Macedonian Journal of Chemistry and Chemical Engineering, Vol. **28**, No. 2, pp. 173–179 (2009)
MJCCA9 – 545
ISSN 1857 – 5552
Received: November 13, 2008
UDC: 665.335.2.061.3
Accepted: June 2, 2009

Original scientific paper

EFFECT OF EXTRACTION TECHNIQUES ON YIELD AND COMPOSITION OF SOYBEAN OIL

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This paper investigated the yield and chemical composition of soy seeds oils obtained by different solvents and by different extraction apparatuses with the aim to compare oils yield and composition. The maximal oil yield with trichloroethylene was achieved by the Soxhlet apparatus after 150 minutes of extraction (21.4 g/100 g) and very satisfactory yield of 20.4 g/100 g, with similar chemical composition, was achieved by reflux, only after 60 minutes. During the Tilepape extraction, the contact of oil with air was continuous and more intensive oxidation reactions occurred and γ-linolenic and linolenic acids were not detected. Between some oil fatty acids and some chemical values, the correlation coefficients were performed by the program STATISTICA version 5.0. The results showed a proper correlation between the iodine value and the oleic acid content as well as between the peroxide value and the linoleic acid content.

Key words: soy; oil, extraction; yield; composition

ПРИНОС И СОСТАВ НА СОИНОТО МАСЛО ДОБИЕНО СО РАЗЛИЧНИ ТЕХНИКИ НА ЕКСТРАКЦИЈА

Испитано е влијанието на различни техники на екстракција: рефлукс, Soxhlet и Tilepape, како и влијанието на растворувачите: трихлоретилен, хлороформ, јаглероден тетрахлорид, *n*-хексан и петролетер врз приносот и составот на маслото од соиното семе. Врз база на добиените резултати е заклучено дека максимален принос (21,4 g/100 g) на масло од соиното семе се добива по 150 минути екстракција со примена на Soxhlet-ова екстракција. Со примена на рефлукс-екстракција се добива незначително помал принос од 20.4 g/100 g за пократко време (по 60 минути), а добиеното масло според хемискиот состав е слично на маслото добиено со Soxhlet-ова екстракција. Во текот на Tilepape-екстракцијата реакциите на оксидација се поинтензивни и ова е веројатно причината зошто γ-линоленска и линоленска киселина не се детектирани во маслото добиено со Tilepape-екстракција. Резултатите од статистичката анализа покажуваат директна зависност меѓу содржината на олеинската киселина и јодниот број и меѓу содржината на линоленската киселина и пероксидниот број.

Клучни зборови: соја; масло; екстракција; принос; состав

1. INTRODUCTION

The soybean oil is one of the common vegetable oils that contains a significant amount of unsaturated acids: α-linolenic acid, known as omega-3 acid, linoleic, γ-linolenic and arachidonic acid, known as omega-6, oleic acid known as omega-9 acid, very important in the human nutrition [1–3], saturated acids: palmitic and stearic acid [4], as well as tocopherols and β-carotene [5–7]. The soybean oil is industrially used in the manufacture of paint, oilcloth, printing ink, soap, insecticides and disinfectans, while lecithin and phospolipid, being obtained as by-products, are used as a wetting and stabilizing agent in food cosmetics, pharmaceuthicals, leather, paint, plastic, soap, and detergent industry [8–11]. The Soxhlet extraction with *n*-hexane as a solvent, is a well known method used for oil extraction from different plant seeds [12, 13]. Luque-García and Luque de Castro (2004) [14] developed the Soxhlet extraction assisted in the cardridge by ultrasound to extract oil from oleaginous seeds such as sunflower, rape and soybeen. There are reports that the Soxhlet extraction causes damage to lipids in corn-soy blends and only about half of the present oil was recovered [15]. To the best of our knowledge, the efficiency of reflux and the Tilepape extraction is not investigated yet, and there are no referent data. The purpose of this paper is to investigate yield of soybean seeds oils obtained by different solvents: trichloroethylene, chloroform, carbon tetrachloride, *n*-hexane and petrol ether and to investigate and compare the yield and the chemical composition by different extraction apparatuses: reflux, the Soxhlet and the Tilepape. The chemical composition was performed determining the content of free fatty acids (FFA), mono- (MAG) , di- (DAG) and tri-acilglycerols (TAG) by the HPLC method, the fatty acids composition by GC method and some chemical values. The differences are that during the Soxhlet extraction, the plant material is soaked by the solvent and after certain time the extract overflows in the flask, and during the Tilepape extraction the plant material is continually washed out by the hot solvent condensate which distills from the condenser and the extract is continually overflowing into the flask, without previous soaking of the plant material. The solvent is evaporating from the flask, going out through the condenser, condensating and washing the plant material again and again. In the reflux extraction, the plant material and the solvent are mixed and heated together into the same flask.

2. EXPERIMENTAL

Plant material. Soybean seeds (*Glycine max* L.) were purchased in the local store and milled to the overal particle size of 0.4 mm.

The soybean seeds oil content. The milled soybean seeds (30 g) were put into an Erlenmeyer flask, 300 ml of trichloroethylene was added and extracted for 30 minutes, under reflux and by mixing (200 min^{-1}) at solvent boiling temperature. The extract was separated by using the Buchner funnel under weak vacuum. The plant material was extracted three more times by the same method, the extracts were mixed together and eluted by water in the separation funnel (3 × 30 ml). The eluted extract volume was recorded and an aliquote (3 ml) was taken for the dry residue determination test in order to determine the oil content in soybean seeds.

Dry residue content. Oil extract (3 ml) was put into the disk plate analyzer (Scaltec SMO 01, Scaltec instruments, Germany), poured and dried at 110 ^{o}C to a constant weight. The content of the dry residue was read out on the display.

Oil extraction by different solvents. The soybean seeds (10 g) were extracted by maceration under reflux and mixing (200 min^{-1}) with trichloroethylene, chloroform, carbon tetrachloride, *n*-hexane and petrol ether, at the plant material to the solvent ratio of 1:10 w/v, at solvent boiling temperature, for periods of 10, 15, 30, 60 and 90 minutes. Separate samples were taken for individual extraction periods. The extract was separated by filtration under vacuum and an aliquote of the extract (3 ml) was taken for the dry residue determination test.

Oil extraction by different techniques. The oil from soybean seeds (10 g) was extracted by a trichloroethylene at the plant material to the solvent ratio of 1:10 w/v and at solvent boiling temperature. Standard apparatuses for reflux, the Soxhlet and the Tilepape extraction were used. After extraction, the solvent was evaporated under vacuum, when the oil residue remained while the reflux extract, after the extraction was separated by filtration under vacuum, and then evaporated. An aliquote of the extract obtained by the reflux, the Soxhlet or the Tilepape extract (3 ml) was taken for the dry residue determination test. The experiments were performed in triplicate.

IR analysis. The IR spectra were recorded at the room temperature on the Bommem MB-100 (Hartman & Brunn) Michelson FTIR spectrometer by the method of a thinlayer film between the KBr cell, with resolution of 2 cm^{-1} and the wave number range of 400–4000 nm.

HPLC analysis. For the HPLC analysis, Holčapek *et al.* (1999) [16] modified HPLC method was used. The equipment consisted of an Agilent 1100 High Performance Liquid Chromatograph, equipped with a degasser, a binary pump, a Zorbax Eclipse

XDB-C18 column (4.4 m × 150 mm × 5 μm) and an UV/ViS detector. The flow rate of the binary solvent mixture (methanol, solvent A, and 2-propanol/*n*-hexane, 5:4 by volume, solvent B) was 1 ml/min with a linear gradient (from 100% A to 40% A+ 60% B in 15 min). The column temperature was held constant at 40 °C. The components were detected at 205 nm.

The mono-, di- and tri-acilglycerols were identified comparing retention times of the lipid components to retention times of standards. The samples of the reaction mixture were dissolved into a mixture of 2-propanol : *n*-hexane (5:4 v/v) and filtered through 0.45 μm Millipore filters.

GC analysis. For the GC analysis, fatty acids, methyl esters were prepared and the HP 5890 Series II Gas Chromatograph, HP with FID detector, and integrator 3396 A HP were used. The column was ULTRA 2 (25 m × 0.32 mm × 0.52 μm), with the injector temperature of 320 °C, and the injector volume of 0.4 μl. The carrier gas was helium at a constant flow rate of 1 ml/min.

The flame ionisation detector was at 350 °C and the split ratio was 1:20. The oven temperature was initially 120 ⁰C and was maintained at 120 °C, for 1 min, then increased by 15 °C/min until 200 °C, increased by 3 °C/min until 240 °C, increased by 8 °C/min until 300 °C and maintained at 300 °C for 15 min. Fatty acids were identified comparing the retention times of the lipid components with retention times of standards.

*Chemical properties***.** Refractive index, acids, saponification, iodine and peroxide values of oils obtained by different apparatuses, were analyzed by using the standard procedures. The Abbe refractometer AR3D (Krüss optronic, Germany) was used for measuring the refractive index. Determinations were done in triplicate for each analysis.

Statistical analysis. The statistical analysis, the correlation matrices and determination of the correlation coefficients were performed by doing the program STATISTICA version 5.0.

3. RESULTS AND DISCUSSION

Plant material. The used plant material had the moisture content of 8.7 %. The oil content was 21.2% and the protein content was 46,7% (N × 6,25), based on absolute dry seeds.

*The influence of solvent and extraction apparatuses on soybean oil extraction***.** The results of the solvent influence on the soybean oil extraction are shown in Table 1. The yields were obtained after 60 minutes of extraction by using reflux.

The results indicate the highest degree of extraction (DE) of 96.8%, calculated from the oil content in the seeds, and the highest oil yield of 20.4 g/100g based on dry seeds was achieved by using trichloroethylene as solvent. It is not negligible, that by using *n*-hexane only 68.7% of oil was extracted, and the oil yield was lower than 14.9 g/100g. The investigations of the influence of extraction apparatures and techniques on oil yield and composition are performed by trichloroethylene and reflux, the Soxhlet or the Tilepape apparatuses.

The maximal oil yield with trichloroethylene was still with the Soxhlet apparatus, but according to the extraction time and the small difference on DE and oil yield (OY), the OY obtained by reflux after only 60 minutes instead 150 minutes of extraction by Soxhlet, is very satisfactory.

Table 1

The results of the oil extraction by reflux and different solvents and by different apparatuses and trichloroethylene, after 60 and 150 minutes

Solvent	DE (%)	OY (g/100g)	OY D (g/100g)
Trichloroethylene	96.8	18.3	20.4
Chloroform	75.7	14.3	16.1
Carbon tetrachloride	74.1	14.2	15.9
n-Hexane	68.7	13.3	14.9
Petrol ether	43.4	8.2	9.2
Soxhlet*	99.3	18.8	21.4
Tilepape*	98.1	18.9	20.8

DE – degree of oil extraction calculated based on the seeds oil content; OY – oil yield; OY D – oil yield regard to dry seeds; * – after 150 minutes

*Oil composition and characterization***.** IR spectra of oil obtained by reflux, the Soxhlet and the Tilepape apparatuses are similar for each other. There are identification peaks of the major functional groups: on 3009 cm^{-1} from ν(CH) vibrations of unsaturated compounds, on 1746 cm^{-1} from ν(C=O) ester type bond, on 1652 cm^{-1} from ν(C=C) vibrations of unsaturated fatty acids, on 1162 cm^{-1} from scelet type ν(C=C) vibrations of sterols and on 712 cm^{-1} from δ (C=O) vibrations (Fig. 1).

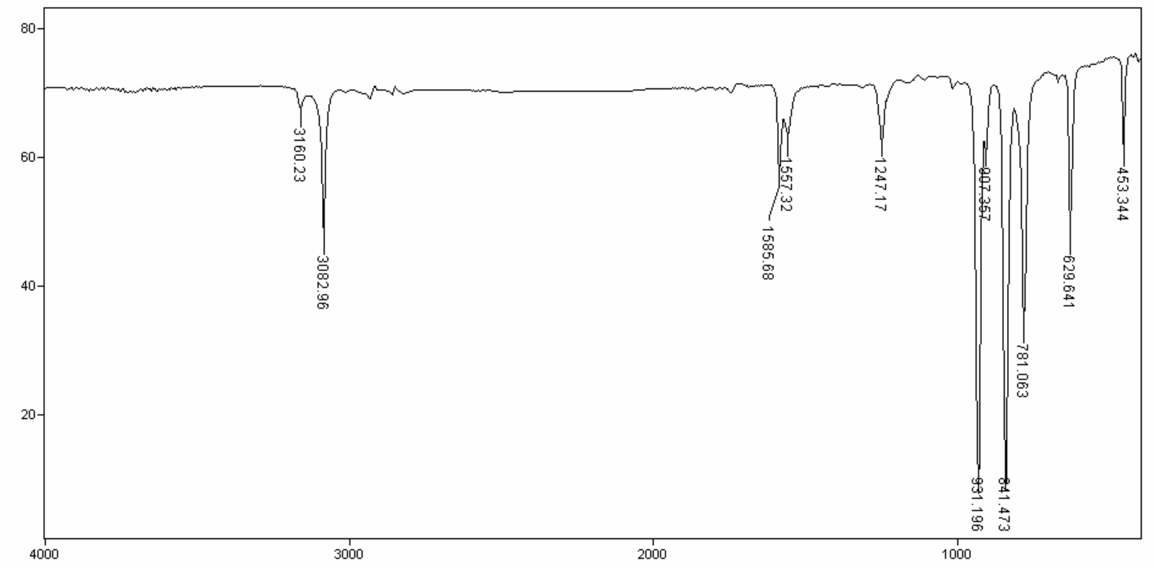

Fig. 1. IR spectrum of oil obtained by reflux and trichloroethylene as solvent, after 60 minutes of extraction

Figure 2 shows the HPLC analysis of oil obtained by trichloroethylene and by using reflux and Table 2 shows the results of the HPLC investigations of oil extracts obtained by different apparatuses. The content of components is determined by measuring the peak area at 1.76 min for free fatty acids, the peak area at 2.15 min for methyl esters, the peaks area in the range of 3.44 – 4.58 min for monoacilglycerol, the peaks area in the range of 5.28 – 8.68 min for diacilglycerols and the peaks area in the range of 10.91–15.81 min for triacilglycerols. The content of free fatty acids is variable in the range from 25.5% obtained by reflux, up to 27.5% obtained by Tilepape. Oil obtained by reflux had similar content of free fatty acids, as well as content of other components: methyl esters, monoacilgycerols, diacilglycerols and triacilglycerols compared to those obtained by Soxhlet: the differences in content are less than 1%. The sample was measured in triplicate and the values of the standard deviation were between 0.01 and 0.30%.

The fatty acids composition was investigated by the GC analysis and the results of the analysis are shown in Table 3.

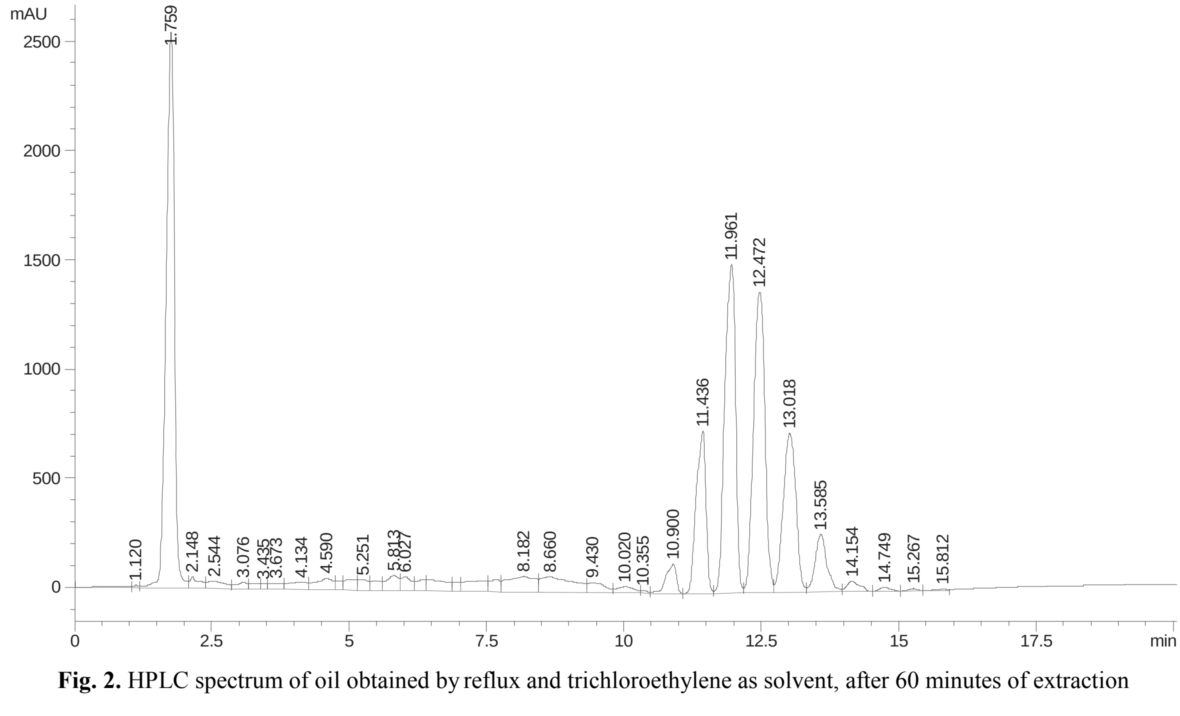

Fig. 2. HPLC spectrum of oil obtained by reflux and trichloroethylene as solvent, after 60 minutes of extraction

Table 2

HPLC results of oil obtained by different apparatuses and trichloroethylene as solvent

Apparatuses	FFA (%)	ME (%)	MAG (%)	DAG (%)	TAG (%)
Reflux	25.5	1.7	0.6	2.1	70.1
Tilepape	27.5	0.7	1.1	2.5	68.2
Soxhlet	26.9	0.4	0.3	1.6	70.9

Table 3

GC analysis of oil obtained by different apparatuses and trichloroethylene as solvent

Acid	Retention time	Content (%)		
		Reflux	Soxhlet	Tilepape
Myristic ($C_{14:0}$)	8.85±0.11	0.01±0.01	0.14±0.08	-
Palmitic ($C_{16:0}$)	10.76±0.12	10.85±0.6	10.40±0.9	7.57±0.6
γ-Linolenic ($C_{18:3}$)	11.95±0.04	0.09±0.02	1.03±0.02	-
Linoleic ($C_{18:2}$)	14.08±0.90	54.23±1.2	61.19±1.3	54.98±1.2
Oleic ($C_{18:1}$)	14.10±0.09	13.57±0.8	13.40±0.6	27.62±0.8
Linolenic ($C_{18:3)}$	14.20±0.09	12.93±0.9	5.98±0.9	-
Stearic ($C_{16:0}$)	14.47±0.12	5.63±0.8	5.47±0.6	4.28±0.7
Nonadecanonic ($C_{19:0}$)	17.47±0.05	0.32±0.09	0.31±0.08	0.55±0.07
Nondetermined	18.01±0.04	0.32±0.11	0.32±0.10	-
Arachidic ($C_{20:0}$)	18.58±0.05	0.60±0.1	0.58±0.09	0.55±0.09
Behenic ($C_{22:0}$)	22.90±0.04	0.48±0.06	0.49±0.09	0.30±0.09
Non determined	25.96±0.03	0.16±0.06	0.17±0.04	2.33±0.03
Lignocerinic ($C_{24:0}$)	27.23±0.03	0.01±0.01	0.02±0.01	0.55±0.01
Non determined	34.42±0.03	0.17±0.06	0.18±0.05	0.42±0.05
TU		**80.81±1.2**	**81.50±1.3**	**82.60±1.2**
TS		**17.90±0.8**	**17.41±0.9**	**13.79±0.7**
ND		**0.65± 0.11**	**0.67±0.10**	**2.75±0.05**
TU/TS		**4.64**	**4.68**	**6.98**

TU – content of total unsaturated fatty acids; TS – content of total saturated fatty acids;
ND – content of nondetermined components; TU/TS – ratio of total unsaturated to total saturated fatty acids content

The oils contained linoleic ($C_{18:2}$), α-linolenic ($C_{18:3}$), oleic ($C_{18:1}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$), arachidonic ($C_{20:4}$), behenic ($C_{22:0}$), nonadecanoic ($C_{19:0}$), γ-linolenic, lignocerinic, myristic ($C_{14:0}$) and several nondetermined components. In all extracts, inependently of the apparatuses used, the main component was linoleic acid with the content over 50%. The biggest content of linoleic acid was in the oil obtained by the Soxhlet (61.2%). The content of palmitic and oleic acids was over 10%, in oils obtained by reflux and the Soxhlet while oleic acid content in the oil obtained by the Tilepape was over 27%. The linolenic acid is considered to be unstable acid because it is easily oxidized [7, 17]**.** As during the Tilepape extraction, the extract is continually overflowing in the flask and the contact with the air was continuous, probably the oxidation reaction had occurred, and linolenic acid was not present in this extract. Some components such as myristic, γ-linolenic acid and nondetermined component with retention time of 18.01 minutes, with content of 0.01 and 0.1%, 0.09 and 1% and 0.32%, presented in oil obtained by reflux and Soxhlet, respectively, were not found in the oil obtained by the Tilepape. Other components are detected in all three investigated oils and their content is very similar, so the application of different apparatuses slightly, although changes the oil fatty acids composition. The share of total unsaturated fatty acids is the highest in the oil obtained by the Tilepape and the lowest in the oil obtained by reflux. The results show, the total content of all nondetermined components are the highest in the oil obtained by Tilepepe. Probably, the nondetermined components are the result of the oxidation reaction, which is more significant during the Tilepape extraction. The ratio of TU to TS fatty acids is similar in oils obtained by reflux and the Soxhlet (4.6–4.7), while this ratio is the highest in the oil obtained by the Tilepape, where it was nearly 7. These results are supported with the highest peroxide value.

The oils are characterized by chemical values which are relevant to their composition and the results are presented in Table 4. Investigated extracts had the saponification value in the range of 190 – 194, the acid value of 2.8 – 3.3%, the iodine value of 131 – 135, the peroxide value of 6.9 – 7.2 milliequivalents/kg and refractive index (n_D^{20}) of 1.4734 – 1.4785.

Statistical analysis results. The correlation coefficients and the respective significance levels were obtained between some oil fatty acids and some chemical values and the data are presented in Table 5. The sample sizes were nine (*N* = 9, three extraction techniques and three determinations) and the correlations with absolute value 0.8 and above 0.8, were considered.

Table 4

Chemical values of oil obtained by different apparatuses and trichloroethylene as solvent

Apparatuses	Acid value	Refractive index	Saponification value	Iodine value	Peroxide value
Reflux	2.80	1.4734	190.4	131.0	6.89
Tilepape	3.29	1.4736	194.6	134.7	7.20
Soxhlet	3.31	1.4785	193.9	133.6	6.90

Table 5

Correlation matrix for the oil fatty acids content and some chemical values obtained for data of three extraction techniques (reflux, Soxhlet, Tilepape) and three determinations (N=9)

	FFA	TAG	γ-Linolenic	Linoleic	Oleic	Linolenic	Palmitic	IV	PV
FFA	1								
TAG	–0,49	1							
γ-Linolenic	0.67	**–0.89**	1						
Linoleic	0.73	**–0.91**	**0.98**	1					
Oleic	0.16	0.52	–0.57	–0.45	1				
Linolenic	–0,65	–0.03	0,02	–0.11	**–0.83**	1			
Palmitic	–0.14	–0.54	0.61	0.50	**–0.99**	**0.82**	1		
IV	0.26	0.31	–0.29	–0.22	**0.81**	**–0.80**	0.29	1	
PV	0.70	–0.76	0.72	**0.81**	–0.08	–0.41	0.09	0.29	1

FFA – free fatty acid; TAG – triacilglycerols; IV – iodine value; PV – peroxide value. Correlations are significant at $p \leq 0.05$

The high γ-linolenic and high linoleic content goes along with the low triacilglycerols content (TAG) and the high linoleic acid content goes along the high γ-linolenic acid content. The high linolenic and high palmitic acid content is associated with the small oleic content, while the high palmitic acid content is associated with the high linolenic content. Between the iodine value and the oleic acid content as well as between the peroxide value and the linoleic acid content, there is a proper correlation. Also, the high linolenic acid content causes low iodine value. Further, it appears that the iodine and the peroxide value do not depend on the other presented fatty acids content.

4. CONCLUSION

By oil extraction from soybean seeds by reflux, the maximum yield was achieved by using trichloroethylene as solvent. By using trichloroethylene oils are obtained by using different apparatuses: reflux, the Soxhlet and the Tilepape. The application of different apparatuses slightly changes the oil composition. The reflux apparatuses can be used for soybean oil obtainment as the method with shorter extraction time and satisfactory oil yield and quality. The correlation coefficients showed proper correlations between the iodine value and the oleic acid content as well as between the peroxide value and the linoleic acid content.

Acknowledgement: This work was supported under the project 19048 by the Ministry of Science of the Republic of Serbia.

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