Cold Pressing and Supercritical CO$_2$ Extraction of Hemp (*Cannabis sativa*) Seed Oil

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In the past few decades, the *Cannabis sativa* L. hemp variety has been unfairly neglected because of its similarity to the illegal kind *Cannabis indica* used as a narcotic. The objective of this study was to evaluate the process of oil extraction from *Cannabis sativa* seeds by cold pressing, followed by extraction with supercritical CO$_2$. In the pressing experiments, the response surface methodology was conducted in order to study the effects of temperature, frequency, and nozzle size on oil recovery and quality parameters. The optimal condition for obtaining the highest oil recovery (23.34 %) and the best oil quality within the experimental range of the variables studied was at temperature of 60 °C, frequency of 20 Hz, and nozzle of ID 6 mm. The residual oil (10.33 %) in the press cake was extracted totally by supercritical CO$_2$ in a newly designed supercritical fluid extraction system. Oregano essential oil was the most effective in protecting the oil from oxidative deterioration.

Key words:
hemp seed oil, cold pressing, supercritical CO$_2$ extraction, optimization, oxidative stability

Introduction

Hemp (*Cannabis sativa* L.) is an annual plant that has been bred for centuries and its main products are hemp seeds and fibers. Fibers from the stalk are still used in production of durable fabrics and specialty papers, such as paper money, tea bags, canvas, linen, cigarette paper, and other strong, thin papers used to make thick books, like the Holy Bible[1]. Hemp production was prohibited due to its chemical drug component THC (δ-9-tetrahydrocannabinol)[2]. Since 1996, hemp varieties containing less than 0.3 % THC can be grown. According to EU legislation, hemp with less than 0.2 % THC can be grown[3]. Hemp seed has been used as a food and feed. The oil has been used for manufacturing printer's ink, for wood preservation, and production of soaps and detergents. The seed contains 25–35 % lipids, 20–25 % protein and 20–30 % carbohydrate, 10–15 % insoluble fibers, and an array of minerals[4]. Hemp oil contains polyunsaturated fatty acids (PUFAs). Among them are several essential fatty acids like linoleic and α-linolenic acid [3]. Hemp seed oil has the perfect ratio (3:1) between two essential fatty acids (linoleic and linolenic acid)[2]. γ-Linolenic acid has a positive effect on patients suffering from rheumatoid arthritis, atopic dermatitis and allergies[5]. Thus, the oil is ideal for human consumption and for preparing different kinds of body oils and creams, due to good absorption through the skin[6]. Tocopherols, which are present in hemp oil, may reduce the risk of cardiovascular diseases, cancer, and age-related macular degeneration. They have an antioxidant function and prevent oxidation of unsaturated fatty acids[3].

The objectives of this work were threefold: (i) to investigate the effects of process parameters during cold pressing of hemp seeds on the oil recovery and oil quality using response surface methodology (RSM); (ii) to recover the residual oil from the press cake using supercritical CO$_2$ in the newly designed supercritical fluid extraction system, and (iii) to investigate the influence of different natural antioxidants and their concentration on the oxidative stability of hemp seed oil.

Material and methods

Chemicals

Rosemary extract Oxy.Less CS, green tea extract, and pomegranate extract were supplied from Naturex (France). Olive leaf extract was supplied from Exxentia (Spain).

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Essential oils of oregano (*Origanum vulgare*), basil (*Ocimum basilicum*), mint (*Mentha piperita*), thyme (*Thymus serphylum*) and winter savory (*Satureja Montana*) were produced by steam distillation according to the standard Ph. Jug. IV procedure[7].

The purity of CO_2 used for extraction was 99.97 % (w/w) (Messer, Osijek, Croatia). All other chemicals and reagents were of analytical reagent grade.

Plant material preparation

The hemp oil used in this study was produced from seeds of *Cannabis sativa* obtained from the family farm Organica Vita (Vraneševci, Croatia) in October 2013. The hemp oil was obtained by pressing using different process conditions. The seeds were pressed in a screw expeller SPU 20 (Senta, Serbia). After pressing, the temperature and mass of the cold pressed oils were measured, after which the oil was centrifuged. The sedimented solids were recovered and solid percentage of the oils was calculated by weight difference.

Determination of initial oil and water content

The initial oil content in hemp seeds was measured by automatic extraction systems Soxterm by Gerdhart with *n*-hexane. 5 g of hemp seeds was extracted with 120 mL solvent, until totally depleted. The whole process took 2 hours and 14 minutes at 180 ºC. The measurement was performed in duplicate. The average of the initial oil content for two replicates was 33.34 ± 0.11 %. Cake residual oil (CRO) was also determined by automatic extraction systems Soxterm.

Moisture content of the seeds (8.09 ± 0.08 %) was determined according to AOAC Official Method 925.40[8].

Determination of particle size distribution of hemp seeds with sieving

After pressing, the cake was ground and sieved for 20 minutes using a vertical vibratory sieve shaker (Labortechnik Gmbh, Ilmenau, Germany). About 200 g were used at each sieving. The raw material size distribution was determined using a nest of 9 sieves of aperture sizes 1.4, 0.8, 0.63, 0.5, 0.4, 0.315, 0.2, 0.1 and 0.05 mm. The mass of fragments remaining on each sieve was used to calculate the distribution of fragments, which was then normalized in respect of the total mass. For evaluation of sieve analysis results, the Rosin-Rammler-Bennet (RRB) distribution was chosen. The percentage by mass of particles (*R*) greater than screen size (*d*) is given as:

$$R = 100 \cdot \exp\left[-\left(\frac{d}{d_0} \right)^n \right] \tag{1}$$

where d_0 represents the particle size corresponding to the 36.8th percentile of the cumulative probability distribution (size constant), and *n* controls the shape of the distribution (uniformity coefficient). The function of the sum of sieve residue (*R*) was fitted to the experimental data by changing the representative particle size d_0 and the uniformity coefficient *n*, minimizing the sum of the mean square error using *STATISTICA 8.0* software (Stat Soft Inc., USA).

The average particle size was determined to be 0.382 mm ±0.15.

Oil quality parameters

Free fatty acids (FFA), iodine value, and saponification value were determined according to AOAC Official Methods 940.28, 920.185, and 920.160[9]. Peroxide value (PV) of oil samples was determined according to ISO 3960[10]. PV was expressed as mmol O_2 kg^{-1} of oil. Insoluble impurities (II) were determined according to ISO 663[11]. *p*-Anisidine value (AV) was determined according to ISO 6885[12]. Totox value was calculated as 2PV+AV[13]. All these determinations were carried out in triplicate.

Determination of chlorophyll *a*, chlorophyll *b*, and total carotene content

For determination of chlorophyll *a* and *b*, and total carotene content, the modified method of Dere et al.[14] was used. The weighted sample, having been added diethyl ether (50 mL for each gram) was dissolved in an ultrasonic bath for one minute. It was then homogenized for 30 seconds with homogenizer, and again in the ultrasonic bath for one minute. The homogenate was centrifuged for 10 minutes at 3000 rpm. The supernatant was separated and the absorbances measured at 400 – 700 nm in an UV spectrophotometer. Chlorophyll *a* showed the maximum absorbance at 660 nm, chlorophyll *b* at 642.5 nm, and total carotene at 470 nm. All analyses were repeated three times. The amount of these pigments was calculated according to the formulas given below (Eqs. 2–5):

$$\text{Chlorophyll } a = 9.93 \cdot A_{660} - 0.78 \cdot A_{642.5} \tag{2}$$

$$\text{Chlorophyll } b = 17.60 \cdot A_{642.5} - 2.81 \cdot A_{660} \tag{3}$$

$$\text{Chlorophyll } a+b = 7.12 \cdot A_{660} - 16.80 \cdot A_{642.5} \tag{4}$$

$$\text{Total carotene} = (1000 \cdot A_{480} - 0.52 \cdot Chl\ a - 7.25 \cdot Chl\ b) / 226 \tag{5}$$

Following the calculation of concentration (mg L^{-1}), the amount of pigment in the oil was calculated as micrograms of pigment per gram of oil (Eq. 6):

$$c = c_1 \cdot V \cdot R / G \quad (6)$$

where:

c – amount of pigment in oil ($\mu g\ g^{-1}$)

c_1 – concentration of pigment (mg L^{-1})

V – initial volume (mL)

R – dilution (if any)

G – measured oil mass (g)

Determination of oxidative stability

The oxidative stability was determined by rapid oil oxidation test – Schaal or Oven Test (63 °C)[15]. The influence of the addition of natural antioxidants, namely rosemary extract, green tea extract, olive leaf extract, and pomegranate extract in concentrations of 0.1 % and 0.2 %, and essential oils of oregano, basil, mint, thyme, and winter savory in the concentration of 0.05 %, on the oxidative stability of hemp oil were monitored. The result of oil oxidation was expressed as PV during 4 days of the test. All determinations were carried out in duplicate.

Experimental design

Box-Behnken design, which includes three variables and three factorial levels, was chosen in this study[16]. The ranges for the variables, namely nozzle size (6–12 mm), temperature (60–100 °C), and frequency (20–40 Hz), were selected to approximate the optimal conditions for cold pressing of hemp oil. Coded and uncoded levels of the independent variables and the experimental design are given in Table 1. Coded value 0 stands for the centre point of the variables, and is repeated for experimental error. Factorial points are coded as ±1.

Table 1 – *Coded and uncoded levels of independent variables used in the RSM design*

Independent variable	Symbol	Level		
		Low (–1)	Middle (0)	High (+1)
Nozzle (mm)	X_1	6	9	12
Temperature (°C)	X_2	60	80	100
Frequency (Hz)	X_3	20	30	40

Experimental data were fitted with second-order response surface model (Eq. 7) with the following form:

$$Y = \beta_0 + \sum_{i=1}^{k}\beta_i X_i + \sum_{i=1}^{k}\beta_{ii} X_i^2 + \sum_{\substack{i=1 \\ i<j}}^{k-1}\sum_{j=2}^{k}\beta_{ij}X_i X_j \quad (7)$$

where Y is investigated response, β_0, β_i, β_{ii}, β_{ij} are constant coefficients of intercept, linear, quadratic, and interaction terms, respectively; X_i and X_j are coded independent variables.

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 $p = 0.05$.

Handmade supercritical fluid extraction (HM-SFE) system

The pre-pressed material resulting from the calculated optimum oil pressing conditions was extracted with CO_2 in a newly designed HM-SFE system at pressure of 25 MPa and temperature of 45 °C with a CO_2 mass flow rate of 1.2 kg h^{-1}. The extracts were collected at 1.5 MPa and 25 °C. The schematic diagram of the newly constructed apparatus used for supercritical fluid extraction is given in Fig. 1.

Materials used for the construction of the HM-SFE system are stainless steel AISI 316Ti and AISI 304. All additional connection tubing parts are of the same grade of material. Extraction and separator vessels were properly tested at safety factor of 1.5. Extraction vessel was tested at working pressure 50 MPa and separator vessel at 3 MPa.

The HM-SFE system was constructed and assembled by Đuro Đaković Aparati d.o.o. (Slavonski brod, Croatia), which performed material durability and pressure tests for the vessels. Extractor was tested at working pressure 50 MPa with safety factor 1.5, and separator was tested at working pressure of 4 MPa also with safety factor 1.5. Extraction vessel is made from stainless steel bar (AISI 304) O.D. 100 mm and height 500 mm. A stainless steel rod is drilled (center hole) with a Ø 40 mm bore for 400 mm, so the volume of extractor is 500 mL. The upper inside part of the extraction cell was polished to plug well gaskets. The cap of extraction cell was designed to hold a plug connected to the extraction cell through a trapezoidal thread. The plug, patented by the manufacturer of the HM-SFE system, has O-ring seals in two places. The plug has a built-in filter element that should prevent withdrawal of material. The filter element has the ability to filter particles of 2 microns nominal and 10 microns absolute (Norman Ultraporous 4202T-6T-2M). The lower part of extraction cell was also drilled and prepared for quick connection with R ½″ connector with O-ring seal. High-pressure seamless tubes of
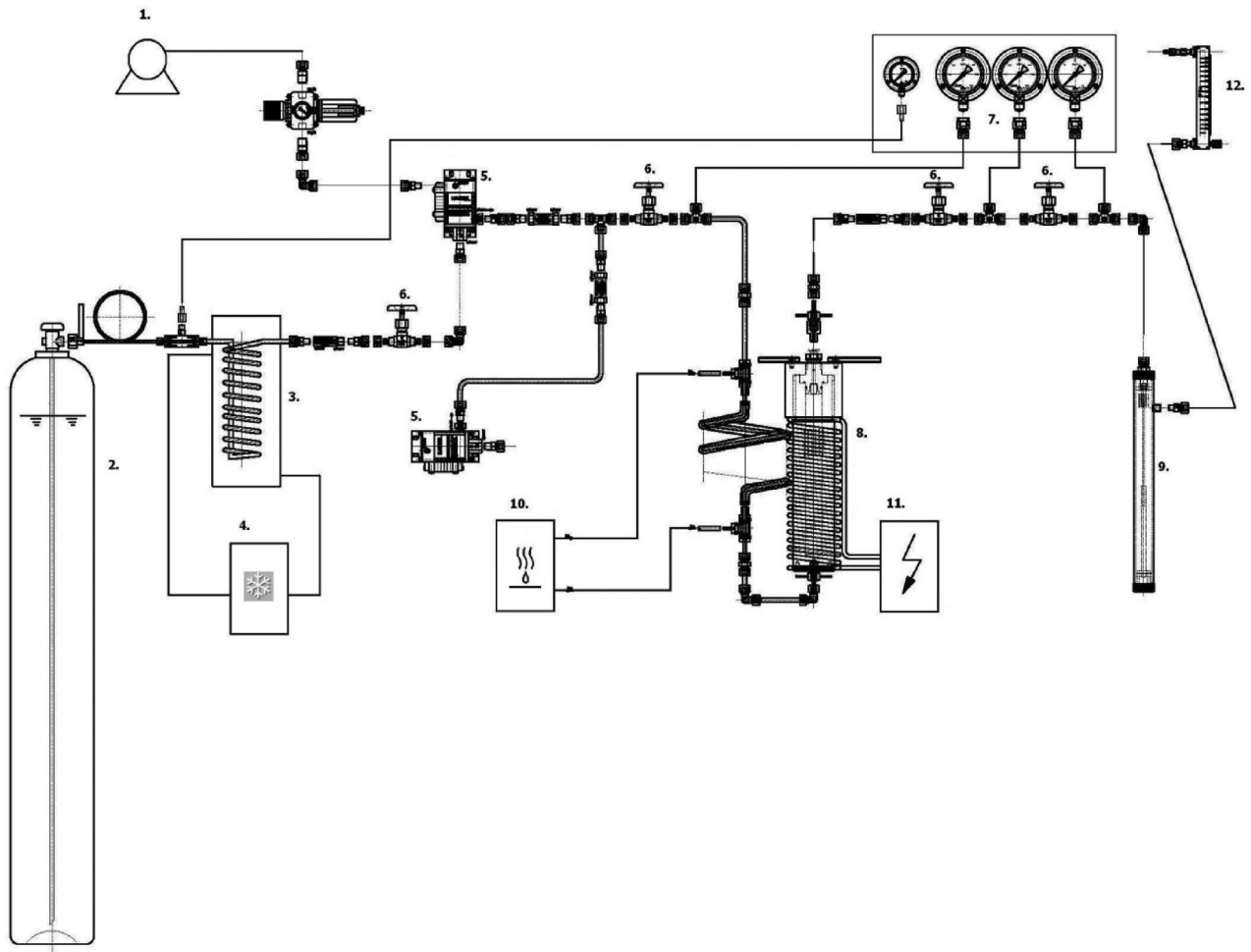
F i g . 1 – *Handmade supercritical fluid extraction system: 1. Compressor; 2. CO₂ Tank; 3. Stainless steel coil; 4. Cooling bath; 5. Air driven fluid pump Haskel MS-71; 6. Valves (B-HV); 7. Manometers; 8. Extraction vessel; 9. Separator vessel; 10. Water bath; 11. Centralized system glass fiber heater; 12. Flow meter*

dimensions 10 x 2 mm are connected to each other by Ermeto couplings (flat, knees, tees). The used high-pressure valves were provided by the same company that produces Ermeto couplings (model B-HV). Pressure extraction cell is controlled by two WIKA manometers (model 212.20) 60 MPa and one WIKA manometer (model 212.20) 4 MPa for pressure in separator.

Extraction cell is heated with a glass fiber electric heater, controlled by a centralized system and Solid State Relays (SSR). Temperature is controlled by means of a PID regulator set to maximum temperature of 80 ºC with lag delay compensation, due to the large mass of the extraction cell. Temperature measurements and regulation of the extraction cell is performed using an integrated temperature sensor within the cell and additional temperature sensor measuring output gas temperature. The input CO_2 line towards the extraction cell is preheated using a heat exchanger powered by a water heating system. The temperature of the input gas is regulated by measuring three temperatures: water temperature alongside the water heater, water input temperature in the heat exchanger, and the output CO_2 temperature that exits the heat exchanger. The temperature is regulated using standard PID regulator, taking into account the differential temperatures of water lines and output gas line. Pressure in the separator is regulated by means of an electromechanical solution for controlling pressure valve, with a pressure sensor working as a feedback.

The pump used to pressurize liquid CO_2 is Haskel® MS-71. Liquid CO_2 is precooled through the SS coil at –18 °C, cooled by ethylene glycol/ethanol cooling bath. A check valve is located after the pump to prevent eventual CO_2 flow disorders. Prior to the input extraction vessel, CO_2 is preheated through a stainless steel double coil at the temperature of extraction. After the extraction vessel, the high pressure is reduced by a high-pressure valve (B-HV) to the desirable pressure. Valves and tubing are heated to a temperature of 0 °C due to high-pressure drop. Flow of CO_2 is controlled through Matheson FM-1050 (E800) flow meter.

Results and discussion

Cold pressing experiments

Hemp seed oil was obtained by cold pressing using different process parameters (nozzle size, temperature, and frequency). The oil recovery and quality parameters of obtained cold pressed hemp oil were monitored after different experiments (Table 2) and the process was optimized using RSM.

In all the experiments, only a small percentage (0.071–0.094 %) of moisture was found in the obtained oils. Primary oxidation processes in the oil mainly form hydroperoxides, which are measured by the PV. In general, the lower the PV, the better the quality of the oil[17]. In this study, the average PV of all experimental runs was 1.95 mmol O$_2$ kg^{-1}. Borhade[18] published that peroxide value in produced hemp oil was 7.2 Meq O$_2$ kg^{-1} (3.6 mmol O$_2$ kg^{-1}), while Teh and Birch[19] had a PV of hemp oil 1.94 Meq O$_2$ kg^{-1} (0.97 mmol O$_2$ kg^{-1}). It is very important that cold pressed oils are low in moisture content and FFA to maintain the quality and shelf life of the oils[19]. In this study, FFA content ranged from 2.49 to 2.66 %, while Teh and Birch[19] published that FFA content was 0.89. Abramovič and Abram[20] published also high value of FFA in came-

lina oil and assumed that the increase in FFA was probably caused by hydrolytic activity of lipolytic enzyme during preparation of the seeds for oil production. It can also be assumed that drying conditions of harvested seeds were rigorous and contributed to the increase in FFA. The oil temperature in all experimental runs in this study ranged from 41 to 49 °C and the content of insoluble impurities was between 0.33 and 0.49 %. According to obtained results, it was concluded that the produced oil is of satisfactory quality as defined by the Ordinance on Edible Oils and Fats NN 41/2012.

Table 3 shows the regression coefficients obtained by fitting experimental data to the second-order response models for investigated responses. The coefficients are related to coded variables. The first-order term of nozzle size (X_1) had a significant effect ($p<0.05$) on the volume of obtained oil and on the amount of residual oil in the press cake. The first-order term of temperature (X_2) had a significant effect on oil volume, oil temperature, and free fatty acid content, while frequency (X_3) had a significant effect on oil volume, insoluble impurities and the amount of cake residual oil. The second-order term of nozzle size (X_1^2) had a significant effect ($p < 0.05$) on oil volume and insoluble impurities. The second-or-

Ta b l e 2 – *Experimental matrix and values of the observed response*

Run	Nozzle (mm)	Temperature (°C)	Frequency (Hz)	Cold press oil volume (mL)	Oil after centrifugation (mL)	Oil temperature (°C)	FFA (%)	II (%)	CRO (%)
1	12.00	100.00	30.00	260	210	49	2.60	0.33	13.20
2	9.00	100.00	20.00	275	225	48	2.64	0.49	10.22
3	12.00	80.00	20.00	260	222	42	2.66	0.43	10.42
4	6.00	60.00	30.00	285	220	41	2.57	0.36	11.17
5	12.00	60.00	30.00	235	180	42	2.50	0.42	13.34
6	9.00	80.00	30.00	255	215	44	2.62	0.44	10.50
7	9.00	100.00	40.00	255	190	48	2.61	0.41	13.38
8	9.00	60.00	20.00	265	215	43	2.49	0.44	10.52
9	9.00	60.00	40.00	250	188	43	2.51	0.46	13.71
10	9.00	80.00	30.00	255	215	44	2.63	0.44	12.32
11	9.00	80.00	30.00	255	215	42	2.63	0.44	13.08
12	9.00	80.00	30.00	253	210	44	2.62	0.47	12.57
13	6.00	80.00	20.00	275	225	45	2.58	0.48	9.96
14	9.00	80.00	30.00	260	219	43	2.63	0.45	12.51
15	12.00	80.00	40.00	235	188	44	2.61	0.43	14.16
16	6.00	80.00	40.00	270	220	48	2.59	0.38	12.10
17	6.00	100.00	30.00	280	225	49	2.61	0.48	10.60

FFA – Free fatty acids; II – Insoluble impurities; CRO – Cake residual oil

Table 3 – *Estimated coefficient of the second-order polynomial equation*

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3$$

Term	Coefficient[a]	Cold press oil	Oil after centrifugation	Oil temperature	FFA	II	CRO
Intercept	β_0	255.60*	214.80*	43.40*	2.63*	0.45*	12.20*
X_1	β_1	−15.00*	−11.25*	−0.75	0.003	−0.011	0.91*
X_2	β_2	4.38*	5.88*	3.13*	0.041*	0.004	−0.17
X_3	β_3	−8.13*	−12.63*	0.63	0.001	−0.020*	1.53*
X_1^2	β_{11}	4.07*	1.60	0.55	0.003	−0.035*	−0.21
X_2^2	β_{22}	5.32*	−7.65*	1.30	−0.059*	−0.015	0.090
X_3^2	β_{33}	0.33	−2.65	0.80	−0.019	0.017	−0.33
$X_1 X_2$	β_{12}	7.50*	6.25	−0.25	0.015	−0.052*	0.11
$X_1 X_3$	β_{13}	−5.00*	−7.25*	−0.25	−0.015	0.025*	0.40
$X_2 X_3$	β_{23}	−1.25	−2.00	0.000	0.003	−0.025*	−0.008

X_1: nozzle size; X_2: temperature; X_3: frequency.
*Significant at $p \leq 0.05$

der term of temperature (X_2^2) had a significant effect on oil volume and free fatty acids, while frequency (X_3^2) had no significant effect on any of the investigated responses. The interactions between the nozzle size and temperature had a significant effect on oil volume, and insoluble impurities, the same as inter-

actions between the nozzle size and frequency, while interactions between temperature and frequency had a significant effect only on insoluble impurities.

The ANOVA results for the modeled responses are reported in Table 4 (*F*-test and probability). Joglekar and May[21] suggested that for a good fit of

Table 4 – *Analysis of variance (ANOVA) of the modeled responses*

Source	Sum of squares	Degree of freedom	Mean square	*F*-value	*p*-value
Cold press oil volume					
Recovery					
Model	3015.02	9	335.00	28.10	0.0001
Residual	83.45	7	11.92		
Lack of fit	56.25	3	18.75	2.76	0.1760
Pure error	27.20	4	6.80		
Total	3098.47	16			
Oil volume after centrifugation					
Recovery					
Model	3236.48	9	359.61	11.29	0.0021
Residual	223.05	7	31.86		
Lack of fit	182.25	3	60.75	5.96	0.0588
Pure error	40.80	4	10.20		
Total	3459.53	16			
Oil temperature					
Recovery					
Model	98.43	9	10.94	4.39	0.0320
Residual	17.45	7	2.49		
Lack of fit	14.25	3	4.75	5.94	0.0591
Pure error	3.20	4	0.80		
Total	115.88	16			

| Source	Sum of squares	Degree of freedom	Mean square	*F*-value	*p*-value
Free fatty acids					
Recovery					
Model	0.032	9	0.003	4.59	0.0286
Residual	0.006	7	0.0008		
Lack of fit	0.006	3	0.002	59.72	0.0009
Pure error	0.0001	4	0.00005		
Total	0.038	16			
Insoluble impurities					
Recovery					
Model	0.028	9	0.003	10.75	0.0025
Residual	0.002	7	0.0003		
Lack of fit	0.001	3	0.0004	2.60	0.1895
Pure error	0.0007	4	0.0002		
Total	0.030	16			
Cake residual oil					
Recovery					
Model	26.93	9	2.99	4.58	0.0286
Residual	4.57	7	0.65		
Lack of fit	0.66	3	0.22	0.23	0.8745
Pure error	3.91	4	0.98		
Total	31.51	16			
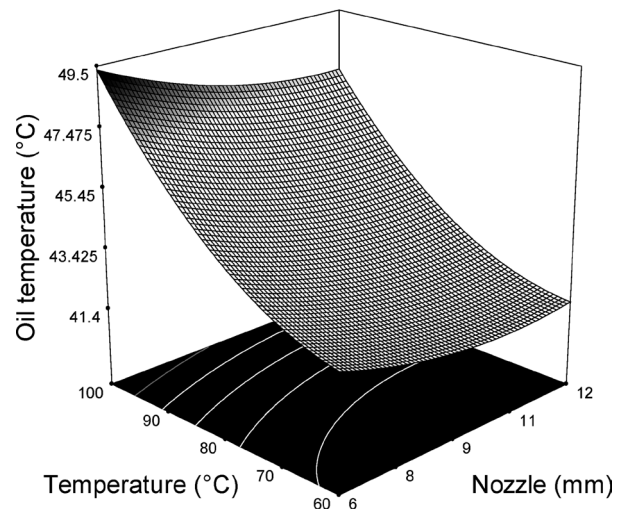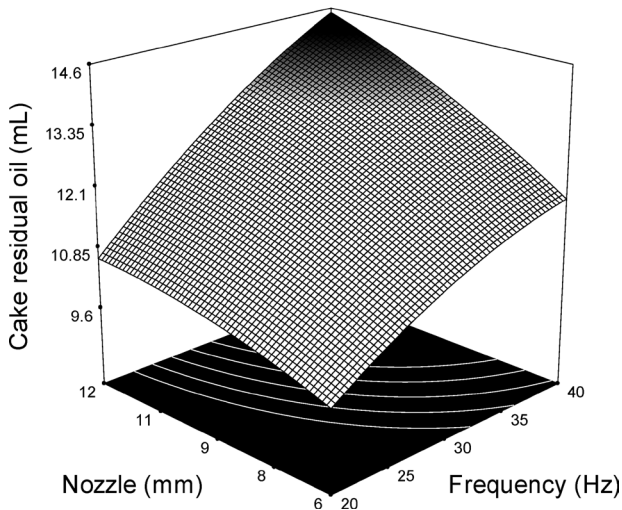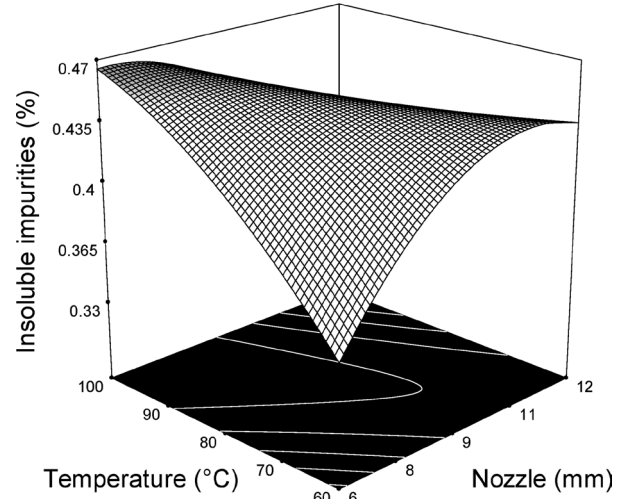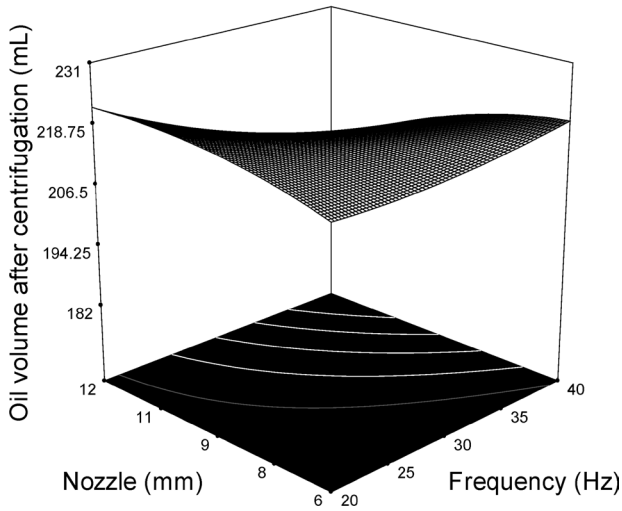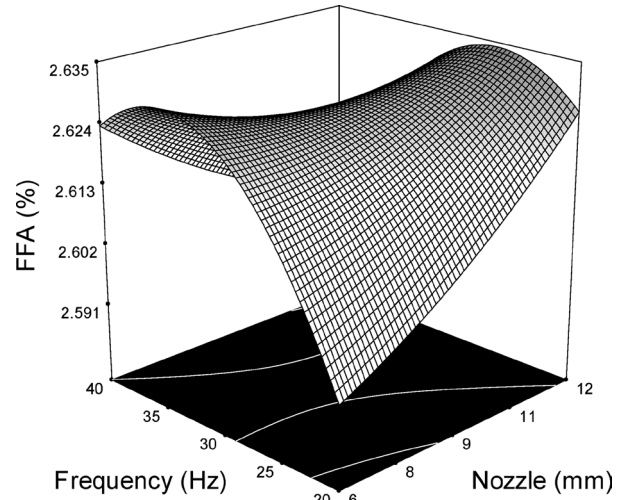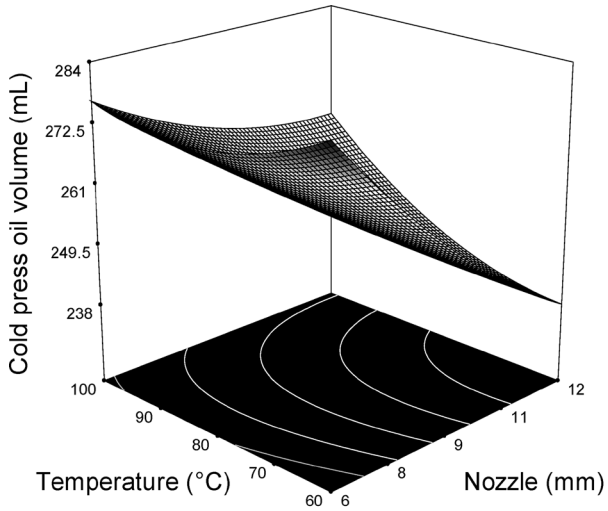
Fig. 2 – *Response surface plots showing the effects of investigated variables on oil recovery*

Fig. 3 – *Response surface plots showing the effects of investigated variables on oil quality*

a model, R^2 should be at least 0.80. In our study, the R^2 values for all investigated response variables were higher than 0.85, indicating the adequacy of the applied regression models. 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 best results were obtained for cold press oil volume (R^2 was 0.973 and *p*-value 0.0001).

The best way to express the effect of cold pressing parameters on oil recovery and oil quality within the investigated experimental range was to generate response surfaces of the model (Figs. 2–3). Fig. 2. shows that the amount of obtained cold press oil significantly decreases with the larger nozzle size. The frequency parameter had a significant effect on the amount of residual oil in the cake. When the lower frequency was used, a smaller amount of oil in cake was measured. Fig. 3. shows the influence of pressing conditions on the quality of the obtained oil. It can be seen that the oil temperature is significantly influenced with increasing temperature of the output press head. Temperature of the obtained oil proportionally increases with temperature of the output press head. FFA increased with increase in nozzle size from 6 to 12 mm, while frequency has a double effect on FFA. Using frequencies from 20 to 30 Hz, the FFA content increased, while a further increase in frequency decreased the FFA. It significantly increased with higher temperature and nozzle size from 6 to 10 mm, while with nozzle size of 10 to 12 mm a decrease in the investigated matter was observed. The ANOVA showed that the models were acceptable and could be used for optimization of the pressing parameters with respect to oil recovery and quality.

Optimization of screw pressing of hemp seed oil

Optimization is an essential tool in food engineering for the efficient operation of different processes to yield a highly acceptable product. During optimization of cold pressing process, several response variables describe the oil quality characteristics and influence on oil recovery. Some of these variables need to be maximized, while others need to be minimized. The goal of this research was to find the best process parameters for the cold pressing of hemp seed oil. Applying response surface methodology, the optimum cold pressing conditions were obtained: temperature of output press head 60 °C, frequency of 20 Hz, and nozzle of ID 6 mm. Cold press oil volume was calculated to be 285.3 mL, oil temperature 42.5 °C, free fatty acids 2.51 %, insoluble impurities 0.39 %, cake residual oil 9.97 %, which is in very close agreement with experimental obtained data.

In the obtained hemp seed oil at calculated optimum cold pressing conditions, the iodine value, saponification value, and moisture content were also determined. Moisture content was determined to be 0.075 %. Iodine value was 155 g I$_2$ 100 g^{-1} oil, and saponification value was 205 mg KOH g^{-1} oil. Kostić et al.[22] published that iodine value in hemp oil was 153 g I$_2$ 100 g^{-1} and saponification number was 189.8, while iodine value and saponification number determined by Borhade[18] in hemp oil was 163.5 g I$_2$ 100 g^{-1} oil and 190.2 mg KOH g^{-1} oil, which is in close agreement with our results. *p*-Anisidine value (AV) in the obtained cold press hemp oil in this study was 1, and Totox value was calculated to be 2.8, indicating that the obtained cold press oil is of good quality because *p*-anisidine value is less than two and Totox value is less than four[17]. Oomah et al.[2] determined *p*-anisidine value at different varieties of hemp oil and AV was from 0.88 to 3.41, and Teh and Birch[19] determined AV of hemp oil to be 0.62.

Oxidative stability of hemp seed oil

Oxidative stability of cold pressed hemp oil with and without the addition of natural antioxidants is shown in Fig. 4. The stability of oil is deter-
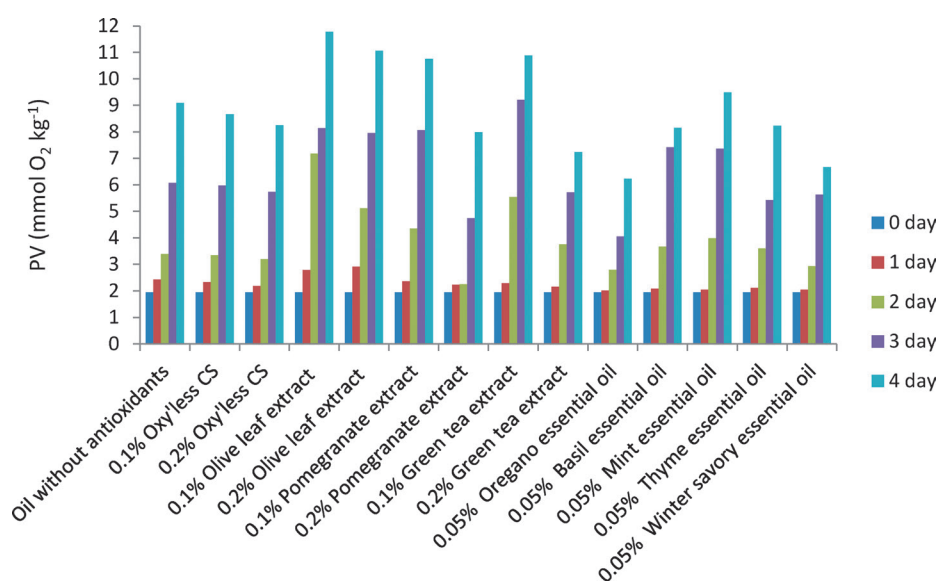

F i g. 4 – *Influence of natural antioxidants on oxidative stability of hemp seed oil*

mined by the accelerated oxidation test, the Schaal oven test (63 °C). Hemp oil without added antioxidants (control sample) after four days of the test had a peroxide value (PV) 9.09 mmol O$_2$ kg^{-1}. The addition of the essential oil of oregano (in concentration of 0.05 %) in hemp oil achieved the best stability of oil to oxidative deterioration (PV 6.23 mmol O$_2$ kg^{-1} after four days) in relation to the use of other investigated natural antioxidants. Winter savory essential oil (0.05 %) effectively protected this oil against oxidative deterioration, PV after four days of the test was 6.67 mmol O$_2$ kg^{-1}. Green tea extract (0.2 %) lead to a decrease in the stability of the hemp oil, in relation to hemp oil with the essential oil of oregano, after 4 days the PV was 7.24 mmol O$_2$ kg^{-1}. The addition of OxyLess CS rosemary extract in concentrations of 0.1 % and 0.2 % did not significantly affect the stability of hemp oil; the value of PV was slightly lower than in the control sample. The stability of hemp oil was further reduced by the addition of antioxidants, respectively, pomegranate extract (0.2 %), basil essential oil (0.05 %) and thyme essential oil (0.05 %), but stability of hemp oil was better than stability of hemp oil with addition of rosemary extract. However, the addition of olive leaf extract (0.1 % and 0.2 %), pomegranate extract (0.1 %), green tea extract (0.1 %) and mint essential oil (0.05 %) did not affect the stability of hemp oil to oxidative deterioration, on the contrary, PV was higher than the control sample after 4 days of the test.

Extraction of residual oil from press cake with CO$_2$

The cake obtained from cold pressing at optimal conditions was subjected to extraction with supercritical CO$_2$ in a newly designed supercritical fluid extraction system (Fig. 1). Residual oil in optimal press cake determined by extraction with *n*-hexane was 10.33 %. Fig. 5 shows that after 3.5 h of extraction, the residual oil from press cake was totally extracted by supercritical CO$_2$. For comparison of the results, the material after supercritical
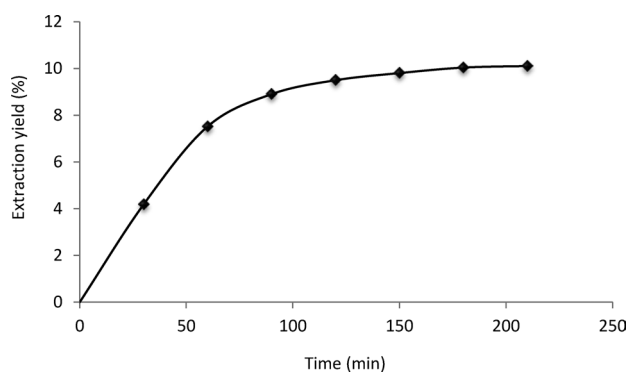
CO$_2$ extraction was subjected to Soxhlet extraction and the obtained results indicated that only 0.39 % of the oil was retained in that material. The PV of oil obtained by cold pressing (CP) at optimal pressing conditions, and oil obtained by supercritical fluid extraction (SFE) was compared. The PV value of oil obtained by supercritical CO$_2$ was slightly higher (2.98 mmol O$_2$ kg^{-1}) than PV obtained in cold press oil. It is assumed that the higher temperature of output press head resulted in the higher temperature of cake, resulting in the raised PV in residual oil cake, which can be attributed to higher temperature of cake. Furthermore, the obtained hemp oil has an intensive green color due to chlorophyll content. Thus, the content of chlorophyll a and chlorophyll b, as well as total carotene content were determined in both oils and compared (Table 5). It can be seen that the hemp oil obtained by supercritical CO$_2$ has three times higher chlorophyll content and four times higher total carotene content compared to cold press oil. Teh and Birch[19] published that chlorophyll content as mg of pheophytin a/kg in cold pressed hemp oil was 75.21.

Table 5 – *Chlorophyll and carotene content of hemp oil*

Hemp oil	Chl *a* (µg g^{-1})	Chl *b* (µg g^{-1})	Chl *a+b* (µg g^{-1})	Car (µg g^{-1})
CP	59.22	39.45	98.60	31.46
SFE	193.50	35.39	228.79	125.37

CP – Cold Pressing; SFE – Supercritical Fluid Extraction

The obtained results show that homemade supercritical fluid extraction system permits the obtaining of extracts in an inexpensive way. Similar conclusions were published by Castro-Vargas et al.[23], where they explained in detail that the obtained extraction yields and composition were very similar to those obtained by commercial supercritical fluid extraction system.

Conclusion

The results of this study suggest that screw press conditions have great influence on oil quality and oil recovery. The quality of produced hemp seed oil was satisfactory, and optimum pressing conditions for obtaining the highest oil recovery and the best oil quality were at temperature 60 ºC, frequency 20 Hz, and nozzle size of ID 6 mm. The influence of different natural antioxidants (in concentration of 0.05 %, 0.1 % and 0.2 %) on the oxidative stability of hemp oil was investigated, and the essential oil of oregano in concentration of 0.05 % was the most efficient in protecting the

F i g . 5 – *Extraction of cake residual oil with CO$_2$ using HM-SFE system*

hemp oil against oxidative deterioration. Supercritical CO_2 extraction of oil from press cake was carried out with a handmade supercritical fluid extraction system, where the oil was extracted almost completely (only 0.39 % of the oil remained in the cake after extraction). Low temperatures of extraction, reduced energy consumption, high product quality, and absence of solvent in extracts are only a few of the numerous advantages that the supercritical fluid extraction technique can provide as opposed to traditional methods of extraction. Because of the oil's high quality, as well as the high content of high-quality components in hemp seed and its oil that positively affect human health, this study is very significant given the small number of published studies on hemp seed oil.

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