HPLC Analysis of Whole Soybean (*Glycine max* L.) Seed Oil

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Abstract: Soybean oil is among the most common vegetable oils containing a significant amount of unsaturated fatty acids, tocopherols and triacylglycerols as the major component of soybean total lipids. An examination was made of oil extraction from whole soybean seed using different solvents: carbon tetrachloride, trichloroethylene, chloroform, hexan and diethylether. The maximum oil yield was achieved by trichloroethylene and was 23.1 g/100 g of dry seed, the extraction degree being 87.93% relative to the oil content in the plant material used. The obtained oil contained 26.9% of free fatty acids, 0.4% of methyl esters, 0.3% of monoacylglycerols, 1.6% of diacylglycerols, and 70.9% of triacylglycerols. The oil chemical and physical properties were characterized by the saponification value of 225.4, acid value of 2.24, iodine value of 140, peroxide value of 19.2 mmol/kg and the refractive index (n\(_D\)) of 1.4712.

Key words: soybean seed, oil, extraction, HPLC analysis, characterization

Introduction

Soybean is a member of the (*Fabaceae*) pea family of vegetables. It is a native plant of China currently being cultivated in many parts of the world. Apart from containing high quality proteins, essential amino acids and isoflavones, the soybean has a high content of lipids (14-27%) (Liu 1999). Soybean oil is one of the most common vegetable oils containing a significant amount of unsaturated acids: \(\alpha\)-linolenic acid, known as omega-3 acid, linoleic, \(\gamma\)-linolenic and arachidonic acid, known as omega-6, oleic acid known as omega 9 acid, being very important in human
nutrition, as well as saturated acids: palmitic and stearic acid (Bressani 1972; Olguin et al., 2003; Bond et al., 2005). Soybean oil also contains tocopherols which are partially removed during soybean oil bleaching (Ortega-Garcia et al., 2005; Ming-Hong and Chun-Lin 2004; Yoshida et al., 2006). Yoshida et al. (2006) found that triacylglycerols were the major component of soybean total lipids, representing 92% of cotyledons and 70% of axis or seed coat. Conventional Soxlet apparatus is usually used to extract the lipid content from different plant seeds (Pomeranz and Meloan 1994; Garcia-Ayuso et al., 1999). García-Ayuso et al. (Leque-Garcia and Luque de Castro 2004) have developed the microwave-assisted Soxhlet extraction technique to accelerate extraction of the fat content in cheese while Luque-García and Luque de Castro (Godwin and Mercer 1983) have developed Soxhlet extraction assisted in the cardridge by ultrasound to extract the total fat content from oleaginous seeds such as sunflower, rape and soybeen. By using n-hexane as solvent only free lipids (oil) are extracted while structural and protective lipids, such as phospholipids, glycolipids, lipoproteins etc., are extracted only after their hydrolysis by boiling alcohol such as ethanol and methanol (Holčapek et al., 1999).

The oil extraction from whole soybean seed was investigated in this study. Different solvents including carbon tetrachloride, trichlorethylene, chloroform, hexane and petroleum ether were used to obtain maximum oil yields. The HPLC oil analysis was used to quantify mono-, di- and tri-glycerols in the obtained extract. The oil was characterized by chemical values.

**Materials and Methods**

**Plant material.** The soybean seeds (*Glycine max* L.) used in the study were purchased at a local store. The whole seeds were milled to an overall particle size of 0.5 mm.

**The soybean seed oil content.** The soybean seeds (30 g) were put into an erlenmayer flask, 300 ml of trichlorethylene was added and extracted for 30 minutes, under reflux and by mixing (200 min⁻¹) at mixture boiling temperature. The extract was separated by using Buchner funnel under weak vacuum. The plant material was extracted three more times by the same method, the extracts being mixed together and eluted in a separation funnel (3 x 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 the used plant material.

**Dry residue content.** The extract (3 ml) was put into the disk plate analyzer (Scaltec SMO 01, Scaltec Instruments, Germany), poured and dried at 110 °C to a constant weight. The content of dry residue was read 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 carbon tetrachloride, chloroform, trichlorethylene, hexane or petroleum ether, at a plant material to solvent ratio of 1:10 w/v, at solvent boiling temperature and 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.

**HPLC analysis.** For the HPLC analysis, a modified HPLC method was used (Holčapek et al., 1999). The equipment included Agilent 1100 High Perfo-
formance Liquid Chromatograph, equipped with a degasser, a binary pump, a Zorbax Eclipse XDB-C18 column (4.4 m x 150 mm x 5 µm) and a UV/Vis detector. The flow rate of the binary solvent mixture (methanol, solvent A, and 2-propanol/n-heksane, 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. Mono-, di- and tri-acylglycerols were identified by comparison of the retention times of the lipid components with those 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.

**Chemical properties.** The refractive index, acid, saponification, iodine and peroxide values of the oil obtained by trichloroethylene during a 60-minute extraction process were determined by standard procedures. The Abbe refractometer AR3D (Krüss Optronic, Germany) was used to measure the refractive index.

**Results and Discussion**

**Plant material.** The oil content was 18.3 g/100 g based on the moisture content of 10.7% (TL) i.e. of 23.2% based on the dry seed and protein content of 52.3% (N x 6.25).

**The effect of the solvent on the oil total lipid extraction kinetics.** The results of the effect of the solvent mixture on the oil extraction kinetics with different solvents, at solvent boiling temperature are shown in Fig. 1. The results indicate that the highest oil yield was obtained with trichloroethylene after a 90-minute extraction, amounting to 16.1 g/100 g i.e. 18.0% based on the dry seed, the extraction degree being 87.93% of the oil content in the used plant material.

![Figure 1. The kinetics of soybean seed oil extraction with various solvents](image-url)
**HPLC analysis and oil characterization.** Fig. 2 shows the HPLC analysis of soybean seed oil. The content of free fatty acids was 26.9%, that of methyl esters 0.4%, monoacylglycerols - 0.3%, diacylglycerols - 1.6%, and triacylglycerols - 70.9%. The content of the components was determined by measuring the peak area at 1.76 min for free fatty acids, peak area at 2.152 min for methyl esters, peak areas in the 3.445-4.580 min, 5.276 - 8.677 min and 10.907-15.815 min range for mono-, di- and triacylglycerols, respectively. Triacylglycerols make up the major portion (over 70%) of the extract, methyl esters, mono- and diacylglycerols being present at lowest concentrations. The sample was measured in duplicate. The standard deviation values ranged from 0.01 to 0.30%.

The oil obtained was gold-yellow in colour and had a characteristic soybean odour. The oil dried was characterized by the saponification value of 194.40±0.6, the acid value of 2.81±0.03, the iodine value of 191.11±0.4, the peroxide value of 34.62±0.03 mmol/kg and the refractive index \( (n_D^{15}) \) of 1.4734±0.01. Determinations were performed in duplicate for each analysis.

**Conclusions**

Maximum oil yield was achieved by trichloroethylene and was 23.1 g/100 g, the extraction degree being 87.93% relative to the oil content of the used plant material. The obtained oil contained 26.9% of free fatty acids, 0.4% of methyl esters, 0.3% of monoacylglycerols, 1.6 % of diacylglycerols, and 70.9% of triacylglycerols. The oil chemical and physical properties were characterized by the saponification
value of 225.4, the acid value of 2.24, the iodine value of 140, the peroxide value of 19.2 mmol/kg and the refractive index (n_{D}^{15}) of 1.4712.

References


HPLC ANALIZA ULJA CELOG SEMENA SOJE (Glycine max L.)

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Rezime

Ulje soje je jedno od najpoznatijih biljnih ulja. Sadrži veliku količinu nezasićenih masnih kiselina, tokoferol i triacilglicerole kao glavnu komponentu ukupnih lipida. U radu je ispitana ekstrakcija ulja iz samlevenog celog semena soje primenom različitih rastvarača: ugljentetrahlorida, trihloetilena, hloroforma, heksana i dietiletara. Najbolji prinos ulja od 23,1 g/100 g suvog semena ostvaren je sa trihlor-etilenom kao rastvaračem, što čini stepen ekstrakcije od 87.93% u odnosu na sadržaj ulja u korišćenom biljnom materijalu. U dobijenom ulju sadržaj slobodnih masnih kiselina bio je 26,9%, metil estara-0,4%, monoacilglicerola-0,3%, diacilglicerola-1,6%, i triacil-glicerola-70,9%. Saponifikacioni broj ispitivanog ulja bio je 225,4, kiselinski stepen 2,24, jodni broj 140, peroksidni broj 19.2 mmol/kg i indeks refrakcije ($n_0^{15}$) 1,4712.