No single test can thus be expected to reflect the total decomposition pattern or to determine, accurately and precisely, a sharp "endpoint" beyond which the frying oil is to be rejected. The accuracy and/or precision obtained if two tests are used, as proposed above, will naturally depend on the accuracy and precision of these two tests.

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REFERENCES

1. Nelson, R.C., *Instruments and Control Systems 36*:117 (1963).
2. Billek, G., G. Guhr and J. Waibel, *J. Amer. Oil Chem. Soc. 55*:728 (1978).
3. Paradis, A.J., and W.W. Nawar, *Ibid. 58*:635 (1981).
4. Peled, M., R. Gutfinger and M. Letan, *J. Sci. Food Agric. 26*:1655 (1975).
5. Meltzer, J.B., E.N. Frankel, T.R. Bessler and E.G. Perkins, *J. Amer. Oil Chem. Soc. 58*:779 (1981).
6. Bhalerao, V.R., J.G. Endres and F.A. Kummerow, *Ibid. 38*:689 (1961).
7. Official and Tentative Methods of the American Oil Chemists' Society, Vols. I and II, Champaign, IL, Methods Ca5a-40 and Cc9a-48.

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Indian Ricebran Lecithin

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In view of the high potential of ricebran oil in India, lecithins recovered from crude and dewaxed Indian ricebran oil were analyzed and different classes characterized with the objective of effectively utilizing this valuable by-product. Lipid classes and individual phospholipid components were identified and estimated. Dewaxing was found to have a considerable effect on composition of the derived lecithin. The lecithin obtained from crude or dewaxed Indian ricebran oil consisted mainly of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and triglycerides, along with carbohydrates, free fatty acid, sterols and waxes (in case of crude oil). The major fatty acids of individual phospholipids were found to be palmitic, oleic and linoleic. Analytical characteristics of ricebran lecithin were shown to be comparable to local soybean lecithin. It can be expected that the gummy materials in the oil, presently lost with the soapstock during refining, could find important applications.

Some 0.15 million tons of ricebran oil are recovered in India annually, and the untapped potential is about four times this figure (1). The present potential of ricebran lecithin (which amounts to roughly 1% of the oil) is approximately 6,000 tons; this will rise with the planned increases in rice production. The objective of this investigation was to assess the suitability of upgrading this highly valuable by-product of ricebran oil to edible grade. Since all lecithin used in India is imported, this locally produced product would be attractive.

Egyptian workers have studied the phospholipid range (1.2-1.94%) of local solvent extracted ricebran oils and composition of the isolated lecithin (2). Fatty acid compositions of total phospholipid and cephalin and lecithin fractions were determined.

The objective of the present study was to determine more completely the composition of ricebran lecithin. Lecithin obtained from crude and dewaxed ricebran oil of Indian origin was analyzed for the different lipid classes present. Each class was identified by different techniques and estimated. Since phospholipids constitute the most important class, the phospholipid components were further resolved, identified and quantified using TLC techniques.

Fatty acid compositions of the total lecithin and each individual phospholipid also were determined. Finally, analytical characteristics of ricebran lecithin were determined and compared with those obtained with local soybean lecithin and with U.S. specifications.

MATERIALS AND METHODS

A commercial ricebran oil[1] obtained through Hindustan Vegetable Oils Corporation Ltd. was used throughout the investigation and had a phosphatide content of 0.6% (AOCS Method) (3).

The oil drawn from ambient temperature storage (30-33 C) was centrifuged to remove impurities like sludge, mucilage, tissue particles and fibers.

For dewaxing, the clarified oil was mixed with n-hexane in 1:1 ratio (v/v) and kept at 10 C with occasional mild stirring for 4 hr, filtered free of wax, and the oil desolventized (4).

TABLE 1

Composition of Ricebran Lecithin (wt %)

	From crude oil	From dewaxed oil
Phosphatidylcholine	20.4	23.1
Phosphatidylethanolamine	17.8	20.2
Phosphatidylinositol	5.8	6.6
Other phosphatides	9.4	10.8
Triglycerides	39.2	35.2
Wax	3.1	—
Carbohydrates, sterols, FFA	4.0	3.8
Moisture	0.3	0.3

[1]Characteristics of the oil: acidity, expressed as % oleic acid, 8.4; color (1/4" cell, Lovibond), 1.2 R/18.0Y; fatty acid composition, myristic and lower, 0.5, palmitic, 16.5, stearic, 1.5, oleic, 44.5, linolenic, 36.0, linolenic, 0.1, others, 0.9 (wt %).

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The clarified and dewaxed oil was next heated to 50–60 C, hot water (95 C) added (the amount being close to the phosphatide content, max. 2%, v/v) and stirring continued at 250–300 rpm for 10–15 min at the same temperature. Hydrated phosphatides were removed by centrifugation of the oil-water mixture at 2000 rpm (RCF × g = 700) for 10 min.

Composition of lecithin obtained from ricebran oil. The crude lecithin (50–60 mg) was separated into lipid classes by preparative thin layer chromatography (TLC) on silica gel G in band form using hexane-diethyl ether-acetic acid (80:20:1) (5). The components were identified by comparing R_f values against standard samples and by reaction with various spray reagents. Identified components were eluted with chloroform:methanol:ether (1:1:1, v/v/v), desolventized and weighed. Compositions of the crude lecithins obtained from both crude and dewaxed oil are shown in Table 1.

Separation and identification of phospholipids. The acetone-insoluble portion was precipitated from the crude lecithin by the standard method of Cocks and van Rede (6). The precipitated phospholipids were further purified by column chromatography.

Identification of resolved phospholipids using spray reagents. The isolated phospholipids were resolved by silica gel TLC of the mixture using either (a) chloroform-methanol-water (65:25:4); (b) chloroform-methanol-28% ammonia (65:25:5), or (c) chloroform-methanol-acetone-acetic acid-water (6:2:8:2:1). Spots were visualized using the following spray reagents: (a) iodine vapor, revealing all lipid materials; (b) ammonium molybdate-percholoric acid reagent, specific for phospholipids; (c) ninhydrin specific for amino-groups, and (d) Dragendorff's reagent, specific for cholines (Fig. 1). The phospholipids were identified from the response of the spots to the spray reagents and by comparing their R_f values with literature data (5).

Identification of phospholipids from hydrolyzed products. Different phospholipid classes were subjected to strong acid hydrolysis for the liberation of bases and glycerol or inositol, so as to have further confirmation regarding identity of the phospholipids. The hydrolytic products were identified using TLC and compared with literature R_f values.

Quantitative separation. Phospholipids were separated into individual classes by preparative TLC (Silica gel G, 0.5 mm, $CHCl_3$-MeOH-H_2O, 65:25:4). Bands were visualized with iodine vapor, scraped off from TLC plates, and extracted using solvent (2:1 $CHCl_3$-MeOH). The solutions were filtered, desolventized and phospholipid components determined gravimetrically (Table 2).

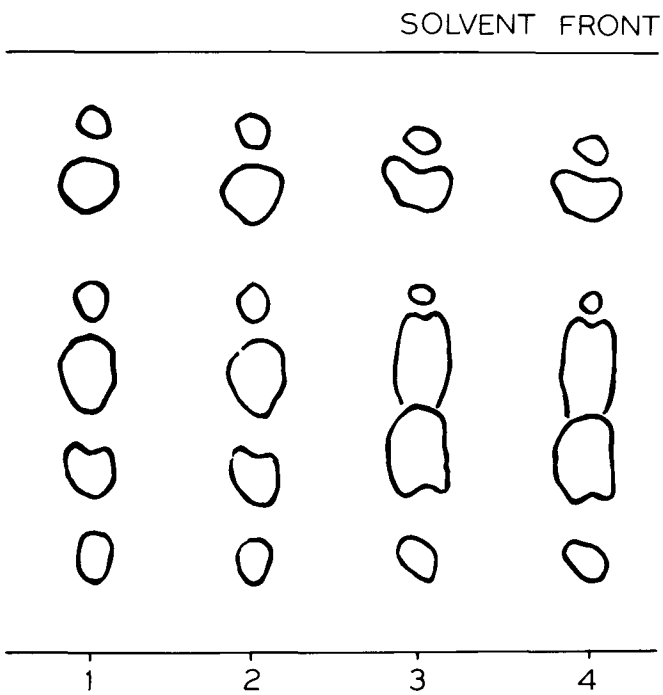

FIG. 1. Thin layer chromatogram of phospholipids. Spots 1 and 2, dewaxed ricebran oil; spots 3 and 4, Indian soybean oil. Solvent system 65:25:4, chloroform-methanol-water. Ammonium molybdate detection agent; charring, 120 C.

TABLE 2

Individual Phospholipid Composition of Phosphatides (wt %)

	Ricebran phospholipids	Indian soy phospholipids[a]
Phosphatidylcholine	38.0	34.8
Phosphatidylethanolamine	33.2	23.6
Phosphatidylinositol	10.9	33.8
Phosphatidylglycerol	8.6	4.5
Lysolecithin	5.7	2.8
Phosphatidic acid	3.6	1.5

[a]Unpublished data.

TABLE 3

Fatty Acid Composition of Ricebran Oil Phospholipids

	Fatty acids (wt %)				
	16:0	18:0	18:1	18:2	18:3
Phosphatidylcholine	46.0	4.8	38.3	10.4	0.5
Phosphatidylethanolamine	24.7	2.6	17.4	55.0	0.3
Phosphatidylinositol	27.9	3.2	20.8	47.9	0.2
Phosphatidylglycerol	25.1	3.5	20.4	50.5	0.5
Lysolecithin	47.2	4.9	31.6	15.0	1.3
Phosphatidic acid	11.8	4.3	39.8	39.7	4.4

TABLE 4

Fatty Acid Compositions of Commercial Ricebran and Soybean Lecithin

	Fatty acids (wt %)				
	16:0	18:0	18:1	18:2	18:3
Ricebran	18.1	4.0	42.8	33.6	1.5
Soybean	10.8	3.9	24.2	54.8	6.3

TABLE 5

Characteristics of Ricebran Lecithin and Indian Soybean Lecithin

Analysis	Indian ricebran	Indian soybean	U.S. soy lecithin specification
Acetone-insoluble (%)	63.0	65.0	62 (min)
Moisture (%)	0.3	0.5	1 (max)
Benzene-insoluble (%)	0.1	0.1	0.3 (max)
Acid value, expressed as % oleic acid	20.8	27.0	30.0 (max)
Color	Comparable to imported soybean lecithin		10 (max) on Gardner scale
Viscosity, poises at 25 C	Matches that of imported soy lecithin		150 (max)

Fatty acid composition. Crude lecithin and its various phospholipid components were converted into methyl esters of fatty acids by treatment with 0.5M sodium methoxide in methanol for about 30 min at 50 C (7).

Fatty acid compositions were determined using a Varian 3700 gas-liquid chromatograph with flame ionization detector. A 6-ft by 1/8-inch stainless steel column packed with 3% EGSS-X supported on Aeropak 30(80/100) was used. Oven temperature was maintained at 160 C. Injector and FID temperatures were 200 C and 230 C, respectively. Carrier gas (N_2) flow rate was 25 ml/min. The weight percentage of different fatty acids was calculated with a computer data system CDS 111 (Tables 3 and 4).

Analytical evaluation. Acetone-insoluble and benzene-insoluble materials, moisture, and acid value were determined for the lecithin product obtained from dewaxed ricebran oil by published methods (6). Corresponding values for lecithin produced from Indian soybean oil were compared. Color and viscosity of the product were compared with those of an imported soy lecithin sample (Table 5).

RESULTS AND DISCUSSION

Dewaxing of crude ricebran oil has a considerable effect on composition of the lecithin product derived by hydration. Crude undewaxed oil yields a lecithin containing about 3% wax (Table 1), which is absent in the lecithin obtained from dewaxed oil. Further, the triglyceride content is slightly greater (39.2%) in the lecithin from crude oil than that (35.2%) from dewaxed oil. The total phospholipid content is considerably (7.3%) lower in the lecithin derived from crude oil. The lecithin product from dewaxed oil has increased phospholipid proportions: phosphatidylcholine, 23.1%; ethanolamine, 20.2%, and inositol, 6.6%. The appearance of lecithin improves considerably by dewaxing the oil.

Phosphatidylcholine (lecithin) (38:0%) and ethanolamine (cephalin) (33.2%) are the major phospholipids present (Table 2). Lesser phospholipids present are phosphatidylinositol (10.9%), phosphatidylglycerol (8.6%), lysolecithin (5.7%) and phosphatidic acid (3.6%). The phosphatidylinositol content of ricebran lecithin is 10.9%, compared to 33.8% in Indian soy lecithin (unpublished data).

The major fatty acids in all the phospholipid classes are palmitic, oleic and linoleic (Table 3). Palmitic acid is high in phosphatidylcholine and lysolecithin, followed by oleic acid. The linoleic acid contents of phosphatidylethanolamine, phosphatidylinositol and phosphatidylglycerol are in the range of 47.9 to 55.0%, and those of oleic acid 17.0 to 20.8%. Phosphatidic acid contains less palmitic acid than the other fractions, about 40% each of oleic and linoleic acids and 4.4% of linolenic acid, a considerably higher figure than in the other fractions (0.3–1.3%) (Table 4). This commercial ricebran lecithin has a higher proportion of saturated acid (22.1%) than does Indian soy lecithin (14.7%). The other major differences in fatty acid composition are higher percentage of oleic acid (42.8%, 24.2%) and lower percentage of linoleic acid (33.6%, 54.8%) in ricebran than in soy lecithin. The lower level of linolenic acid (1.5%, 6.3%) should give ricebran lecithin greater resistance to autoxidation and development of off-flavors than soy lecithin.

The analytical characteristics of Indian ricebran and soy lecithins are comparable. Both conform to U.S. specifications for soy lecithin.

It appears that the gummy materials containing valuable phosphatides, which presently are lost in the soapstock when crude ricebran oil is refined, have potential to be upgraded to commercial lecithin for useful food and non-food applications.

REFERENCES

1. Achaya, K.T., *Proceedings of Seminar on Technology and Product Mix Forecast—Oils and Fats in 2000 A.D., and 39th Annual Convention, Oil Technologists' Association of India,* Dec. 1983, p. 53.
2. El-Shattory, Y., L.H. Fade and H.M. El-Khalafy, *Grasas Aceites 9*:309 (1979).
3. *AOCS Official and Tentative Methods,* Champaign, IL, 1979, Method No. Cal2-55.
4. Bhattacharyya, D.K., and A.C. Bhattacharyya, *Proceedings of the Seminar Organized by Oil Technologists' Association of India (Southern Zone) on Ricebran Oil Status and Prospects,* Regional Research Laboratory, Hyderabad, Aug. 13, 1983, p. 57.
5. Kates, M., *Techniques of Lipidology,* North Holland Publishing Company, Amsterdam, 1975, p. 436.
6. Cocks, L.V., and C. van Rede, *Handbook of Oils and Fats Analysis,* Academic Press, New York, 1966, p. 137.
7. Christie, W.W., in *Topics in Lipid Chemistry,* Vol. 3, edited by F.D. Gunstone, Logos Press Ltd., London, 1972, p. 191.
8. National Soybean Processors' Association, *Yearbook and Trading Rules,* NSPA, Washington, D.C., 1976, p. 60.

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