Fatty Acid Composition of Oil Obtained from Soybeans by Extraction with Supercritical Carbon Dioxide

Stela JOKIĆ[1], Rezica SUDAR[2], Sandra SVILOVIĆ[3], Senka VIDOVIĆ[4], Mate BILIĆ[1], Darko VELIĆ[1] and Vlatka JURKOVIĆ[2]

[1]Faculty of Food Technology, University of J. J. Strossmayer in Osijek, Osijek, Croatia; [2]Agricultural Institute Osijek, Croatia; [3]Faculty of Chemistry and Technology, University of Split, Split, Croatia; [4]Faculty of Technology, University of Novi Sad, Novi Sad, Serbia

Abstract

Jokić S., Sudar R., Svilović S., Vidović S., Bilić M., Velić D., Jurković V. (2013): **Fatty acid composition of oil obtained from soybeans by extraction with supercritical carbon dioxide**. Czech J. Food Sci., **31**: 116–125.

Soybean oil fractions were obtained by collecting the extract at different time intervals during supercritical carbon dioxide extraction. The extraction was performed at the following temperatures: 313, 323, and 333 K, and at pressures of 300, 400, and 500 bar. The triacylglycerol composition and concentration of fatty acids in soybean oil fractions was evaluated. The fatty acid and triacylglycerol compositions of soybean oil obtained with supercritical carbon dioxide was compared with the soybean oil extracted with *n*-hexane. The extraction temperature and pressure, did not influence the fatty acids compositions which, however, differed in different fractions collected at different time intervals. The concentrations of myristic, palmitic, linoleic, and linolenic fatty acids of soybean oil were the highest in the first fraction and then decreased, while the concentrations of stearic and oleic acids showed the opposite trend. The solubility of all fatty acids increased with the pressure from 300 to 400 bar at constant temperature, while in the interval from 400 to 500 bar the solubility decreased with long chain fatty acids (C20–C24).

Keywords: supercritical carbon dioxide extraction; fractions; soybean oil; triacylglycerols

Supercritical fluid extraction in food processing has received an increased attention because of its advantages over other conventional extraction techniques, such as: a higher diffusion coefficient and a lower viscosity of supercritical fluids than are those of organic solvents; higher mass transfer rates of solutes into supercritical fluids; rapid penetration of supercritical fluids into the matrices pores, which improves the extraction efficiency; and selectivity during the extraction through the manipulation of system pressure and temperature affecting the solubility of various components in supercritical fluids. Furthermore, supercritical carbon dioxide extraction is a promising environmentally friendly, safe, and cost-efficient alternative technique. The operation costs of supercritical carbon dioxide extraction are low, however, due to the applied high pressure the investment costs are high. A further disadvantage of using supercritical solvents might be the solute solubility when compared to organic solvents, because it is less effective (Rozzi & Singh 2002).

Supercritical carbon dioxide extraction is a relatively new technique studied for oilseed processing. Vegetable oils are the vital part of human nutrition. Soybean oil is recognised, among the most common vegetable oils, as oil that contains significant amounts of unsaturated fatty acids: α-linolenic acid known as omega-3 acid, linoleic acid known as omega-6 acid, oleic acid known as omega-9 acid;

and also tocopherols and triacylglycerols as the major components of soybean total lipids (Olguin *et al*. 2003; Bond *et al*. 2005; Nikolić *et al*. 2008).

In recent years, the analysis of fatty acids has gained importance because of their nutritional and health implications (Sahena *et al*. 2009). Fractionation of oil in terms of its fatty acid composition is very important for producing products with physical or nutritional properties of interest to the food industry (Soares *et al*. 2007). Oil of various characteristics could be produced through a simple fractionation process, i.e. just by collecting the oil at various time or mass intervals (Hassan *et al*. 2000). The fractionation effect of supercritical extraction of seed oil is caused by a large disproportion in solubility of triglycerides in supercritical carbon dioxide. Generally, the solubility of triglycerides depends upon the density of carbon dioxide, which can be manipulated through the pressure variation. Snyder *et al*. (1984) found that the fractionation of soybean oil in supercritical carbon dioxide extraction at 550 bar and 50°C only occurred in the last 10–15% of the oil extracted in which the composition of fatty acid in triglycerides varied.

In our earlier papers (Jokić *et al*. 2011a,b), we studied the solubility behaviour of soybean oil and main fatty acids in supercritical carbon dioxide in the pressure range of 100–300 bar. Furthermore, the experimental data were correlated using different empirical equations. In this study, supercritical carbon dioxide was used as a solvent in the extraction of soybean oil in the pressure range of 300–500 bar and temperatures between 313 K to 323 K. The fractionation of soybean oil into various fractions was carried out by collecting the extracted oil at various time intervals. The fatty acid and TAG compositions of each fraction were determined. The solubility behaviour of soybean oil fatty acids in supercritical carbon dioxide under the extraction conditions investigated was correlated using an empirical model.

MATERIAL AND METHODS

Material. Supercritical carbon dioxide extraction was performed on the soybean cultivar Ika created at the Agricultural Institute Osijek in Croatia in 2009. The initial oil content was measured by traditional laboratory Soxhlet-extraction with *n*-hexane. About 30 g of ground soybeans was extracted with about 250 ml solvent, until totally depleted. The whole process took 16 hours. The measurement was done in triplicate. The average oil content in soybeans for three replicates was 20.08 ± 0.14%. The moisture content was determined by oven drying to constant weight at 105°C (AOAC 2000) and expressed in percentage (11.02 ± 0.11%). Reagent-grade *n*-hexane (J.T. Baker, Milan, Italy) was used for laboratory Soxhlet-extraction. Commercial carbon dioxide (Messer, Novi Sad, Serbia) was used for laboratory supercritical fluid extraction. FAME mix C14–C24 (AOCS Standard 3; Restek, USA) was used.

Determination of particle size distribution of ground soybeans by sieving. Soybeans were ground and sieved using a vertical vibratory sieve shaker (Labortechnik GmbH, Ilmenau, Germany) for 20 minutes. About 200 g loading was 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 after sieving was used to calculate the distribution of fragments, which was then normalised in respect of the total mass. For the evaluation of the sieve analysis results, the Rosin-Rammler-Bennet (RRB) distribution (Allen 1981) was chosen. The percentage by mass of particles (R) greater than screen size (d) is given as: missing equation

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

where:

d_0 – particle size corresponding to the 36.8th percentile of the cumulative probability distribution (size constant)

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, minimising the sum of the mean square error using STATISTICA 8.0 software (Stat Soft Inc., USA).

Supercritical carbon dioxide extraction. The experiments were performed on the laboratory-scale high pressure extraction plant (HPEP, NOVA-Swiss, Effertikon, Switzerland) given in detail elsewhere (Jokić *et al*. 2011a,b; Vidović *et al*. 2011). The main plant parts and properties, according to the manufacturers specifications, were: the diaphragm type compressor (with pressure range

up to 1000 bar), extractor with internal volume 200 ml (P_{max} = 700 bar), separator (with internal volume 200 ml, P_{max} = 250 bar), and maximum CO_2 mass flow rate of 5.7 kg/hour.

The ground soybean sample of 120 g was placed into the extractor vessel. The extracts were collected in previously weighed glass tubes. The amounts of extract obtained at regular intervals of time were established by weight using a balance with a precision of ± 0.00001 g. Separator conditions were 15 bar and 298 K.

The extraction process was carried out in different extraction conditions (pressure and temperature) until the extraction yield became constant. Different fractions, depending on the extraction conditions, were obtained by collecting the extract every two hours during the extraction process. At the pressure of 400 bar the extraction process was carried out over a period of 8 h, and for every set of temperature (313, 323, or 333 K) four different fractions were obtained: F1 after 2 h of extraction, F2 after 4 h, F3 after 6 h, and F4 after 8 h of extraction. Similarly, at the pressure of 300 bar and temperature of 313 K, the extraction process proceeded out for 12 h, so six different fractions were prepared (F1–F6). At the pressure of 500 bar and temperature of 313 K, the extraction process was the shortest, 6 h, so three different fractions were prepared (F1, F2, and F3). The conditions under which the fractionation procedures were performed are given in Tables 1 and 2.

Determination of fatty acid composition of soybean oil. The preparation of methyl esters of fatty acids (FAME) was carried out according to the International Standard ISO 5509:2000 – boron trifluoride (BF_3) method (EN ISO 5509:2000). The fatty acid methyl esters were analysed by using a Shimadzu GC-2010 Plus gas chromatography system, equipped with autosampler, oven, flame ionisation detector, and Lab solution software (version 2.32.00). The separation was performed on column Forte GC 30 m length, 0.25 mm inner diameter and film thickness 0.25 µm. The sample volume injected was 1 µl. The operating conditions were: split ratio 30:1, the inlet temperature set at 498 K, the detector temperature set at 553 K, carrier gas was He at a flow rate of 0.8 ml/minutes. The initial oven temperature was 423 K (held for 7 min), and was then increased to 513 K at a rate of 281 K/min, held for 1 min, and finally increased to 523 K at 523 K/min and held at that temperature. Total analysis time was 25 minutes. Fatty acids were separated according to carbon atoms and number of double bonds and were identified by comparing their retention times to standards.

Table 1. Fatty acid composition of soybean oil extracts/fractions obtained by supercritical CO_2 (SC-CO_2) at constant pressure and by soxhlet extraction

SC-CO_2 extraction*		Fatty acids (%)									
T (K)	fraction	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0	C22:1	C24:0
313	F1	0.076^{a}	11.562^{a}	4.141^{a}	21.240^{a}	55.758^{a}	6.314^{a}	0.369^{a}	0.180^{a}	0.297^{a}	0.064^{a}
	F2	0.071^{a}	11.352^{a}	4.347^{a}	21.631^{a}	55.366^{a}	6.259^{a}	0.422^{b}	0.202^{a}	0.283^{a}	0.071^{a}
	F3	0.027^{b}	9.018^{b}	6.752^{b}	25.390^{b}	51.346^{b}	5.268^{b}	0.985^{c}	0.541^{b}	0.501^{b}	0.233^{b}
	F4	0.043^{c}	9.419^{b}	6.800^{b}	24.868^{c}	50.982^{c}	5.209^{b}	1.002^{c}	0.734^{c}	0.592^{c}	0.351^{c}
323	F1	0.075^{a}	11.672^{a}	4.231^{a}	21.472^{a}	55.359^{a}	6.247^{a}	0.378^{a}	0.185^{a}	0.294^{a}	0.067^{a}
	F2	0.073^{a}	11.590^{a}	4.243^{a}	21.478^{a}	55.395^{a}	6.297^{a}	0.412^{b}	0.187^{a}	0.299^{a}	0.067^{a}
	F3	0.042^{c}	10.365^{c}	5.318^{c}	23.282^{d}	53.767^{d}	5.812^{c}	0.610^{d}	0.329^{d}	0.349^{d}	0.124^{d}
	F4	0.037^{c}	9.521^{b}	6.201^{b}	24.470^{c}	52.141^{e}	5.426^{b}	0.872^{e}	0.565^{b}	0.488^{b}	0.290^{e}
333	F1	0.077^{a}	11.802^{a}	4.337^{a}	21.606^{a}	55.166^{a}	6.100^{a}	0.386^{a}	0.192^{a}	0.266^{e}	0.070^{a}
	F2	0.076^{a}	11.727^{a}	4.292^{a}	21.472^{a}	55.458^{a}	6.198^{a}	0.375^{a}	0.182^{a}	0.254^{e}	0.066^{a}
	F3	0.048^{c}	10.539^{c}	4.978^{d}	22.730^{e}	54.527^{f}	6.050^{c}	0.515^{f}	0.275^{e}	0.285^{a}	0.089^{f}
	F4	0.038^{d}	9.306^{b}	6.178^{b}	23.900^{d}	52.463^{e}	5.785^{c}	0.889^{e}	0.639^{f}	0.508^{b}	0.295^{e}
Soxhlet extraction		0.060^{e}	11.083^{a}	4.894^{d}	22.334^{e}	54.350^{f}	6.007^{c}	0.535^{f}	0.298^{e}	0.319^{a}	0.120^{d}

*extraction conditions: P_E = 400 bar, mass flow rate = 0.194 kg/h, d_0 = 0.383 mm; fractions were obtained at different extraction temperature conditions and were collected every two hours – F1 after 2 h; F2 after 4 h; F3 after 6 h; F4 after 8 h; mean values (n = 3) followed by different letters within the same column differ at $P \leq 0.05$, according to Duncan's post-hoc test

Table 2. Fatty acid compositionof soybean oil extracts/fractions obtained by SC-CO_2 at constant temperature

SC-CO_2 extraction*		Fatty acids (%)									
P (bar)	fraction	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0	C22:1	C24:0
300	F1	0.079^{a}	11.740^{a}	4.108^{a}	21.191^{a}	55.788^{a}	6.236^{a}	0.359^{a}	0.178^{a}	0.256^{a}	0.064^{a}
	F2	0.078^{a}	11.697^{a}	4.080^{a}	21.181^{a}	55.757^{a}	6.383^{a}	0.354^{a}	0.171^{a}	0.237^{a}	0.062^{a}
	F3	0.079^{a}	11.722^{a}	4.094^{a}	21.151^{a}	55.788^{a}	6.346^{a}	0.356^{a}	0.170^{a}	0.232^{a}	0.062^{a}
	F4	0.066^{b}	11.531^{a}	4.115^{a}	21.371^{a}	55.883^{a}	6.269^{a}	0.349^{a}	0.169^{a}	0.195^{b}	0.061^{a}
	F5	0.039^{c}	10.465^{b}	4.853^{b}	23.003^{b}	54.699^{b}	5.989^{a}	0.469^{b}	0.213^{b}	0.296^{c}	0.075^{b}
	F6	0.028^{d}	8.995^{c}	6.711^{c}	25.370^{c}	51.460^{c}	5.288^{b}	0.889^{c}	0.552^{c}	0.489^{d}	0.227^{c}
400	F1	0.076^{a}	11.562^{a}	4.141^{a}	21.240^{a}	55.758^{a}	6.314^{a}	0.369^{a}	0.180^{a}	0.297^{c}	0.064^{a}
	F2	0.068^{b}	11.352^{a}	4.347^{a}	21.631^{a}	55.366^{a}	6.259^{a}	0.422^{b}	0.202^{b}	0.283^{c}	0.071^{b}
	F3	0.027^{d}	9.018^{c}	6.752^{c}	25.390^{c}	51.346^{c}	5.268^{b}	0.925^{c}	0.541^{c}	0.501^{d}	0.233^{c}
	F4	0.043^{c}	9.219^{c}	6.800^{c}	24.868^{d}	50.982^{d}	5.209^{b}	1.002^{c}	0.734^{d}	0.592^{e}	0.351^{d}
500	F1	0.074^{a}	11.379^{a}	4.035^{a}	21.048^{a}	56.071^{a}	6.494^{a}	0.379^{a}	0.171^{a}	0.291^{c}	0.058^{a}
	F2	0.067^{b}	11.243^{a}	4.107^{a}	21.351^{a}	55.968^{a}	6.358^{a}	0.388^{a}	0.181^{a}	0.254^{a}	0.063^{a}
	F3	0.037^{c}	10.255^{b}	6.130^{d}	24.863^{d}	51.703^{c}	5.162^{b}	0.745^{d}	0.437^{e}	0.400^{f}	0.170^{e}

*extraction conditions: T_E = 313 K, mass flow rate = 0.194 kg/h, d_0 = 0.383 mm; fractions were obtained at different extraction pressure conditions and were collected every two hours – F1 after 2 h; F2 after 4 h; F3 after 6 h; F4 after 8 h; F5 after 10 h; F6 after 12 h; mean values (n = 3) followed by different letters within the same column differ at $P \leq 0.05$, according to Duncan's post-hoc test

Fatty acids were quantified based on the peak area by the method of area normalisation.

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 $P < 0.05$. The data were expressed as mean values of three replicates.

High Performance Liquid Chromatography (HPLC) analysis of soybean oil Triacylglycerols (TAG) was conducted by the IUPAC method (1986), using a Perkin-Elmer High Performance Liquid Chromatography system series 200 equipped with isocratic pump, refractive index detector, and TotalChrom Navigator (HPLC software). The separation was performed on two serial connected PE Pecosphere C18 columns (83 × 4.6). The analysis was carried out with acetone/acetonitrile (70:30) as the mobile phase. Standard and oil samples (5%) were dissolved in HPLC-grade acetone and 20-µl aliquots were injected onto the column and eluted at a flow rate of 2.5 ml/minute. Furthermore, triacylglycerols were identified by comparing their retention times to standards. The data were expressed as mean values of three replicates.

Solubility data correlation. The solubility of the soybean oil fractions with different fatty acids concentrations was determined using a gravimetric method. Three sets of pressure (300, 400, and 500 bar) were evaluated at the extraction temperature of 313 K. The carbon dioxide mass flow rate ($_{m_f}$) of 0.194 kg/h was sufficient to ensure the saturation while the solubility was no longer flow rate dependent. The collection of oil was done at definite time intervals during the extraction. The amount of oil was measured gravimetrically and the amount of each fatty acid was determined after GC analysis of each extract.

Empirical solubility model proposed in our earlier study (Jokić 2011a) was used for correlating the solubility behaviour of fatty acids in soybean oil:

$$\ln S = c_0 + c_1 P + c_2 P^2 + c_3 PT + c_4 T \quad (2)$$

where:

c_0–c_4 – model constants
P – pressure (bar)
T – temperature (K)

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

$$\text{AARD} = \frac{1}{n} \sum_{i=1}^{n} \left| \frac{S_{exp} - S_{cal}}{S_{exp}} \right| \times 100 \quad (3)$$

where:

S_{exp} – experimental solubility data
S_{cal} – calculated solubility value

RESULTS AND DISCUSSION

Soybean oil was fractionated via supercritical carbon dioxide extraction under different extraction conditions and fatty acids composition of each obtained fraction was determined by GC/FID analysis. Table 1 shows the variation of fatty acid composition in each fraction of soybean oil collected every two hours during the extraction process at the temperature interval from 313–333 K and constant pressure of 400 bar. Fatty acid profiles of soybean oil extracted by *n*-hexane were also analysed and are presented in the same table. Fatty acid composition is a major determinant of oil quality. Good quality of oil mainly refers to high percentages of unsaturated fatty acids, usually oleic and linoleic acids. The main fatty acids of the soybean oil obtained by supercritical carbon dioxide were: saturated palmitic and stearic acids, and unsaturated oleic, linoleic, and linolenic acid.

The results indicate that the soybean oil is rich in polyunsaturated fatty acids (PUFA). The most abundant unsaturated fatty acid in soybean oil was linoleic acid, in amounts higher than 50% in all the fractions analysed. Oleic acid, belonging to monounsaturated fatty acids (MUFA), was the second most abundant unsaturated fatty acid in soybean oil, amounting from around 21% to around 25%. According to the data obtained, the third unsaturated fatty acid, and that is very important, was polyunsaturated linolenic acid, in amounts from around 5% to around 6%. The most dominant saturated fatty acid was palmitic acid. In all soybean oil fractions analysed, no fatty acid with the chain length shorter than C14 and no fatty acids with chain length longer than C24 were detected.

The represented results (Table 1) show that there were statistically significant differences (ANOVA, Duncan's post-hoc test $P < 0.05$) in the fatty acids compositions between the obtained soybean oil fractions during different extraction time collection intervals. Higher concentrations of unsaturated fatty acids (linoleic and linolenic acids) as well as of saturated palmitic acid were obtained in earlier fractions, while the concentrations of oleic and stearic acids increased from the first to the last fractions. Long chain fatty acids (C20–C24), present in small amount, increased from fraction F1–F4. The variation of the fatty acids concentrations in the fraction obtained after first two hours of extraction at various temperatures and at the pressure of 400 bar was not significant and remained almost stable.

Table 2 shows the variation in fatty acid composition in each fraction of soybean oil collected every two hours during the extraction process at the pressure interval from 300–500 bars at constant temperature of 313 K. With regard to the extraction pressure, different effects on the total extraction time were observed and, consequently, different numbers of fractions were collected. The extraction process at 300 bar lasted 12 h until the last extraction period, was achieved, where the mass transfer occurred mainly by diffusion in the bed and inside the solid substratum particles. The extraction process carried out at 500 bar lasted 6 hours. Furthermore, it is a well known fact that with the pressure increase, the amount of extracted oil increases. This phenomenom is caused by the enhancement of the solvent density with the extraction pressure. Similar results were published by other researchers (Louli *et al*. 2004; Wang *et al*. 2007; Rubio-Rodríguez *et al*. 2008). Comparing the fatty acids profiles in the obtained soybean oil fractions at different temperature intervals (313–333 K) and different pressure intervals (300–500 bar), it can be concluded that the temperature had a greater statistically significant influence on the fatty acid composition compared to that of the pressure. Furthermore, the last fractions obtained at different pressures showed, highly statistically significant differences compared to the first collected oil fractions. A similar trend in the concentration of fatty acids from the first to the last fractions was observed both in Tables 1 and 2, regardless of whether it was due to changes in temperature or pressure during the extraction process. Higher concentrations of linoleic, linolenic, and palmitic acids were obtained in earlier fractions, while the concentrations of oleic and stearic acids were higher in later fractions. The same trend was observed for all pressures investigated.

Fattori *et al*. (1987) fractionated the lipids in canola seed extracts using supercritical carbon dioxide and reported that later fractions were richer in C22 and C24 fatty acids than the earlier fractions. Ragunath *et al*. (1993) studied the fractionation of fatty oil constituents of C6, C12, C16, and C18:1 using supercritical carbon dioxide at 40–80°C and 300 bar. The authors reported that the solubility of the components increased with the pressure while at a constant pressure the solubility decreased as the carbon number increased. Fatouh *et al*. (2007) showed that supercritical carbon dioxide extraction

was a useful tool for producing buffalo butter oil fractions that differed extremely in their properties. Fractionation was performed at 50 and 70°C over a pressure range of 109–401 bar. Short chain fatty acids (C4–C8), medium chain fatty acids (C10–C14), and saturated fatty acids decreased from the first to the last fractions, while long chain fatty acids (C16–C18:3) and unsaturated fatty acids increased. Hassan *et al*. (2000) showed the variation of fatty acids composition in each fraction of palm kernel oil collected in the course of extraction at 345 bar and 70°C. The percentages of C8, C10, and C12 fatty acids decreased with time, while the percentages of C16, C18:0, C18:1, and C18:2 fatty acids increased with time. The percentage of C14 fatty acid remained unchanged. Zaidul *et al*. (2006) reported that supercritical carbon dioxide can be applied to fractionate palm kernel oil to decrease C8–C14 levels and concentrate C16–C18:2 fatty acid constituents.

As concerns the solubility, the comparison of the experimental and correlated solubility data of soybean oil fatty acids in supercritical carbon dioxide by means of the empirical equation proposed in our earlier study (Jokič *et al*. 2011a) at

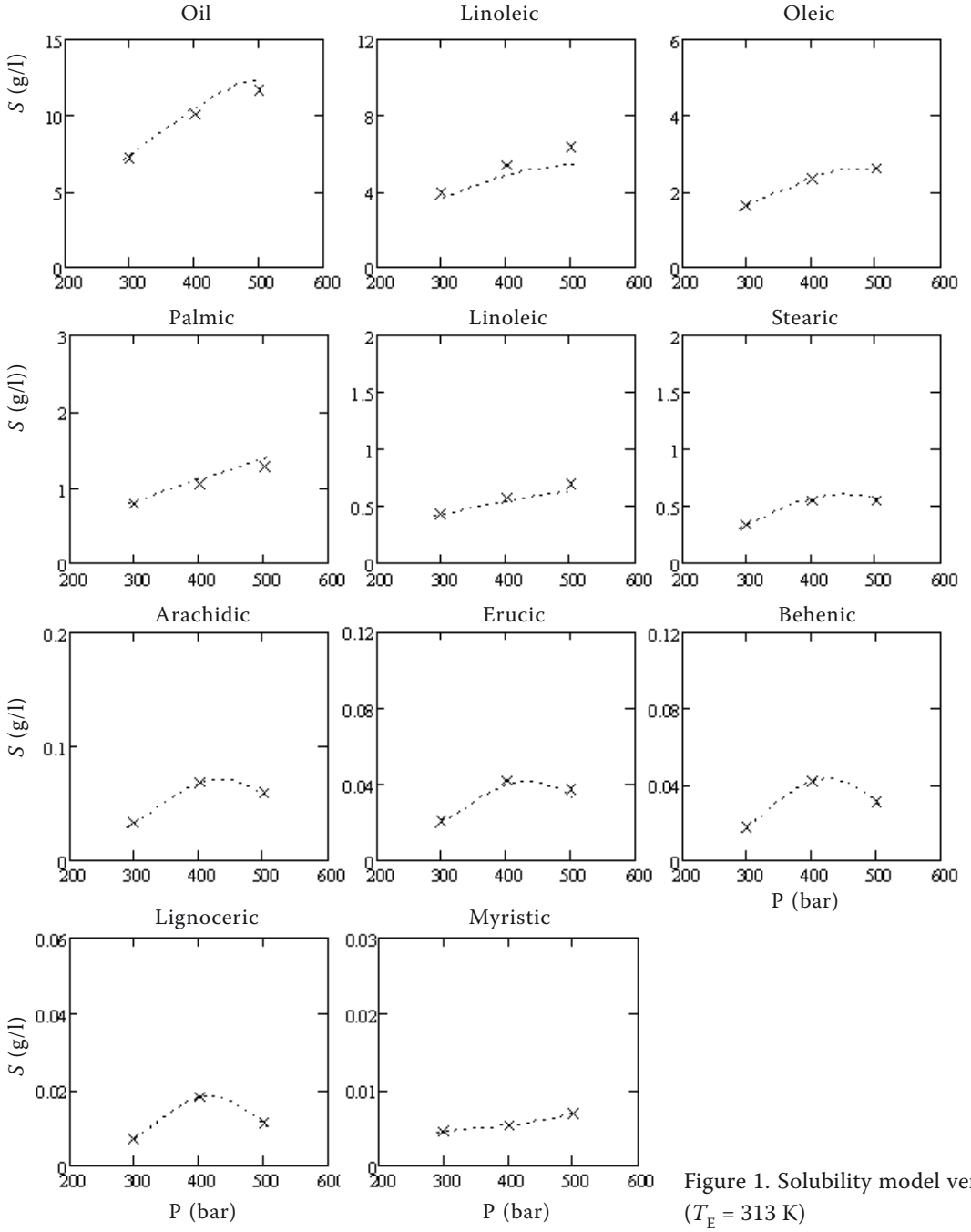

Figure 1. Solubility model verification (T_E = 313 K)

Table 3. Calculated model parameters and deviations

Model parameters	Oil	Linoleic	Oleic	Palmitic	Stearic	Linolenic	Arachidic	Erucic	Behenic	Myristic	Lignoceric
c_0	13.0678	10.99939	10.57168	5.166162	3.535815	5.314862	0.593809	4.392	1.471172	3.829032	0.923839
c_1	0.00883	0.000028	0.003804	0.002745	0.009719	–0.00494	0.030769	0.003489	0.026885	-2.18×10^{-6}	0.033982
c_2	–0.000009	–0.000007	–0.000013	–0.000003	–0.000025	–0.00001	–0.000043	–0.00004	–0.00006	2.17×10^{-6}	–0.000069
c_3	0.000003	0.000024	2.98×10^{5}	0.000007	0.000041	0.000035	0.000021	0.000105	0.000074	1.31×10^{-6}	0.000075
c_4	–0.04213	–0.036179	–0.041016	–0.020962	–0.029107	–0.02402	–0.036231	–0.04937	–0.04862	–0.03050	–0.053942
AARD (%)	4.56	10.27	0.0007	6.55	3.9	5.17	0.95	6.79	1.57	0.048	0.22

Extraction conditions: T_E = 313 K

300, 400, and 500 bar and 313 K is given in Figure 1. It can be seen that the solubility of all fatty acids increased with the pressure from 300 to 400 bar at a constant temperature, while in the interval from 400 to 500 bar the solubility of long chain fatty acids (C20–C24) decreased. Therefore, it can be seen that the correlated solubility matched the experimental values very well for all the examined experimental ranges. It could be also noted that linoleic and oleic acids were more soluble then stearic and palmitic acids, due to the presence of double bonds in their structure.

The constants for the empirical equation (Eq. 2) are given in Table 3 with their average absolute relative deviations (AARD) for all pressure ranges at temperature 313 K. The parameters and the units used in the regressions were solubility in g/l, temperature in K, and pressure in bar in all the equations. It can be seen in Table 3 that the selected empirical model proposed in our earlier study (Jokić 2011a) fitted well the experimental data according to the calculated AARD values. According to the AARD values for all fatty acids (from 0.0007% for oleic acid to 10.27% for linoleic

Table 4. Main triacylglycerols composition of soybean oil extracts/fractions obtained by SC-CO_2 at constant pressure and by soxhlet extraction

SC-CO_2 extraction*		Triacylglycerols (%)						
T (K)	fraction	LnLL	LLL	LLO	LLP	LOO	LOP	LOS
313	F1	7.25^a	22.99^a	16.04^a	15.39^a	7.22^a	12.07^a	5.46^a
	F2	7.32^a	22.65^a	16.35^a	15.27^a	7.65^a	12.38^a	5.87^a
	F3	6.91^b	22.72^a	16.50^b	14.64^b	7.85^b	12.24^a	6.34^b
	F4	5.52^c	19.95^b	17.07^c	12.43^c	9.15^c	13.96^b	7.88^c
323	F1	7.04^a	22.94^a	16.19^a	15.53^a	7.35^a	12.30^a	5.92^a
	F2	7.19^a	23.13^a	16.17^a	15.61^a	7.35^a	12.32^a	5.77^a
	F3	6.41^b	21.28^c	16.63^b	13.56^d	8.16^d	13.11^c	6.75^b
	F4	5.84^c	20.14^b	16.81^c	12.23^c	8.79^c	13.24^c	7.80^c
333	F1	6.81^b	22.17^d	16.07^a	15.50^a	7.25^a	12.32^a	5.73^a
	F2	6.78^b	22.26^d	15.96^a	15.44^a	7.25^a	12.34^a	5.73^a
	F3	6.89^b	22.37^d	16.02^a	15.25^a	7.31^a	12.21^a	6.11^b
	F4	6.75^b	22.37^d	16.15^a	14.18^e	7.98^b	12.79^a	6.46^b
Soxhlet extraction		6.60^b	22.26^d	16.27^a	14.67^b	7.71^b	12.52^a	6.29^b

*extraction conditions: P_E = 400 bar, mass flow rate = 0.194 kg/h, d_0 = 0.383 mm; fractions were obtained at different extraction temperature conditions and were collected every two hours – F1 after 2 h; F2 after 4 h; F3 after 6 h; F4 after 8 h; mean values (n = 3) followed by different letters within the same column differ at $P \leq 0.05$, according to Duncan's post-hoc test; LnLL – linolenodilinolein; LLL – trilinolein; LLO – dilinoleoolein; LLP – dilinoleopalmitin; LOP – linoleooleopalmitin; LOS – linoleooleostearin

Table 5. Main triacylglycerols composition of soybean oil extracts/fractions obtained by SC-CO_2 at constant temperature

SC-CO_2 extraction*		Triacylglycerols (%)						
P (bar)	fraction	LnLL	LLL	LLO	LLP	LOO	LOP	LOS
300	F1	7.05^{a}	22.97^{a}	15.22^{a}	16.22^{a}	6.49^{a}	12.51^{a}	5.57^{a}
	F2	7.15^{a}	22.92^{a}	14.94^{b}	16.48^{a}	6.35^{a}	12.56^{a}	5.51^{a}
	F3	7.12^{a}	22.98^{a}	14.88^{b}	16.61^{a}	6.29^{a}	12.57^{a}	5.52^{a}
	F4	7.11^{a}	23.20^{b}	15.07^{a}	16.01^{b}	6.37^{a}	12.71^{a}	5.57^{a}
	F5	6.37^{b}	22.37^{b}	16.17^{c}	14.63^{c}	7.19^{b}	13.92^{b}	6.47^{b}
	F6	5.49^{c}	19.95^{c}	16.36^{c}	12.67^{d}	8.19^{c}	14.75^{c}	8.43^{c}
400	F1	7.25^{a}	22.99^{a}	16.04^{c}	15.39^{e}	7.22^{b}	12.07^{d}	5.46^{a}
	F2	7.32^{a}	22.65^{a}	16.35^{c}	15.27^{e}	7.65^{e}	12.38^{d}	5.87^{a}
	F3	6.91^{b}	22.72^{a}	16.50^{d}	14.64^{c}	7.85^{e}	12.24^{d}	6.34^{b}
	F4	5.52^{c}	19.95^{c}	17.07^{e}	12.43^{d}	9.15^{f}	13.96^{b}	7.88^{d}
500	F1	7.57^{a}	23.62^{a}	16.33^{c}	15.43^{e}	7.28^{e}	11.94^{e}	5.44^{a}
	F2	7.49^{a}	23.46^{b}	16.05^{c}	15.25^{e}	7.24^{b}	11.82^{e}	5.46^{a}
	F3	5.37^{c}	19.55^{d}	16.89^{e}	12.92^{d}	9.08^{f}	14.16^{b}	8.16^{d}

*extraction conditions: T_E = 313 K, mass flow rate = 0.194 kg/h, d_0 = 0.383 mm; fractions were obtained at different extraction pressure conditions and were collected every two hours – F1 after 2 h; F2 after 4 h; F3 after 6 h; F4 after 8 h; F5 after 10 h; F6 after 12 h; mean values (n = 3) followed by different letters within the same column differ at $P \leq 0.05$, according to Duncan's post-hoc test; LnLL – linolenodilinolein; LLL – trilinolein; LLO – dilinoleoolein; LLP – dilinoleopalmitin; LOP – linoleooleopalmitin; LOS – linoleooleostearin

acid), a very good agreement between the calculated and the experimental solubility data for fatty acids can be observed.

The analysis of triacylglycerols composition (TAG) of soybean oil extracted by *n*-hexane and supercritical carbon dioxide was performed using reversed phase high performance liquid chromatography. The application of this method resulted in a successful separation of triacylglycerols in 15 min, with a very simple sample preparation (Sudar *et al*. 2003). The chromatogram of each injected sample showed 17 individual triacylglycerol peaks and their concentrations were calculated from the peak areas. The major TAGs were LLL (trilinolein), LLO (dilinoleoolein), LLP (dilinoleopalmitin), and LOP (linoleooleopalmitin). Jokić *et al*. (2010) reported that in soybean oil obtained by supercritical CO_2 higher contents were obtained of triacylglycerols with unsaturated fatty acids (linoleic and linolenic acid), while the extraction with organic solvent resulted in increased contents of triacylglycerols with saturated fatty acids (palmitic and stearic acid). This is associated with the solubility of certain components in supercritical carbon dioxide, and can be explained by the fact that TAGs with higher unsaturation dissolve better in supercritical carbon dioxide than TAGs with lower unsaturation, or that the lighter TAGs are more soluble than the heavier ones (Davarnejad *et al*. 2008). The influence of temperature on the TAG composition of oil fractions during different extraction time collection intervals is given in Table 4. It can be seen that the major TAG was LLL (19.95–22.37%), followed by LLO (15.96–17.07%), LLP (12.23–15.52%) and LOP (12.07–13.96%). The contents of LnLnLn, LnLnL, LnLnO, LnLnP, LnLO, PLnP, OOO, OOP, OOS, and SOP were relatively low in all extracts analysed (less than 4%). The content of LnLL (linolenodilinolein) varied from 5.52% to 7.32%, LOO from 7.22% to 9.15%, and LOS (linoleooleostearin) from 5.46% to 7.88%. The results given in Table 4 confirm the results obtained in Table 1 which shows the fatty acid compositions in the extracts obtained at different extraction temperatures and collected during the extraction. Higher concentrations of linoleic, linolenic, and palmitic acids were obtained in previously collected fractions, whereas the concentrations of oleic and stearic acids increased in the fractions collected later. A similar trend in

the composition of triacylglycerols from the first to the last collected fractions of soybean oil can be seen in Table 5 which shows the influence of extraction pressure on the TAG composition in the collected fractions as follows: LLL (18.48–23.62%), LLO (14.88–17.07%), LLP (12.43–16.61%) and LOP (11.82–15.11%). Similar data for soybean oil triacylglycerols composition was reported by Neff *et al*. (1992), Holčapek *et al*. (2003), Sudar *et al*. (2003), with specific differences due to the use of different soybean cultivars.

CONCLUSIONS

The goal of food research has usually been to increase the yield and productive efficiency, but it should be also more focused on improving the nutrient profile of food products. The present study has shown that supercritical carbon dioxide can be used to fractionate soybean oil to decrease palmitic, linoleic and linolenic fatty acids concentrations and to increase stearic and oleic acids concentrations. The solubility data for fatty acids of soybean oil were successfully correlated by the empirical model used. The average absolute relative deviation (AARD) ranged from 0.0007% to 10.27% for the applied extraction conditions. Supercritical fluid extraction as an environmentally friendly method can be an alternative method to the conventional extraction of fatty acids.

Acknowledgement. The authors greatly acknowledge Agricultural Institute Osijek for financial support of this research.

References

Allen T. (1981): Particle Size Measurement. 3rd Ed. Chapman and Hall, London.

AOAC (2000): Official Methods of Analysis. No. 925.40. 17th Ed. Association of Official Analytical Chemists, Washington.

Bond B., Fernandez D.R., Vanderjagt D.J., Williams M., Huang Y.S., Chuang L.T., Millson M., Glew R.H. (2005): Fatty acid, amino acid and trace mineral analysis of three complementary foods from Jos, Nigeria. Journal of Food Composition and Analysis, **18**: 675–681.

Davarnejad R., Kassim K.M., Zainal A., Sata S.A. (2008): Phase equilibrium study of triacylglycerols from crude palm oil using CO_2. World Applied Sciences Journal, **5**: 68–75.

EN ISO 5509 (2000): Animal and vegetable fats and oils – Preparation of methyl esters of fatty acids. European Committee for Standardization, Brussels.

Fatouh A.E., Mahran G.A., El-Ghandour M.A., Singh R.K. (2007): Fractionation of buffalo butter oil by supercritical carbon dioxide. LWT-Food Science and Technology, **40**: 1687–1693.

Fattori M., Bulley N.R., Meisen A. (1987): Fatty acid and phosphorus content of canola seed extracts obtained with supercritical carbon dioxide. Journal of Agricultural and Food Chemistry, **35**: 739–743.

Hassan M.N., Rahman N.N.A., Ibrahim M.H., Omar A.K.M. (2000): Simple fractionation through the supercritical carbon dioxide extraction of palm kernel oil. Separation and Purification Technology, **19**: 113–120.

Holčapek M., Jandera P., Zderadička P., Hruba L. (2003): Characterization of triacylglycerol and diacylglycerol composition of plant oils using high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. Journal of Chromatography A, **1010**: 195–215.

IUPAC (1986): Standard Methods for the Analysis of Oils, Fats and Derivatives. No. 2.325. 7th Ed. Blackwell Scientific Publications, Oxford.

Jokić S., Zeković Z., Vidović S., Sudar R., Nemet I., Bilić M., Velić D. (2010): Supercritical CO_2 extraction of soybean oil: process optimisation and triacylglycerol composition. International Journal of Food Science and Technology, **45**: 1939–1946.

Jokić S., Svilović S., Zeković Z., Vidović S., Velić D. (2011a): Solubility and kinetics of soybean oil and fatty acids in supercritical CO_2. European Journal of Lipid Science and Technology, **113**: 644–651.

Jokić S., Svilović S., Zeković Z., Vidović S. (2011b): Mathematical modelling of soybean oil solubility in supercritical carbon dioxide. International Journal of Food Science & Technology, **46**: 1031–1037.

Louli V., Folas G., Voutsas E., Magoulas K. (2004): Extraction of parsley seed oil by supercritical CO_2. The Journal of Supercritical Fluids, **30**: 163–174.

Nikolić N., Stanković M., Todorović Z., Lazić M., Nikolić G. (2008): HPLC analysis of whole soybean (*Glycine max* L.) seed oil. Acta Agriculturae Serbica, **25**: 3–8.

Neff W.E., Selke E., Mounts T.L., Rinsch W., Frankel E.N., Zeitoun M.A.M. (1992): Effect of triacylglycerol composition and structures on oxidative stability of oils from selected soybean germplasm. Journal of the American Oil Chemists' Society, **69**: 111–118.

Olguin M.C., Hisano N., D'Ottavio E.A., Zingale M.I., Revelant G.C., Calderari S.A. (2003): Nutritional and antinutritional aspect of an Argentinean soy flour

assessed on weanling rats. Journal of Food Composition and Analysis, **16**: 441–448.

Ragunath B., Yamane S., Inomata H., Adschiri T., Arai K. (1993): Phase equilibria of supercritical CO_2 fatty oil component binary systems. Fluid Phase Equilibria, **83**: 183–192.

Rozzi N.L., Singh R.K. (2002): Supercritical fluids and the food industry. Comprehensive Reviews in Food Science and Food Safety, **1**: 33–44.

Rubio-Rodríguez N., De Diego S.M., Beltrán S., Jaime I., Sanz M.T., Rovira J. (2008): Supercritical fluid extraction of the omega-3 rich oil contained in hake (*Merluccius capensis–Merluccius paradoxus*) by-products: Study of the influence of process parameters on the extraction yield and oil quality. The Journal of Supercritical Fluids, **47**: 215–226.

Sahena F., Zaidul I.S.M., Jinap S., Karim A.A., Abbas K.A., Norulaini N.A.N., Omar A.K.M. (2009): Application of supercritical CO_2 in lipid extraction – A review. Journal of Food Engineering, **95**: 240–253.

Snyder J.M., Rossell J.M., Friedrich J.P., Christianson D.D. (1984): Effect of moisture and particle size on the extractability of oils from seeds with supercritical CO_2. Journal of the American Oil Chemists' Society, **61**: 1851–1856.

Soares B.M.C., Gamarra F.M.C., Paviani L.C., Goncalves L.A.G., Cabral F.A. (2007): Solubility of triacylglycerols in supercritical carbon dioxide. The Journal of Supercritical Fluids, **43**: 25–31.

Sudar R., Jurković Z., Vratarić M., Sudarić A., Duvnjak T. (2003): Triacylglycerols composition of oil in OS soybean cultivars. European Food Research and Technology, **217**: 115–119.

Vidović S., Mujić I., Zeković Z., Lepojević Ž., Milošević S., Jokić S. (2011): Extraction of fatty acids from *Boletus edulis* by subcritical and supercritical carbon dioxide. Journal of the American Oil Chemists' Society, **88**: 1189–1196.

Wang H., Suo Y., Wang X., Li Y., You J., Zhao X. (2007): Extraction of *Nitraria tangutorum* seed oil by supercritical carbon dioxide and determination of free fatty acids by HPLC/APCI/MS with fluorescence detection. Separation and Purification Technology, **56**: 371–377.

Zaidul I.S.M., Norulaini N.N.A., Omar A.K.M., Smith Jr. R.L. (2006): Supercritical carbon dioxide (SC-CO_2) extraction and fractionation of palm kernel oil from palm kernel as cocoa butter replacers blend. Journal of Food Engineering, **73**: 210–216.

Received for publication January 14, 2012
Accepted after corrections August 2, 2012

Corresponding author

Dr Stela Jokić, University of J. J. Strossmayer in Osijek, Faculty of Food Technology, F. Kuhaca 20, 31000 Osijek, Croatia; E-mail: stela.jokic@ptfos.hr