walnut oil the following parameters were analyzed: peroxide value, free fatty acids, insoluble impurities, moisture content, iodine value, saponification value, *p*-anisidine value and Totox value. The residual oil from pressed cake obtained at optimal conditions was extracted with CO_2 with a goal to extract tocopherols residue from walnut after applied screw press process. Content of tocopherols in walnut oil obtained by pressing and oil extracted by supercritical CO_2 were compared.

Keywords: walnut oil, screw pressing, supercritical CO_2 extraction, oil quality, tocopherols, optimization

Introduction

Walnut seeds are a good source of nutrients, particularly proteins and essential fatty acids. They contain high level of oil (52-70 %) with excellent fatty acid balance (Crews et al., 2005). Their major constituents are triglycerides, in which monounsaturated (mainly oleic acid) and polyunsaturated fatty acids are present in high amounts. Regarding the antioxidant potential, nuts are an excellent source of tocopherols and polyphenols (Crews et al., 2005; Martínez et al., 2006).

The amount and quality of extracted walnut oil are crucial for determining feasibility of commercial production. Recently, there has been interest in the use of continuous, mechanical screw presses to recover oil from oilseeds, but screw pressing will not extensively replace solvent extraction in commodity oilseeds. The main reason is lower proportion of collected oil. Benefits of screw pressing is providing a simple and reliable method of processing small batches of seed (Martínez et al., 2008). Conventional solvent extraction produces low-quality oil that requires extensive purification operations while screw pressing does not require the use of organic solvent and is able to retain bioactive compounds such as essential fatty acids, phenolics, flavonoids and tocopherols in the oils (Teh and Birch, 2013).

Oil extraction using supercritical fluids is an alternative method to replace conventional industrial process such as pressing and solvent extraction as it offers a number of advantages, including the absence of solvent residue and better retention of aromatic compounds (Salgın and Salgın, 2006; Martínez et al., 2008; Sovilj, 2010). Supercritical fluids provide high solubility and improved mass transfer rates and process can be manipulated by changing the main process parameters. Carbon dioxide (CO_2) as an environmentally-friendly solvent is mainly used as the extraction agent in supercritical fluid extraction. Extracts obtained using CO_2 as the extraction solvent are solvent-free / without any trace of toxic extraction solvents, and are thereby highly valued (Brunner, 2005; Martínez et al., 2008; Sahena et al., 2009; Temelli, 2009).

Supercritical fluid extraction is still relatively new and is not widely used on the commercial scale for the extraction of edible oils mainly due to very high investment costs for equipment. But when considering industrial application, it is essential to provide research on the fundamentals of the supercritical processes, and according to that the aim of this work was to optimize the extraction process of walnut oil using screw pressing and employing supercritical CO_2 to recover the residual oil from pressed cake.

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Materials and methods

Material

Walnut samples were collected during the 2013 from Slavonian area (Croatia). Moisture content of the walnuts (6.43 % ± 0.09 %) was determined according to AOAC Official Method 925.40 (2000). The walnut oil was obtained by pressing of 0.5 kg of walnuts per each experiment using different process conditions. The pressing were performed in a screw expeller SPU 20 (Senta, Serbia). After pressing, the volume of screw pressed oils and their temperature were measured and after that the oil was centrifuged. The sedimented solids were recovered and solid percentage of the oils was calculated by weight difference.
The purity of CO_2 used for extraction was 99.97 % (w/w) (Messer, Osijek, Croatia). α-, β-, γ- and δ-Tocopherols were purchased from Supelco (Bellefonte, USA) and Dr. Ehrenstorfer (Augsburg, Germany). Potassium hydroxide was supplied by Kemika (Zagreb, Croatia).

Organic solvent extraction

The initial oil content in walnut samples was measured by automatic extraction systems Soxterm by Gerdhart with *n*-hexane. 5 g of ground walnut samples was extracted with 120 mL solvent, until totally depleted according to HRN ISO 6492 (2001). The whole process took 2 h and 45 min, at 180 °C. The measurement was done in duplicate. The average of the initial oil content for two replicates was 63.28 ± 0.23 %. Cake residual oil was also determined by automatic extraction systems Soxterm.

Oil quality parameters

Free fatty acids, iodine value and saponification value were determined according to AOAC Official Methods 940.28, 920.185 and 920.160 (1999). Peroxide value (PV) of oil samples was determined according to ISO 3960 (1998) and was expressed as mmol O_2/kg of oil. Insoluble impurities were determined according to ISO 663 (1992). *p*-Anisidine value (AV) was determined according to ISO 6885 (2006). Totox value was calculated as 2PV+AV (Hamilton and Rossell, 1986). All these determinations were carried out in triplicate.

Analysis of tocopherols

Preparation of samples for GC-MS analysis has been provided by saponification 0.5 g of sample in 50 ml of potassium hydroxide, and then by extraction unsaponifiable components using diethyl ether as extraction solvent.
For analysis of tocopherols Agilent 7890 A GC equipped with Agilent 5975 MSD has been used. For this analysis GC-MS was fitted with HP-5MS (Agilent J&W 19091S-433) column (30m x 0.25 mm ID, 0.25 μm). The temperature of injection port was 250 °C, splitless injection. The temperature of transfer line was 280 °C. The initial oven temperature was set at 200 °C for 3 min, and then programmed as 8 °C/min to 280 °C. The carrier gas was He. MS conditions were: scan (45 to 450 amu), threshold 100 MS quad 150 °C, MS source 250 °C. Injected sample volume was 1μl. The identification of components was carried out based on computer matching with NIST 2008 MS library. For qualitative analysis, based on retention times, Supelco (USA) and Dr. Ehrenstorfer (Germany) standard components were used. Quantitative analysis has been provided based on calibration curves. For preparation of calibration curves standard compounds *α*-tocopherol (Dr. Ehrenstorfer Cat No. 1792430), *β*-tocopherol (Supelco Cat No. 46401-U), *γ*-tocopherol (Supelco Cat No. 47785) and *δ*-tocopherol (Supelco Cat No. 47784) were used. Standard compounds were dissolved in *n*-hexane to prepare six different concentration of each standard compound. R^2 for each calibration curve was 0.999. All analyses were performed in triplicate.

Experimental design

Box-Behnken design which includes three variables and three factorial levels was chosen in this study (Bas and Boyacı, 2007). The ranges for the variables, namely nozzle size (8, 10, 12 mm), temperature (70, 85, 100 °C) and frequency (20, 30, 40 Hz) were selected to approximate the optimal conditions for screw pressing of walnut oil. Coded and uncoded levels of the independent variables and the experimental design are given in Table 1. Coded value 0 stands for centre point of the variables and repeated for experimental error. Factorial points are coded as ±1.

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

Independent variable	Symbol	Level		
		Low (-1)	Middle (0)	High (+1)
Nozzle (mm)	X_1	8	10	12
Temperature (°C)	X_2	70	85	100
Frequency (Hz)	X_3	20	30	40

Second-order polynomial equation was used to express the investigated responses (*Y*) after pressing, namely the volume of screw press oil after centrifugation (ml), oil temperature (°C) and residual oil in pressed cake (%) as a function of the coded independent variables, where X_1, X_2, …, X_k are the independent variables affecting the responses *Y*'s; β_0, β_j (*i*= 1, 2,..., *k*), β_{ii} (*i*= 1, 2,..., *k*), and β_{ij} (*i*= 1, 2,..., *k*; *j*=1, 2,.. , *k*) are regression coefficients for intercept, linear, quadratic, and interaction terms, respectively; *k* is the number of variables.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{\substack{i=1 \\ i<j}}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j \quad (1)$$

Statistical analysis was performed using RSM software Design-Expert®, v.7 (Stat Ease, Minneapolis, USA). The results were statistically tested by the analysis of variance (ANOVA) at the significance level of p=0.05. The adequacy of the model was evaluated by the coefficient of determination (R^2) and model *p*-value. Mathematical models were established to describe the influence of single process parameter and/or interaction of multiple parameters on each investigated response. Response surface plots were generated with the same software and drawn by using the function of two factors, and keeping the other constant.

Supercritical CO_2 extraction

The experiment was performed in supercritical fluid extraction system explained in detail elsewhere (Moslavac et al., 2014) with some new modifications that are manifesting on the reduction in the number of valves and installation of heat exchangers for the separator. The new process scheme for supercritical extraction system is given in Fig. 1.

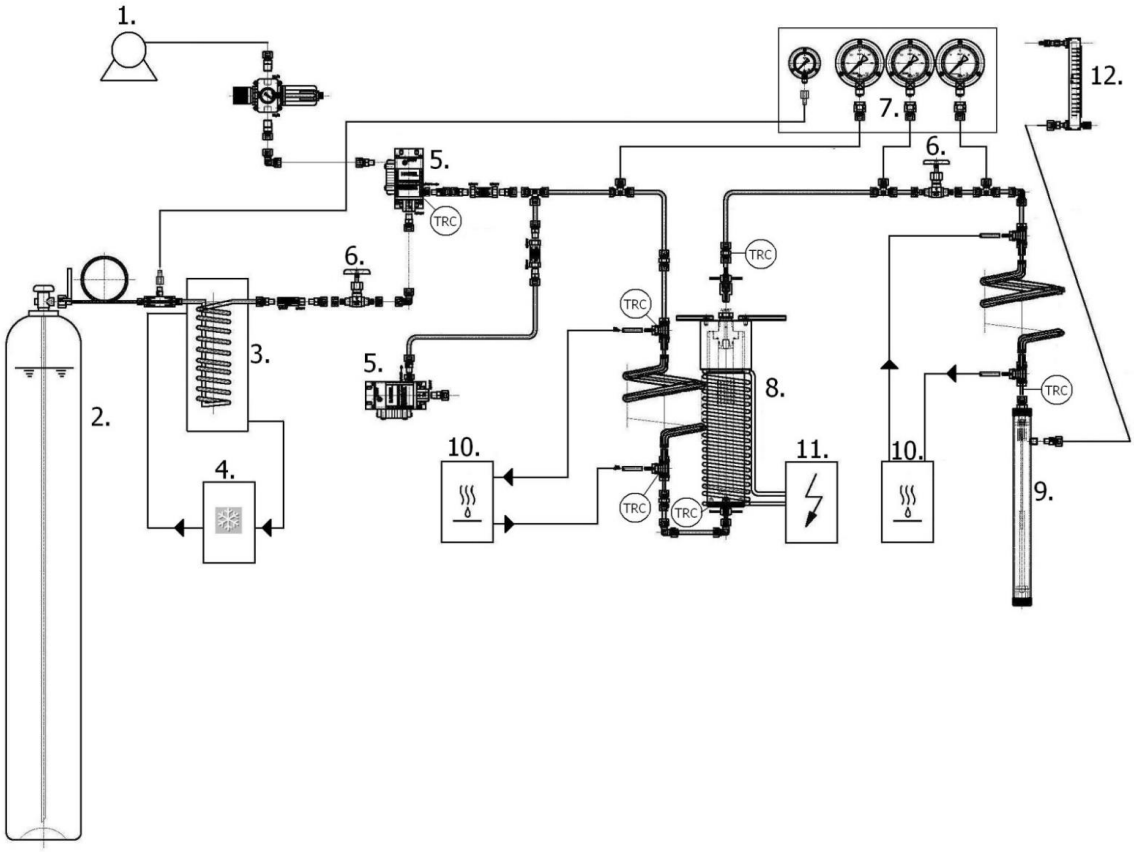

Fig. 1. Experimental set-up of supercritical fluid extraction system
1. Compressor; 2. CO_2 Tank; 3. Stainless steel coil; 4. Cooling bath; 5. Air driven fluid pump Haskel MS-71; 6. Valves (B-HV); 7. Manometers; 8. Extraction vessel; 9. Separator vessel; 10. Water bath; 11. Centralized system glass fiber heater; 12. Flow meter

The pressed cake of 100 g was placed into an extractor vessel. The extracts were collected in previously weighed glass tubes. The amount of extract obtained at regular intervals of time was established by weight using a balance with a precision of ± 0.0001 g. Extraction was performed at the following conditions: pressure of 30 MPa, temperature of 40 °C and a CO_2 mass flow rate of 1.73 kg/h. Separator conditions were 1.5 MPa and 25 °C.

Results and discussion

Optimization of screw pressing of walnut seeds

The oil from walnut seeds were obtained by screw pressing using different process parameters. Effects of temperature, nozzle size and frequency on recovery and temperature of walnut oil were studied by RSM and experiments were performed according to the Box-Behnken design (Table 2).

Table 2. Experimental matrix and values of the observed response

Run	Nozzle (mm)	Temperature (°C)	Frequency (Hz)	Pressed cake amount (g)	Screw pressed oil (ml)	Oil volume after centrifugation (ml)	Oil temperature (°C)	Cake residual oil (% of total)
1	12	85	40	212.70	320	270	53	17.57
2	8	100	30	181.10	360	280	56	8.52
3	10	70	20	161.10	380	260	41	8.73
4	12	70	30	176.80	360	260	50	15.56
5	10	85	30	193.90	350	275	50	14.44
6	8	70	30	188.40	370	295	45	9.78
7	10	85	30	201.70	340	275	53	14.09
8	12	100	30	191.26	330	255	55	12.31
9	10	85	30	203.90	330	265	52	13.01
10	10	85	30	200.10	340	270	51	14.23
11	10	85	30	201.30	340	280	54	12.71
12	12	85	20	204.10	330	290	45	10.43
13	10	70	40	268.20	265	215	43	10.95
14	8	85	40	196.10	350	275	52	12.91
15	10	100	40	181.90	370	275	58	11.58
16	8	85	20	184.60	360	285	53	7.95
17	10	100	20	185.90	360	305	57	9.05

In Table 3 is given the estimated coefficients of the second order polynomial equation for the most important responses (volume of screw press oil after centrifugation, oil temperature and residual oil in pressed cake). In order to avoid difficulties in the beginning of pressing experiments with the used screw expeller and to increase efficiency, the head of the screw press was preheated to a temperature between 70 and 100 °C. The pressing conditions had influence also on the amount of residual oil in press cake. The value of residual oil in press cake was in the range from 7.95 to 17.57 % depending on applied pressing parameters. In Table 3 it can be seen which process parameter had statistically significant effect on investigated responses. The nozzle size (X_1) and frequency (X_3) had significant effects ($p<0.05$) on the volume of obtained oil and on the amount of cake residual oil. The temperature of screw press head (X_2) had significant effect on temperature of screw press oil. The regression coefficients (related to coded variables) are obtained by fitting experimental data to the second order models for investigated responses.

The ANOVA results for modeled responses are reported in Table 4. Joglekar and May (1987) suggested that for a good fit of a model, R^2 should be at least 0.80. In our study, the R^2 values for these response variables were higher than 0.80, indicating the adequacy of the applied regression models. Table 4 shows the test statistics for the model (F-test and probability) of oil recovery and oil temperature. The probability (p-value) of all regression models was below 0.05, which means that there was a statistically significant multiple regression relationship between the independent variables and the response variable. The lack of fit, which measures the fitness of the model, resulted in no significant F-value ($p>0.05$) in terms of the response variables studied, indicating that the model was sufficiently accurate for predicting the response variations. The ANOVA showed that the models were acceptable and could be used for optimization the pressing parameters with respect to oil recovery and oil temperature.

Table 3. Estimated coefficient of the second order polynomial equation

Term	Coefficient[a]	Oil volume	Oil temperature	Cake residual oil
Intercept	β_0	273.00*	52.00*	13.70*
X_1	β_1	-16.25*	-0.37	2.09*
X_2	β_2	10.63	5.88*	-0.44
X_3	β_3	-21.88*	1.25	2.11*
X_1^2	β_{11}	16.62	0.25	-0.0008
X_2^2	β_{22}	-17.12	-0.75	-2.15*
X_3^2	β_{33}	7.87	-1.50	-1.47
X_1X_2	β_{12}	2.50	-1.50	-0.50
X_1X_3	β_{13}	15.00	2.25	0.54
X_2X_3	β_{23}	3.75	-0.25	0.077

[a] $y = \beta_0+\beta_1x_1+\beta_2x_2+\beta_3x_3+\beta_{11}x_1^2+\beta_{22}x_2^2+\beta_{33}x_3^2+\beta_{12}x_1x_2+\beta_{13}x_1x_3+\beta_{23}x_2x_3$

X_1: nozzle size; X_2: temperature; X_3: frequency

*Significant at $p \leq 0.05$

Table 4. Analysis of variance (ANOVA) of the modeled responses

Source	Sum of squares	Degree of freedom	Mean square	*F*-value	*p*-value
Oil volume					
The recovery					
Model	10364.49	9	1151.61	3.71	0.0490
Residual	2173.75	7	310.54		
Lack of fit	2043.75	3	681.25	20.96	0.0566
Pure error	130.00	4	32.50		
Total	12538.24	16			
Oil temperature					
The recovery					
Model	331.69	9	36.85	4.67	0.0272
Residual	55.25	7	7.89		
Lack of fit	45.25	3	15.08	6.03	0.0576
Pure error	10.00	4	2.50		
Total	386.94	16			
Cake residual oil					
The recovery					
Model	104.37	9	11.60	5.94	0.0141
Residual	13.65	7	1.95		
Lack of fit	11.22	3	3.74	6.14	0.0560
Pure error	2.44	4	0.61		
Total	118.02	16			

Fig. 2 show three-dimensional plots for obtained responses as a function of investigated variables in screw pressing experiments. It can be seen that the amount of obtained oil significantly increased with the increase of temperature up to about 85 °C while further increase in temperature did not cause a significant change in oil volume. Larger nozzle diameter results with a lower amount of the obtained oil. Furthermore, it can be seen that the oil temperature is significantly influenced by used temperature for heating the output press head. The amount of residual oil in cake increased with increasing frequency from 20 to 40 Hz and with increasing nozzle diameter. Pressing temperature had double effect on the amount of residual cake oil: from 70 to 85 °C the amount of residual press cake oil increased with temperature while further increase of temperature show decrease in amount of residual oil.

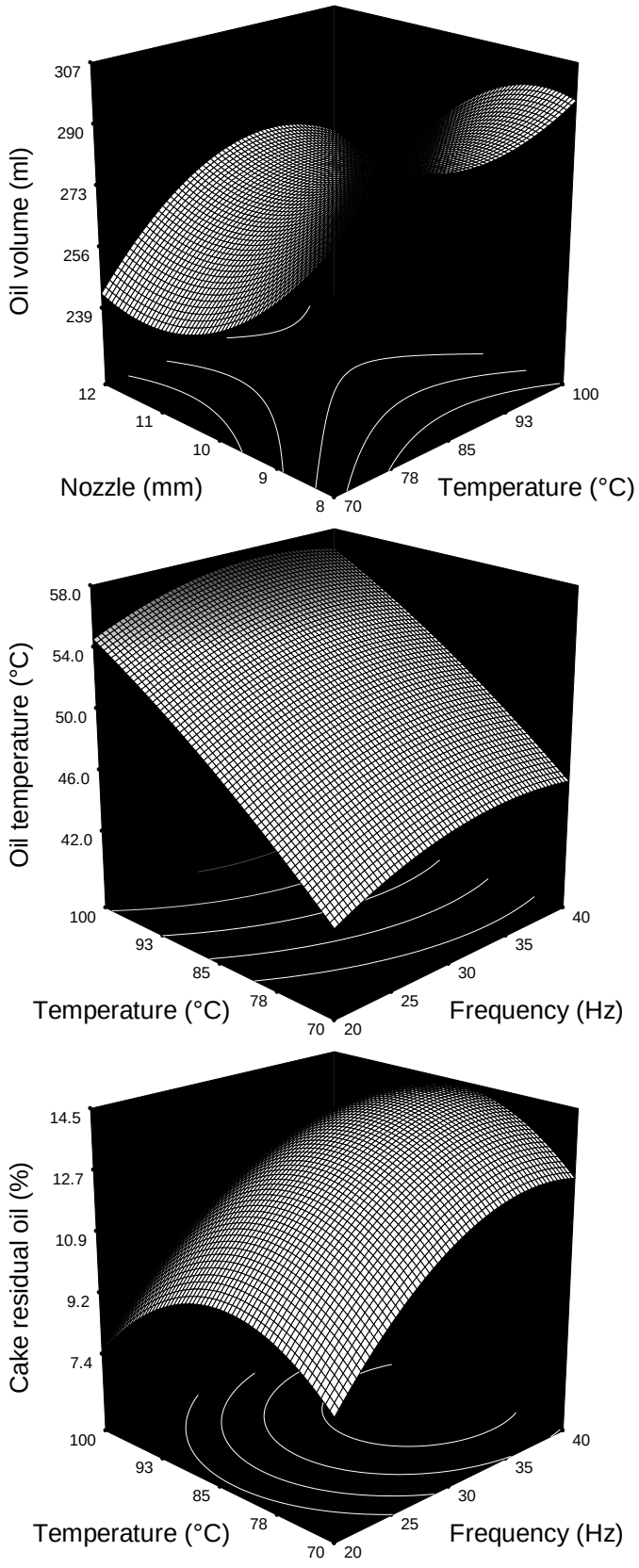

Fig. 2. Three-dimensional plots for obtained responses as a function of investigated variables

By applying desirability function method (Cojocaru et al., 2009) the calculated optimal screw pressing conditions for production of walnut oil were as follows: temperature of 70 °C, frequency of 20 Hz and nozzle of ID 8 mm. At this conditions oil volume was calculated to be 288.9 ml, oil temperature 42.7 °C and cake residual oil 7.95 %, which is in very close agreement with experimental obtained data (measured oil volume 284 ml, oil temperature 44 °C and cake residual oil 7.81 %).

In obtained walnut oil at this optimal screw pressing conditions the quality parameters were determined and listed in Table 5.

Table 5. Physicochemical properties of walnut oil

Properties	**Value**
Iodine value (g I_2/100 g of oil)	161.79
Saponification value (mg KOH/g of oil)	193.00
Peroxide value (mmol O_2/kg of oil)	0.46
Free fatty acids (%)	0.23
Insoluble impurities (%)	0.34
Moisture content (%)	0.11
p-Anisidine value	0.08
Totox value	1.02

Primary oxidation processes in oil mainly form hydroperoxides, which are measured by the peroxide value. The lower the peroxide value, the better the quality of the oil (Frankel, 2005). It is also very important that cold pressed oils are low in moisture content and free fatty acids to maintain the quality and shelf life of the oils (Teh and Birch, 2013). Good quality oil should have *p*-anisidine value of less than two, and Totox value less than four (Frankel, 2005). In obtained walnut oil at optimal pressing conditions in this study, low values of peroxide number, free fatty acids, insoluble impurities, moisture content and *p*-anisidine value show that obtained walnut oil are of very good quality. Free fatty acids and iodine value was very similar than that reported by Patraş and Dorobanţu (2010).

Extraction of residual oil from pressed cake with supercritical CO_2

The cake resulting from pressing at optimal conditions (temperature of 70 °C, frequency of 20 Hz and nozzle ID 8 mm) was extracted with supercritical CO_2 and the obtained kinetic curve for this experiment is shown in Fig. 3.

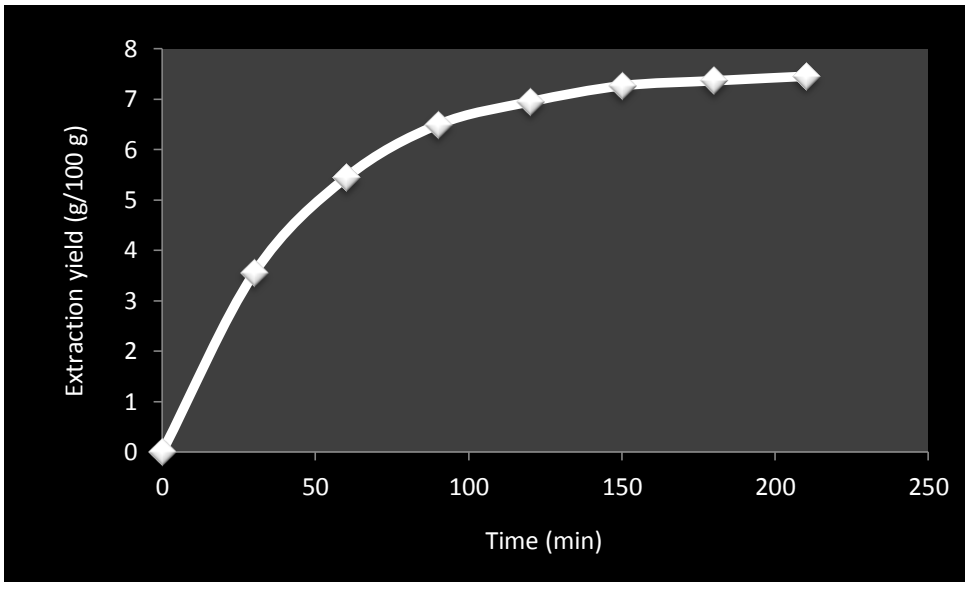

Fig. 3. Extraction of cake residual oil with supercritical CO_2

The amount of residual oil in press cake at optimal conditions was 7.81 % (obtained by Soxhlet extraction). From Fig. 3 it can be seen that after 210 min of extraction almost all residual oil in the pressed cake was extracted by supercritical CO_2. The similar results were obtained in our previous study (Moslavac et al., 2014) where authors investigate the possibility to recover the residual oil from pressed cake obtained after pressing *Camelina sativa* seeds. The shape of obtained extraction curve is the same like in other edible oils obtained by supercritical fluids where the three periods of extraction: rapid extraction period, transition period and slow extraction period are visible.

Tocopherol content of walnut oil

Walnut oil obtained by supercritical CO_2 from pressed cake has tocopherol content similar to those extracted by screw pressing. Measured total tocopherol content was 463.71 mg/l in screw pressed oil and 498.05 mg/l in oil obtained from press cake by supercritical CO_2. The most frequent form of tocopherol was γ-tocopherol (322.83 mg/l in screw pressed oil and 309.32 mg/l in oil extracted by supercritical CO_2) followed by α-tocopherol (140.88 mg/l in screw pressed oil and 188.73 mg/l in oil extracted by supercritical CO_2). Other two tocopherols (*β*- and *δ*-) were not detected. Content of γ-tocopherols in both oil are similar to those found by Savage et al. (1999), Lavedrine et al. (1999) and Oliviera et al. (2002), while amount of α-tocopherols were significantly greater.

Conclusions

This study show that supercritical CO_2 extraction, as a relatively new separation technique, could be used as a very efficient process in recovering the residual walnut oil from pressed cake. When screw press was preheated to the temperature of 70 °C, and applying frequency of 20 Hz and using nozzle of ID 8 mm, the good oil recovery and oil quality were obtained. Regarding the antioxidant potential, walnuts are an excellent source of tocopherols where γ-tocopherol followed by α-tocopherol are dominant.

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