In situ Esterification of Rice Bran Oil with Methanol and Ethanol

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***In situ* esterifications of high-acidity rice bran oil with methanol and ethanol and with sulfuric acid as catalyst were investigated. In the esterification with methanol, all free fatty acids (FFA) dissolved in methanol were interesterified within 15 min, and it was possible to obtain nearly pure methyl esters. The amount of methyl esters obtained from a given rice bran was dependent on the FFA content of the rice bran oil. In the esterification with ethanol, it was not possible to obtain pure esters as in methanol esterification, because the solubilities of oil components in ethanol were much higher than those in methanol.**

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

Rice bran, which is obtained as a by-product in the milling of brown rice kernel to yield the familiar white rice, has an oil content that varies from 12 to 25%, depending on the variety of rice and the degree of milling (1-3). Oil extracted from bran may have low or high acidity, depending on the conditions and duration of storage. The rapid increase of free fatty acids (FFA) in the bran after milling has been recognized as a serious problem for rice bran oil industries. The principal cause of oil deterioration in the bran during storage is the activity of lipase enzyme in the presence of moisture.

The esterification of high-acidity oils, such as rice bran oil, presents a problem because these materials contain, in addition to triglycerides, varying amounts of FFA, as well as mono- and diglycerides. Attempts to prepare methyl esters with acidic catalysts and excess methanol afford rapid and essentially complete conversion of FFA, but slow alcoholysis of the glycerides. On the other hand, alcoholysis of oils in alkali-catalyzed direct esterification of fatty acids is quite slow. For this reason, rapid and efficient conversion of high-acidity oils to methyl esters by a one-step process is not yet practical (4).

The concept of transesterification of sunflowerseed oil *in situ* was described by Harrington and D'Arcy-Evans (5,6), and they demonstrated that significant increases in ester yields could be achieved by such a method because of the following reasons: (i) by subjecting the whole seed to the esterification process, the lipid content of the hull itself could contribute to the overall yield of esters from the seed; and (ii) the esterified lipids, with viscosities and solubilities different from those of triglycerides, could prove easier to recover from the solid residue.

In this study, *in situ* esterifications of high-acidity rice bran oil with methanol and ethanol were investigated because this method offers the advantage that it eliminates the extraction step and, thus, it could be possible to recover the oil directly as a valuable product.

MATERIALS AND METHODS

Rice bran. The rice bran used in this study was obtained from a local rice mill in Istanbul. During the course of this study, the bran was kept in a covered jar at ambient temperature. Oil and moisture contents of bran and FFA contents of the oil were determined weekly (7). The oil content of the bran was determined by extraction in a Soxhlet apparatus with hexane for six hours.

In situ esterification. Rice bran (50 g) was transferred to a flask, and 200 mL of methanol (99.7%) or ethanol (96%) was added. The mixture was refluxed with a catalyst of concentrated sulfuric acid while stirring with a magnetic stirrer for 1 or 4 h. The course of reaction was followed by examination of alcoholic phases by thin-layer chromatography (TLC). At the end of the reaction, the mixture was filtered and the bran was washed with alcohol. After drying at room temperature, the bran was reextracted in a Soxhlet apparatus with hexane to obtain the oil fraction left in the bran.

Most of the alcohol was evaporated from the filtrate, and the mixture was extracted three times with hexane to remove the esterified product. The combined extracts were washed with water, dried over sodium sulfate and evaporated to give the esterified product. The oil left in the bran and the esterified product obtained in each reaction were investigated by TLC. The FFA contents of these oils also were determined.

TLC was performed on glass plates coated with Silica Gel G (Merck, Darmstadt, Germany) and developed in a solvent system of petroleum ether/diethyl ether/acetic acid (90:10:1), vol/vol/vol). Spots were detected by iodine vapor staining (8).

RESULTS AND DISCUSSION

Table 1 shows changes in moisture and oil contents of the bran and FFA percentage of rice bran oils (as oleic acid) during storage.

In situ *esterification with methanol.* As in the first experiments, two *in situ* esterification reactions were carried out for 4 h; one of the mixtures contained 5 mL sulfuric acid as catalyst, the other contained no catalyst. The oil in the bran used in these reactions had an FFA content of 14.5%. Examination of the methanolic aliquots taken each hour for TLC showed that no methyl esters were produced without the catalyst. The main components of the methanol phases were fatty acids with minor amounts of partial glycerides. Practically no triglycerides and wax esters were found in these phases. This observation implies that these components did not dissolve in the methanol but remained in the bran. TLC examination of the reaction mixture in which sulfuric acid was used as catalyst showed that the main components were fatty acid methyl esters with minor amounts of partial glycerides. FFA, triglycerides and wax esters were not detected in these phases. These two reactions showed that it was necessary to use sulfuric acid as catalyst to convert fatty acids into methyl esters. Practically all the FFA dissolved in methanol were esterified, and all triglycerides and wax esters remained in the bran during *in situ* esterification with sulfuric acid.

To determine the effect of the amount of catalyst on the degree of esterification, two experiments with 2.5 mL and

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TABLE 1

Changes of Rice Bran During Storage

Time (d)	Moisture (%)	Oil (%)	Free fatty acid of oil (%)
0	—	18.8	14.2
7	11.8	18.7	32.8
16	12.7	18.9	45.8
21	11.7	18.9	50.8
28	11.6	18.8	55.8
36	12.4	18.6	56.8
42	11.1	18.9	60.3
51	11.9	18.7	63.4
63	12.6	19.0	68.4

TABLE 2

***In situ* Esterification of Rice Bran with Methanol**

Free fatty acid (FFA) of oil (%)	Residual oil in bran		Oil converted to methyl esters (%)
	Amount (%)	FFA (%)	
19.0	76.1	1.02	23.9
34.4	61.2	1.00	38.8
41.6	52.7	1.24	47.3
50.8	45.5	1.58	54.5
57.5	35.1	1.67	64.9
60.3	28.9	3.82	71.1
68.4	14.1	1.89	85.9

5 mL sulfuric acid, respectively, were carried out for 1 h, and reaction courses were followed by TLC examinations of the methanol phases sampled every 15 min. In the sample treated with 2.5 mL sulfuric acid, both FFA and methyl esters were present after 1 h of reaction; however, all the fatty acids in the sample treated with 5 mL H_2SO_4 were converted to methyl esters within the first 15 min of reaction, leaving no FFA in the medium.

According to these findings, the quality and quantity of the methyl ester fraction that can be obtained from a given rice bran seem to be dependent on the FFA content of its oil, because it was observed that practically all of the FFA were dissolved in methanol and esterified, whereas methanol-insoluble triglycerides and other nonpolar components of the oil remained in the bran. Furthermore, the solubility of triglycerides in methanol increases with an increase of the accompanying FFA in the medium (9). Therefore, higher FFA contents in the bran oil lead to increased solubilities in methanol of the triglycerides as well.

To determine the effect of FFA content of oil on esterification, brans with different storage histories were esterified with methanol (Table 2). Reaction time was 1 h and the amount of sulfuric acid was 5 mL for each reaction. The percentages of the oil converted to methyl esters increased with an increase in the FFA content of the oil. According to the TLC examinations, all the methyl ester fractions had nearly the same composition, *i.e.,* they contained only partial glycerides as impurities. The methyl esters obtained from rice bran with 60.3% FFA contained no triglycerides, but when the same bran was dissolved in methanol some of the triglycerides were detected in the methanol phase (Fig. 1). Therefore, it was deduced that triglycerides dissolved in methanol were transesterified under these conditions.

In situ *esterification with ethanol.* The same experiments described above were repeated with ethanol. When esterification was carried out in the absence of sulfuric acid, no ethyl esters were detected in the ethanol phase after 4 h. The components dissolved in ethanol were mainly FFA, triglycerides, partial glycerides and small amounts of wax esters. When 5 mL sulfuric acid was used, most of the FFA were esterified. However, small amounts of fatty acids and triglycerides were not converted into esters even after 4 h. Furthermore, it was not possible to esterify all of the fatty acids and glycerides dissolved in ethanol by increasing the amount of sulfuric acid to 7.5 and 10 mL. The ethyl ester fractions isolated from ethanol phases had FFA contents of 6–7%.

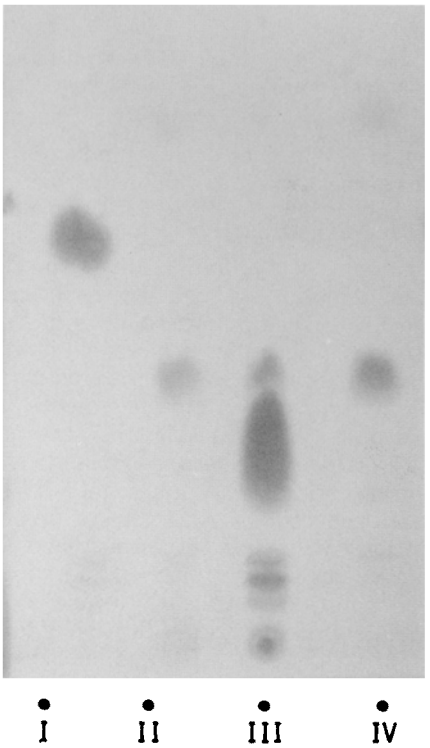

FIG. 1. Typical chromatogram of products obtained from *in situ* esterification of rice bran (603% free fatty acids) with methanol and of methanol-soluble and insoluble fractions of the same bran. Lane I, methyl esters; lane II, residual oil in bran; lane III, methanol-soluble fraction; and lane IV, methanol-insoluble fraction.

Table 3 shows the results obtained from *in situ* esterifications with ethanol of rice brans with different FFA contents. Reaction time was 1 h, and the amount of sulfuric acid was 7.5 mL for each reaction. Unlike the study with methanol, the amount of the residual oil in bran and the percentage of oil converted to ethyl esters were not dependent on the FFA content of bran, because the solubilities of oil components in ethanol were much higher than those in methanol. A typical chromatogram of the fractions obtained from esterification of a rice bran (57.0% FFA) is shown in Figure 2.

By comparing results obtained from methanol and ethanol esterification, it was noted that the relative com-

TABLE 3

***In situ* Esterification of Rice Bran with 96% Ethanol**

Free fatty acid (FFA) of oil (%)	Residual oil in bran		Oil converted to ethyl esters (%)
	Amount (%)	FFA (%)	
19.0	5.7	3.39	94.3
30.3	7.0	7.74	93.0
41.6	2.4	9.56	97.6
57.0	4.7	4.19	95.3
60.3	1.3	10.33	98.7
61.2	8.7	5.49	91.3

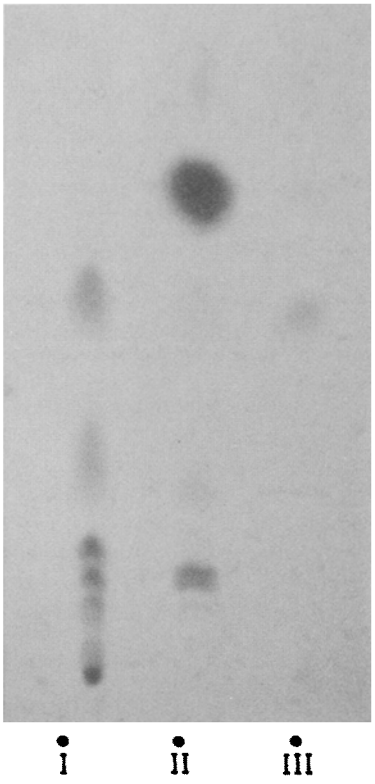

FIG. 2. Typical chromatogram of fractions obtained from *in situ* esterification of rice bran (57.0% free fatty acids) with ethanol. Lane I, rice bran oil; lane II, ethyl ester fraction; and lane III, residual oil in bran.

positions and the amounts of esters that can be obtained from a given rice bran were quite different depending on the solvent. In methanol esterification, it was possible to obtain purer esters, which could not be obtained by conventional one-step esterification processes because methanol selectively dissolves fatty acids.

For low-acidity rice bran oils, *in situ* methanol esterification may also be considered as a deacidification process because all the FFA are removed from the bran as methyl esters, whereas practically all of the triglycerides remain in the bran.

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