Variables Affecting the Yields of Methyl Esters Derived from *in situ* Esterification of Rice Bran Oil

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ABSTRACT: The influence of specific factors on *in situ* methanolic esterification of rice bran oil (RBO) using sulfuric acid catalyst was investigated. Using high-FFA rice bran was found to be the most effective means to increase methyl ester yields. The ester content of the extract increased about 67% when the FFA content of oil was increased from 16.6 to 84.5%. Increasing the reaction time beyond 30 min did not affect yields. Increasing the temperature from 20 to 65°C elevated the FAME yield by about 30%, but increasing the amount of acid catalyst above 5 mL did not enhance yield, and increasing the methanol dose from 200 to 250 mL had a negligible effect.

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KEY WORDS: Esterification, fatty acid, fatty acid methyl esters, *in situ* esterification, rice bran, rice bran oil.

FAME are used, rather than FA, to produce a number of FA derivatives such as fatty alcohols, alkanolamides, α-sulfonated methyl esters, and sucrose esters because FAME are more stable, less corrosive, and more easily fractionated (1). Methyl esters have recently been used as a diesel additive and clean energy source in Europe (2) but are currently not competitive with diesel fuel prices. However, relatively inexpensive raw materials such as soapstock, agricultural wastes, tallow, and high-FFA-containing greases are being used, which may reduce production costs (3–5). In this study, factors affecting FAME production from rice bran oil (RBO), an inexpensive rice co-product, were investigated.

Production costs may be further reduced by *in situ* esterification, i.e., simultaneous oil extraction and methyl esterification. *In situ* esterification was first performed by transesterification of sunflower seed oil with acidified methanol, and it resulted in a significant methyl ester yield (6,7). High-acidity oils, such as rice bran oil (RBO), can readily form FAME during extraction (8), and methanol acts as both an extraction solvent and reagent to produce FAME. In our first paper (8), we described *in situ* esterification of high acidity RBO with methanol and ethanol using an acid catalyst, and ester yields depended on the RBO FFA content. Esterification with ethanol did not produce pure esters, as did methanol esterification. This was probably due to the higher solubility of oil components in ethanol than methanol and thus the greater amounts of nonreacted substrate. In a second study (9) we investigated *in situ* alcoholysis and extraction of soybean oil with methanol, ethanol, *n*-propanol, and butanol. Ethyl, propyl, and butyl esters of soybean FA could be obtained in high yields from *in situ* alcoholysis of soybean oil with these alcohols. Since methanol is a poor solvent for soybean oil, the amount of the oil dissolved in methanol and converted to methyl esters was low after *in situ* alcoholysis.

The objective of this study was to determine the effects of RBO FFA levels, *in situ* esterification time, reaction temperature, amount of catalyst, rice bran moisture, and amount of methanol on the yield and purity of methyl esters.

EXPERIMENTAL PROCEDURES

Materials. Rice bran was supplied by a local rice mill (Bereket Celtik, Istanbul, Turkey) and was sieved to particle size of <0.6 mm. The rice bran was thoroughly mixed and divided into two equal portions. The first portion was stored in a sealed container at room temperature, between 15 and 25°C, for up to 6 mon to increase the oil FFA content. The second portion was stored at 4°C, also in sealed containers, to inhibit FFA formation.

Effect of RBO FFA. Rice bran samples were taken from the stored bran to provide a range of FFA contents (16–85%). The total bran oil was determined in duplicate by Soxhlet extraction of the bran prior to *in situ* esterification. Samples were esterified *in situ* in duplicate according to the method of Özgül and Türkay (8). Rice bran (50 g) was mixed with 200 mL methanol and 5 mL of concentrated sufuric acid and refluxed for 1 h. The mixture was vaccum-filtered, and the retained material was washed with 100 mL methanol and dried overnight at room temperature. The bran was re-extracted in a Soxhlet apparatus with hexane to obtain the residual bran oil. The ratio of the residual oil to the total amount of oil in the brans was calculated for both *in situ* esterification and extraction experiments. The percentage of the oil dissolved in methanol was determined by difference.

Water (100 mL) was added to the methanol phase, and the solution was transferred to a separatory funnel. The aqueous layer was extracted with hexane (3 × 50 mL), and the combined hexane extracts were washed with water. The hexane solution was dried over anhydrous sodium sulfate, filtered, and evaporated to obtain the esterified product.

The FFA content of esterified products and the residual bran oil was determined by standard NaOH titration (10). Oil and ester phases obtained by esterification and in extraction experiments were determined qualitatively by TLC. TLC was performed on glass plates coated with Silica Gel (Merck, Darmstadt, Germany). The solvent system was hexane/diethyl ether/acetic acid (90:10:1, by vol). Three-microliter samples were applied. Visualization was achieved by iodine vapor staining (11).

The ester content of the esterified product was determined by column chromatography. The modified method for the determination of MG, DG, and TG by column chromatography

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was used (12). Silica gel (25 g, 0.5–1.0 mm; Macherey-Nagel Co., Duren, Germany) in hexane was transferred to a column of 18 mm diameter. Esterified product (1 g), dissolved in hexane, was added to attain an eluate flow rate of 2 mL/min. Fractions of 50 mL were collected and confirmed by TLC, and it was determined that only the first 300 mL of eluate contained FAME. The combined eluates were evaporated and weighed.

Effect of in situ *esterification time.* The *in situ* esterification was repeated in duplicate for 0.5, 1, 3, and 5 h with bran containing approximately 75% FFA. The subsequent treatment and analysis described previously were then performed.

Effect of in situ *esterification temperature.* The *in situ* esterification was repeated in duplicate at 20 and 65°C with 200 mL methanol and 5 mL of sulfuric acid for 1 h with bran containing 56% FFA. The subsequent treatment and analysis described previously were then performed.

Effect of amount of catalyst. *In situ* esterification of rice bran with approximately 75% FFA was carried out in duplicate using 5, 7.5, and 10 mL H_2SO_4. Samples were processed and analyzed as before.

Effect of moisture content of rice bran. Rice bran was dried at 45°C to obtain a 2.23% moisture content; in addition, nondried bran samples that contained 13.4% moisture were studied. *In situ* esterification was conducted as described earlier with methanol but using 7.5 mL sulfuric acid. Subsequent treatment and analysis described previously were performed.

Amount of methanol. The study was repeated with both 200 and 250 mL of methanol and 7.5 mL of sulfuric acid using rice bran with approximately 77% FFA.

RESULTS AND DISCUSSION

Effect of FFA content of RBO. Table 1 shows the effect of varying FFA content on the composition of the residual oil after extraction and *in situ* esterification (i.e., simultaneous extraction and esterification) as well as on the ester extracts. *In situ* esterification was much more effective than extraction alone in reducing residual FFA levels. This was particularly noticeable with high initial FFA levels and is probably due to a shift in the chemical equilibrium as solubilized FFA is esterified, causing more FFA to leach from the bran into solution. The residual bran oil content reduced markedly with increased initial FFA levels, as oil is probably lost with hydrolysis and subsequent oxidation. Residual FFA levels are substantially less with *in situ* esterification relative to extraction alone. This shows the effect of esterification to shift the extraction equilibria and thus enhance FFA extraction. However, the residual neutral oil content was higher in bran used for *in situ* esterification, indicating preferential extraction of FFA. The highest methyl ester yields were obtained with the highest FFA content bran. But the methyl esters obtained contained a small amount of FFA as an impurity. The data indicated that high-FFA bran is most suitable for *in situ* esterification. Methyl ester synthesis increased with increase in initial FFA content, confirming our earlier report (8). Furthermore, the purity of methyl esters was greater in oil with higher FFA content.

Table 2 shows the effect of rice bran initial FFA on the lipid content of oil after extraction and *in situ* esterification. There is little difference between the total oil and FFA contents obtained in the two processes. However, the neutral oil level was greater in extracted oil, particularly at high initial FFA levels, but the ratio of FFA/neutral oil was much greater in extracted oil of higher FFA samples, indicating methyl esterification of FFA in the *in situ* process. The synthesis of methyl esters reduces the solubility of neutral oil. Figure 1 shows the results found on a TLC plate following separation of the methyl ester extract and methanol extract after *in situ* esterification and extraction of 37.3% FFA rice bran. Rice bran contained TG, FFA, and partial glycerides and polar lipids (lane II). In the extracted methyl ester fraction (lane III), methyl ester is the main component with TG, FFA (4.12%), and polar lipids. Residual oil after extraction contained TG, wax, FFA (2.01%), polar lipids, and a small quantity of free FAME (lane IV). This is due to insufficient methanol washing. The methanol-soluble fraction after extraction (lane VI) contained mainly FFA, polar lipids, and TG.

TABLE 1
Effect of Rice Bran Oil FFA Content on Extraction and *in situ* Esterification

Procedure	FFA content of initial oil (%)	FFA content of residual oil in bran (%)	Residual bran			Crude methyl ester	
			Oil (%)	FA (%)	Neutral oil (%)	FFA content (%)	Ester content (%)
Extraction	16.6	5.60	64.1	21.62	72.6	—	—
	37.3	6.54	44.0	7.72	65.6	—	—
	45.2	8.00	35.0	6.19	58.8	—	—
	56.0	9.85	28.2	4.98	58.0	—	—
	71.8	33.10	16.6	7.65	39.4	—	—
	84.5	28.90	9.08	3.11	41.7	—	—
In situ esterification	16.6	6.86	69.5	28.72	77.6	6.57	57.6
	37.3	2.01	55.9	3.01	87.4	4.12	71.0
	45.2	1.98	46.6	2.04	83.4	3.09	77.1
	56.0	1.95	39.2	1.37	87.4	4.28	80.5
	71.8	2.44	24.3	0.83	84.2	3.03	83.1
	84.5	4.41	12.2	0.64	75.4	3.65	86.2

TABLE 2
Amount of Oil, FA, and Neutral Oil Dissolved in Methanol According to the FFA Content of Rice Bran Oil

Procedure	FFA content of initial oil (%)	Oil dissolved in methanol (g)			
		Oil	FA	Neutral oil	FA/neutral oil
Extraction	16.6	35.9	13.0	23.9	0.57
	37.3	56.0	34.4	21.6	1.59
	45.2	65.0	42.4	22.6	1.88
	56.0	71.8	53.2	18.6	2.86
	71.8	83.4	66.3	17.1	3.87
	84.5	90.9	81.9	9.0	9.10
In situ esterification	16.6	30.5	11.8	18.7	0.63
	37.3	43.6	36.1	7.5	4.81
	45.2	53.4	44.3	9.1	4.87
	56.0	60.8	55.2	5.6	9.86
	71.8	75.7	71.2	4.5	15.82
	84.5	87.8	84.0	3.8	22.10

TABLE 3
Effect of Time on Extraction and *in situ* Esterification of Rice Bran Oil

Procedure	Time (h)	FFA content of residual oil in bran (%)	Residual bran			Crude methyl ester	
			Oil (%)	FA (%)	Neutral oil (%)	FFA content (%)	Ester content (%)
Extraction	0.5	29.30	18.6	7.59	46.6	—	—
	1	33.10	16.6	7.65	39.4	—	—
	3	33.10	16.9	7.79	40.0	—	—
	5	47.60	12.1	8.02	22.5	—	—
In situ esterification	0.5	2.43	24.7	0.84	85.4	2.75	80.5
	1	2.44	24.3	0.83	84.2	3.03	83.1
	3	2.42	23.1	0.79	80.1	2.70	84.9
	5	3.21	22.1	0.99	76.0	2.91	86.9

Residual oil in bran had TG, wax, FFA, and polar lipids (lane V).

Effect of in situ *esterification time.* Table 3 shows the effect of time on oil and residual bran composition with an initial bran FFA composition of 71.8%. The residual FFA levels increased with extraction time and remained relatively high, >25%. However, *in situ* esterification reduced FFA levels to <4% throughout. Levels of residual oil were greater after *in situ* esterification than after extraction but changed little over time. The natural lipid content did not change after 30 min, indicating that it was not processed beyond this time. However, there was a small increase in ester content with time.

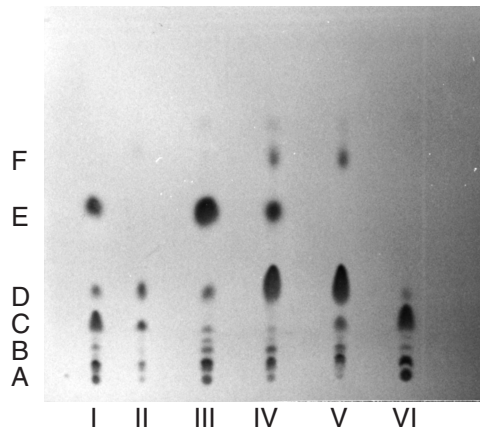

FIG. 1. Typical thin-layer chromatogram of products obtained from extraction and *in situ* esterification of rice bran having 37.3% FFA content with methanol. I, standard; II, rice bran oil; III, extracted methyl ester; IV, residual oil after esterification; V, residual oil after extraction; VI, methanol-soluble fraction after extraction. A, MG; B, DG plus sterol; C, FFA; D, TG; E, FAME; F, wax.

Effect of in situ *esterification temperature.* Table 4 shows the effect of temperature on oil processing methods. The higher temperature significantly reduced the residual bran FFA for each process and produced a much higher FAME yield.

Effect of amount of catalyst. Table 5 shows that increasing the sulfuric acid level above 5 mL did not change the composition of the processed oil of residual bran FFA. Using larger acid doses resulted in bran darkening and an increase in bran stickiness that interfered with filtration operations. This was probably due to bran cell wall disruption by the acid.

Effect of moisture content of rice bran. Table 6 indicates that lipid bran compositions were initially similar, but esterification was most effective in reducing bran FFA, with the oil containing slightly more total oil and much more neutral oil than obtained by extraction. However, moisture content had little effect on the ester content.

Effect of amount of methanol. Increasing the amount of methanol used from 200 to 250 mL did not increase the methyl ester yield or the residual bran FFA after esterification (Table 7). However, increasing the methanol level reduced the total residual oil, neutral oil, and oil FFA after processing.

TABLE 4
Effect of Temperature on Extraction and *in situ* Esterification of Rice Bran Oil

Procedure	Temperature (°C)	FFA content of residual oil in bran (%)	Residual bran			Crude methyl ester	
			Oil (%)	FA (%)	Neutral oil (%)	FFA content (%)	Ester content (%)
Extraction	20	17.70	33.2	10.49	62.1	—	—
	65	9.85	28.2	4.98	58.0	—	—
In situ esterification	20	4.78	39.4	3.37	85.4	22.72	62.2
	65	1.95	39.2	1.37	87.4	4.28	80.5

TABLE 5
Effect of Amount of Catalyst on *in situ* Esterification of Rice Bran Oil

FFA content of initial oil (%)	Amount of H_2SO_4 (mL)	Residual bran		Crude methyl ester	
		Oil (%)	FFA content (%)	FFA content (%)	Ester content (%)
71.8	5	24.3	2.44	3.03	83.1
74.4	7.5	25.2	3.31	2.70	85.8
78.8	10	26.1	3.09	2.48	86.9

TABLE 6
Effect of Moisture Content of Rice Bran on Extraction and *in situ* Esterification of Rice Bran Oil

Procedure	Rice bran			FFA content of residual oil in bran (%)	Residual bran			Crude methyl ester	
	Moisture content (%)	Oil content (%)	FFA content (%)		Oil (%)	FA (%)	Neutral oil (%)	FFA content (%)	Ester content (%)
Extraction	2.23	18.6	58.7	22.40	21.7	8.28	40.8	—	—
	13.40	15.3	57.1	9.45	26.2	4.34	55.3	—	—
In situ esterification	2.23	18.6	58.7	2.71	32.3	1.49	76.1	2.71	77.5
	13.40	15.3	57.1	0.52	38.0	1.68	86.3	4.15	81.7

TABLE 7
Effect of Amount of Methanol on Extraction and *in situ* Esterification of Rice Bran Oil

Procedure	FFA content of initial oil (%)	Amount of methanol (mL)	FFA content of residual oil in bran (%)	Residual bran			Crude methyl ester	
				Oil (%)	FA (%)	Neutral oil (%)	FFA content (%)	Ester content (%)
Extraction	74.4	200	34.00	12.1	5.52	31.2	—	—
	80.2	250	42.05	9.5	4.98	27.8	—	—
In situ esterification	74.4	200	3.31	25.2	1.12	95.2	2.70	85.8
	80.2	250	3.54	15.0	0.66	73.1	2.77	84.3

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