In-situ Alcoholysis of Soybean Oil

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ABSTRACT: *In-situ* alcoholysis of soybean oil with methanol, ethanol, *n*-propanol, and *n*-butanol was investigated, as well as the extraction of the oil with these solvents, to explain the progress of *in-situ* alcoholysis and to determine the parameters that affect this reaction. Because methanol is a poor solvent for soybean oil, the amount of oil dissolved in methanol and converted to methyl esters was low after *in-situ* alcoholysis. Ethyl, propyl, and butyl esters of soybean fatty acids could be obtained in high yields from *in-situ* alcoholysis of soybean oil with these alcohols. *In-situ* alcoholysis proceeded through dissolution and alcoholysis of triglycerides successively, and the overall reaction rate was determined by the extraction and alcoholysis rates. The parameters, affecting yield and purity of the product esters, were mainly those that favor extraction rate.

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Alcoholysis of vegetable oils and animal fats is an important reaction that produces fatty acid alkyl esters, which are valuable intermediates in oleochemistry, and methyl and ethyl esters, which are excellent substitutes for diesel fuel. Soybean and sunflower seed oils have gained much attention lately as renewable energy sources because of their relatively high yield per hectare and widespread production, and alcoholysis of these oils has been studied intensively by numerous investigators (1–3).

In-situ alcoholysis differs in that the oil-bearing material contacts acidified alcohol directly instead of reacting purified oil and alcohol. That is, extraction and alcoholysis proceed in the same process. This means that the alcohol acts both as an extraction solvent and as an esterification reagent.

In-situ alcoholysis of sunflower oil with acidified methanol produces fatty acid methyl esters in yields significantly greater than those obtained from the conventional reaction with pre-extracted seed oil (4,5). *In-situ* esterification of high-acidity rice bran oil with methanol and ethanol were also investigated with sulfuric acid as catalyst, and the main parameter that affected methyl ester yield was the free fatty acid (FFA) content of the oil (6). Because the solubility of triglycerides in ethanol is much higher than that in methanol, the FFA content of the oil did not affect ethyl ester yield. The purity of the esters obtained depended on the alcohol used because the solubilities of the oil components in methanol and ethanol were different.

In this study, *in-situ* alcoholysis of soybean oil with methanol, ethanol, *n*-propanol, and *n*-butanol was investigated as well as extraction of the oil with these solvents. Thus, it was possible to characterize the progress of *in-situ* alcoholysis and to determine the parameters that affect this reaction, thereby producing pure, cheap alkyl esters in high yield.

EXPERIMENTAL PROCEDURES

Materials. Soybeans obtained from an oil extraction plant in Adana in southern Turkey were ground to particle sizes smaller than either 1 or 0.5 mm. Moisture content of the beans was 9.0%. Oil contents of the ground fractions, determined by Soxhlet extraction with hexane for 6 h, were 20.9 and 23.1%, respectively. Acid value of the extracted soybean oil was 2.52 (7).

Methanol (99.7%) and fermentation-derived 96% ethanol were of technical grade and were purchased from Turkish State Monopolies (Istanbul, Turkey). Dehydration of ethanol was achieved by drying over calcium oxide (8). For this purpose, the mixture of 96% ethanol and calcium oxide was refluxed on a waterbath for 6 h, allowed to stand overnight, and then distilled. Water content of distilled ethanol after dehydration was 1.2% by Karl-Fischer titration (9). *n*-Propanol (Riedel de Haen, Hannover, Germany) and *n*-butanol (Merck, Darmstadt, Germany) were of analytical grade.

Methods. For *in-situ* alcoholysis and extraction experiments, 50 g of ground soybean was transferred to a flask, and 150 mL of alcohol was added. For alcoholysis reactions, 6 mL concentrated sulfuric acid also was added to the flask as catalyst. The mixture was refluxed in a thermostatic water bath while it was stirred magnetically. At the end of reaction or extraction period, the mixture was vacuum-filtered on a Buchner funnel, and the filter cake was washed with 200 mL alcohol. After drying overnight at room temperature, the residue was reextracted in a Soxhlet apparatus with hexane to obtain the oil fraction remaining in the beans. The ratio (calculated as percentage) of the residual oil to the total amount of the oil in the beans was calculated for both *in-situ* alcoholysis and extraction experiments. The difference of the per-

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centage of the residual oil in beans from 100 gave the percentage of the oil dissolved in alcohol.

To the filtrate obtained from *in-situ* alcoholysis was added 100 mL of water, and the solution was then extracted three times with hexane (3 × 50 mL). The combined extracts were washed with water, dried over sodium sulfate, and evaporated to give the esterified product. The residual oil in the beans and the esterified product were both examined qualitatively by thin-layer chromatography (TLC). The FFA content and ester content of the esterified product also were determined.

TLC was performed on glass plates coated with Silica Gel G (Merck), and developed in a solvent system of hexane/diethyl ether/acetic acid (90:10:1, vol/vol/vol). Spots were detected by iodine vapor staining (10).

The FFA content of the esterified products was determined by titration with standard NaOH solution and phenolphthalein as indicator (7). The ester content of the esterified products was determined by column chromatography. The method for the determination of mono-, di-, and triglycerides by column chromatography was modified (11): 25 g of silica gel, 0.5–1.0 mm (Macherey-Nagel Co., Duren, Germany) in hexane was transferred to a column of 18 mm diameter. One gram of esterified product, dissolved in hexane, was added to the column, and hexane/diethyl ether (99:1, vol/vol) was added to attain an eluate flow rate of 2 mL/min. Fractions of 50 mL were collected and checked by TLC, and it was determined that the first 300 mL of eluate contained only fatty acid esters of monoalcohols. The combined eluates were evaporated and weighed.

Conditioning of the beans was achieved by heating the ground beans in an oven at 85°C for 1 h. Maceration was performed by regrinding 50 g of ground beans with 50 mL methanol in a blender for 30 min, the slurry obtained being used in the extraction and *in-situ* experiments.

RESULTS AND DISCUSSION

In-situ *alcoholysis of soybean oil with methanol.* Ground soybeans, sieved to particle size between 0.5 and 0.3 mm, were used in the extraction and *in-situ* alcoholysis with methanol for 3 h at 65°C. The results are given in Table 1. Average values obtained in parallel experiments were not used, because some of the results of duplicates were too different.

In the extraction of soybeans with methanol, approximately 9% of the oil in the soybeans dissolved in alcohol, and pretreatment of the beans, such as conditioning and maceration, had little effect on the amount of the oil dissolved in methanol. After *in-situ* alcoholysis, the oil dissolved in methanol was nearly 20% of the total oil, and methyl ester content of this oil was 42%. Conditioning of the ground beans did not affect the amount of the oil dissolved in methanol (Table 1). Maceration before *in-situ* alcoholysis caused an appreciable increase in the amount of this oil (Table 1). With maceration, approximately 40% of the soybean oil was transferred to the methanol phase, and the methyl ester content of this oil reached 55%. Because all reaction conditions, such as volume of methanol, temperature, and time, were the same, the result obtained with maceration suggests that maceration caused breakdown of the cell walls of the soybeans, leaving the oil free and available to react with methanol.

TABLE 1
Extraction and *In-situ* Alcoholysis of Soybean Oil with Methanol

		Oil dissolved in methanol		
Procedure	Pretreatment	Amount (%)	Ester content (%)	Free fatty acid (%)
Extraction	—	9.0		
		8.5		
	Conditioning	11.7		
	Maceration	14.8		
		12.0		
In-situ alcoholysis	—	19.8	41.9	2.00
		21.6		
	Conditioning	20.0		1.52
		20.7		1.52
	Maceration	41.4	56.4	2.8
		39.7	54.6	2.8

In-situ *alcoholysis of soybean oil with 96% ethanol.* Soybeans ground to particle sizes smaller than either 1 or 0.5 mm were extracted and esterified with 96% ethanol separately for 1, 3, and 5 h at 78°C, and the results obtained are given in Table 2.

In the extraction with 96% ethanol, oil dissolved in alcohol was approximately 25–30% of the total oil, regardless of particle size. Increasing the extraction time from 1 up to 5 h did not affect the amount of oil dissolved, indicating that extraction reached equilibrium within one hour (12,13). On the other hand, after *in-situ* alcoholysis, the amount of oil dissolved in ethanol increased with increasing reaction time. This indicates that, as triglycerides dissolved in ethanol were converted to ethyl esters, thus decreasing the triglyceride concentration in the ethanol phase, more triglycerides continued to dissolve. Thus, *in-situ* alcoholysis proceeded through dissolution and alcoholysis of triglycerides successively, and the overall reaction rate was determined by the extraction and alcoholysis rates.

Table 2 shows that the oil dissolved in ethanol was also increased by decreasing the particle size of the ground beans. At the end of 5 h, the amount of oil was 95% of the total for particles smaller than 0.5 mm, while 78% of the total soybean oil transferred to the ethanol phase for particles smaller than 1 mm. This result was probably due to an increase of the extraction rate as the particle size decreased, as previously reported for the extraction of soybean flour with hexane (14).

In Table 2, the results of some replicate experiments were very different. These differences probably arose from the filtration and washing conditions after extraction or alcoholysis. Because the hold-up volumes, i.e., the amount of solution held by the solid after filtration could be high, it was necessary to wash the cake with ethanol. TLC examination showed

TABLE 2
Extraction and *In-situ* Alcoholysis of Soybean Oil with 96% Ethanol

Procedure	Particle size (mm)	Time (h)	Oil dissolved in ethanol amount (%)
Extraction	<1	1	23.3 28.3
		3	29.6
		5	22.3 27.2
	<0.5	1	26.1 29.6
		3	28.8 29.1
		5	24.9 25.5
In-situ alcoholysis	<1	1	50.1 42.8
		3	64.3 65.0
		5	78.3 78.2
	<0.5	1	33.7 47.2
		3	87.8 74.6
		5	94.0 95.5

TABLE 3
***In situ* Alcoholysis of Soybean Oil with Monohydroxy Alcohols**

		Oil dissolved in alcohol		
Procedure	Alcohol	Amount (%)	Ester content (%)	Free fatty acid (%)
Extraction	Methanol	13.1 15.1		
	Ethanol, 96%	28.8[a] 29.1[a]		
	Ethanol, 98.8%	33.2 26.5		
	n-Propanol	92.9 85.4		
	n-Butanol	92.0 90.9		
In-situ alcoholysis	Methanol	21.4 22.8	41.5	1.27
	Ethanol, 96%	70.8 66.3	58.6	7.67
	Ethanol, 98.8%	97.0 96.7	80.7 80.9	5.56 5.50
	n-Propanol	98.9 95.9	84.6	3.74
	n-Butanol	94.5 91.6	78.0	4.50

[a] From Table 2.

that dissolution of triglycerides in ethanol during the washing step caused the differences in results, and it was decided to use methanol as washing solution.

In-situ *alcoholysis with various monohydroxy alcohols.* Soybeans, ground to particle size smaller than 0.5 mm, were extracted and esterified with methanol at 65°C, or 96% ethanol, 98.8% ethanol, *n*-propanol, or *n*-butanol at 78°C for 3 h. After filtration of the mixtures, cakes were washed with 200 mL of methanol to prevent dissolution of triglycerides in cakes. The results are in Table 3.

Because the solubility of triglycerides in monohydroxy alcohols increases with increasing alcohol chainlength, the amount of oil dissolved in alcohol increased with respect to the molecular weight of the alcohols. After *in-situ* alcoholysis, the oil dissolved in alcohol also increased with increasing alcohol chainlength. Under alcoholysis conditions with 98.8% ethanol, *n*-propanol, and *n*-butanol, practically all of the oil present in soybeans was dissolved in the alcohol phases, and 80–85% of these oils were converted to monoesters in 3 h.

The differences between the amounts of oil extracted and the ethyl ester contents of the products obtained with 96 and 98.8% ethanol were mainly due to the water content of ethanol. Ethanol, anhydrous as well as aqueous, is not a good solvent for soybean oil at normal temperatures, but as the temperature increases, the solubility increases until the critical solution temperature is reached, at which point the components become miscible. Critical solution temperatures of soybean oil with 95.4, 98.0, and 99.9% ethanol are 90, 78, and 67°C, respectively, and show the effect of the water content of ethanol on triglyceride solubility (15).

The FFA contents of the esterified products obtained with ethanol, *n*-propanol, and *n*-butanol were higher than that of the crude soybean oil in seeds. This result suggests that a hydrolysis reaction occurred during *in-situ* alcoholysis.

A typical chromatogram of the ester products obtained by the *in-situ* alcoholysis of soybean oil with monohydroxy alcohols is shown in Figure 1. Identification of the spots was performed by comparing the R_f values of the spots with those obtained with the standard mixture, which consisted of monoglycerides, diglycerides, fatty acid, triglyceride, and fatty acid methyl ester. The main component is alkyl ester, with triglycerides, FFA, and partial glycerides also detected in small amounts in these ester products.

Based on these experiments, we conclude that ethyl, propyl, and butyl esters of soybean fatty acids could be obtained directly in high yields by *in-situ* alcoholysis of soybean oil. By increasing reaction temperature and time, and by decreasing the particle size of the soybeans and the water content of ethanol, it was possible to increase the purity of the products. Parameters that affect yield and purity of the product esters from soybean oil were mainly those that favor extraction rate.

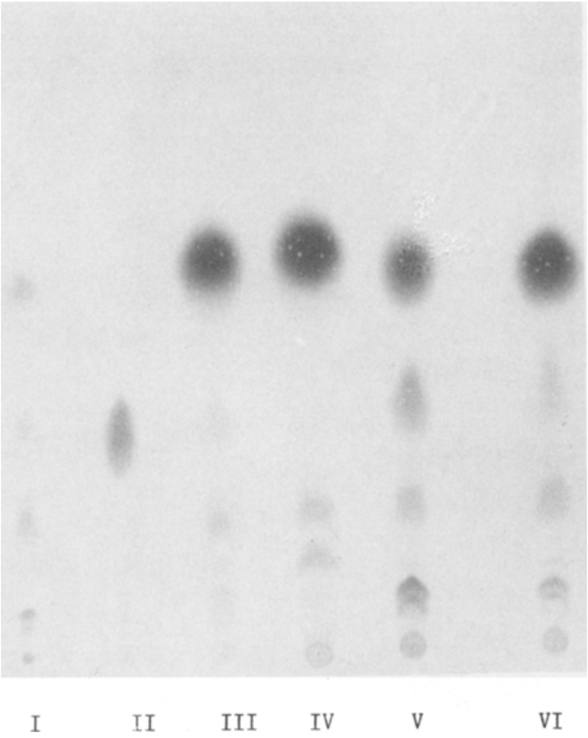

FIG. 1. Typical chromatogram of the esterified products obtained from *in-situ* alcoholysis of soybean oil; I: standard mixture (from bottom, monoglycerides, diglycerides, fatty acid, triglyceride, fatty acid methyl ester), II: soybean oil, III: propyl ester, IV: butyl ester, V: ethyl ester (with 96% ethanol), VI: ethyl ester (with 98.8% ethanol).

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