Fatty Acid Composition of Rice Lipids by Gas-Liquid Chromatography[1]

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Abstract

Gas-liquid chromatographic analysis of the methyl ester of lipids of four rice varieties showed that bran lipids had significantly higher mean contents of linoleic and linolenic acids, but lower contents of myristic, palmitic, palmitoleic, and stearic acids than milled rice lipids. Nine fatty acids were detected. The principal components were oleic, linoleic, and palmitic, which also was confirmed by thin-layer chromatography of the esters.

Introduction

MILLING OF brown rice results in milled rice which has lower contents of protein, lipids, crude fiber, and ash than the original. The amino acid composition of the protein of brown and milled rice has been demonstrated to be different (12). A similar difference in iodine and saponification values of milled rice and brown rice lipids has been noted (19).

Recent advances in gas-liquid chromatography (GLC) have made the fatty acid analysis of the methyl esters of lipids routine. The GLC also more readily separates and detects polyunsaturated acids than previous methods (9,10). As part of a program of physicochemical characterization of the rice kernel, the fatty acid composition of the lipids of the two brown rice fractions, bran and milled rice, of four Asian varieties was determined by GLC. This also is of interest because of the reported nutritional role of polyunsaturated acids in depressing serum cholesterol levels (1).

Experimental

Rough rice of four varieties was dehulled with a McGill Sheller. The resulting brown or dehulled rice was milled with a McGill Mill No. 3 into milled rice and bran (true bran plus polish plus germ) (17). These varieties had been grown under uniform conditions at the Institute during the 1962 rainy season, and consisted of two *indica* varieties, Peta (nonwaxy or nonglutinous) and Malagkit Sungsong Puti (waxy or glutinous), and two *japonica* varieties, Taichung 65 (nonwaxy) and Taichung Glutinous 46. Milled rice was ground to a 40-mesh powder with a Wiley mill. Moisture content of the samples was determined by the loss of wt after 5 hr at 98–100C in a vacuum oven (4). The content of lipids was determined by anhydrous petroleum ether (BP 40.8–56.4C) extraction (4) of the dried samples in a Goldfisch extractor.

The lipids were extracted, especially from bran, as soon as possible after milling. Milled rice powder (1,000 g) and bran samples (100 g) were pre-dried in a vacuum oven at 90C for at least 1 hr. Lipids were extracted from the pre-dried samples in Soxhlet extractors with petroleum ether for at least 8 hr, and the solvent was evaporated from the extract under reduced pressure in a rotary evaporator. The iodine value (I.V.) was determined in duplicate for all samples by the Wijs method (3). Free fatty acids content of the lipids were all below 4% as oleic acid (3).

The fatty acid methyl esters were prepared from the lipids by direct inter-esterification. Lipids (250 mg) were refluxed for 1.5 hr in 25 ml 1.5% reagent grade sodium methoxide in absolute methanol. The mixture was cooled, and excess methoxide was neutralized with 1.5 ml glacial acetic acid. Petroleum ether (30 ml) and 2 ml water were then added to the solution with shaking. The ether layer was separated, and the aqueous methanol phase was re-extracted twice with 30-ml portions of ether. The ether extracts were pooled, and the solvent eliminated by concentration under reduced pressure in a rotary evaporator. Yield: 85–91%.

The methyl ester preparations were separated from unsaponifiable matter by the silicic acid column chromatographic method of Luddy and co-workers (13) with petroleum ether-diethyl ether (99:1 v/v) as eluting solvent. The solvent was evaporated under reduced pressure from the eluate in a rotary evaporator. Yield: 90–93%.

For the GLC analysis, 1–2 μl samples of purified methyl ester preparation were used in tripilcate. The apparatus was a Perkin-Elmer Model 154L Vapor Fractometer with a four-filament (tungsten) thermal conductivity detector attached to a Leeds and Northrup Model G 0-5 mv, 1-second response recorder linked to a Perkin-Elmer Model 194B Automatic Printing Integrator. The column was a 2-m long, 0.25-in. O.D. stainless steel tube packed with 20% diethylene glycol succinate (DEGS) polyester (mol wt ca. 4,000) adsorbed on 60–80 mesh C-22 firebrick. The apparatus was calibrated against the National Institutes of Health's Metabolism Study Section, Standard Mixtures C and D. Conditions of the GLC were: sample injection block temp, ca. 270C; column temp, 203 ± 1C; detector current, 260 ma; detector temp, 335C; helium (Matheson) carrier gas inlet pressure, 21 psig; and gas outlet flow rate, 70 ml per min. The mean analysis time was 24 min.

Fatty acid composition was calculated directly by the peak area method. Preliminary results, using standard fatty acid methyl esters (California Corp. for Biochemical Research) in the approximate ratio found in rice lipids, showed an essentially linear relationship between peak area and wt percentage of the fatty acids. The retention times of the acids closely agreed with reported values on an identical apparatus at 210C (15).

TLC analysis of the methyl esters was used to confirm the GLC data. Siliconized chromatoplates (20 × 20 cm) of 0.25 mm Silica Gel G (E. Merck Ag.) containing 1% soluble starch were prepared and developed with acetonitrile-acetic acid-water (14:2:5 v/v/v) after Malins and Mangold (14). The chromatograms were sprayed with 1% α-dextrin in 30% aqueous methanol, exposed to iodine, and the acids were identified by comparison of R_f values with known fatty acid methyl esters.

A sample of crude rice bran oil (California Rice Growers Assoc., Sacramento) and of edible bran oil (Comet Rice Mills, Houston, Texas) also was analyzed for I.V. and for fatty acid composition by GLC.

[1] Issued as I.R.R.I. Journal Series No. 12.

TABLE I

Contents of Lipids of Rice Bran and Milled Rice

Variety	Lipids content [a]		Bran in brown rice [a]	Brown rice lipids in bran
	Bran	Milled rice		
	%	%	%	%
Peta	19.7	0.39	10.1	85.0
Malagkit Sungsong Puti	23.5	0.34	11.9	90.3
Taichung 65	21.7	0.30	8.88	87.6
Taichung Glutinous 46	20.2	0.40	8.35	82.1
Mean	21.3	0.36	9.81	86.2

[a] % Dry-weight basis.

Results and Discussion

The quantities of lipids in the brown rice fractions (Table I), bran and milled rice, agree with the ranges of 9–22% and 0.3–0.4% of dry matter, respectively, reported by Eckey (7). The bran fraction contained the bulk of the lipids in brown rice, with only 13.8% of the lipids present on the average in the milled rice samples.

The mean I.V. of bran lipids were significantly higher than those of milled rice lipids (Table II). This contrasts with the report of Yamasaki (19) that milled rice had a slightly higher I.V. than brown rice. However, his milled rice sample had higher oil contents, being 0.41–1.48%. The bran lipids of the *indica* rice samples, Peta and Malagkit Sungsong Puti, had lower I.V. than the *japonica* samples.

Fatty acid analysis by GLC of the methyl esters of rice lipids show marked contrast in composition between bran lipids and milled rice lipids (Table II; Fig. 1). Nine acids were detected by GLC, the principal acids being oleic, linoleic, and palmitic. TLC of the methyl esters confirmed the identification of the three main acid components. Minor constituents were lauric, myristic, palmitic, palmitoleic, stearic, and arachidic acids. Less than 0.1% lauric acid was present in the samples. Bran lipids had higher total contents (38.2 ± 0.9%) of polyunsaturated acids, linoleic and linolenic, than milled rice lipids (18.6 ± 0.9%), which is consistent with the lower I.V. noted for the latter samples. However, the latter had higher contents of myristic, palmitic, palmitoleic, and stearic acids. This demonstrates that there exists in the rice kernel a difference not only in the quantity of lipids in the bran and milled rice fractions, but also in the fatty acid composition of these lipids.

The two samples of U.S. rice bran oil (Table III) had higher I.V. but had comparable fatty acid composition by GLC to the experimental Asian bran lipids. An I.V. of 82.9 has been reported for whole rice lipids of a Pakistani rice, which reflects varietal differences in characteristics of rice lipids (2). The discrepancy in I.V. may also in part result from the commercial dewaxing process in industry. This removes the more saturated glycerides.

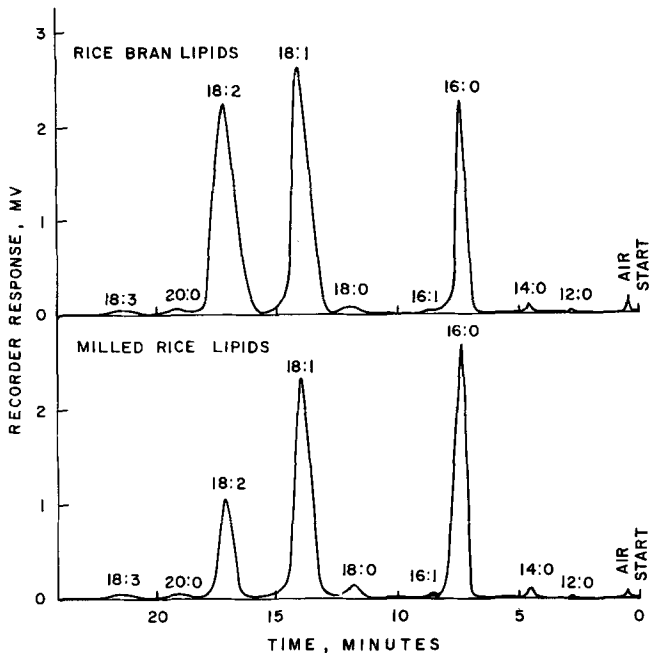

FIG. 1. Chromatogram of methyl esters of rice bran and milled riced lipids. Column 2 m × 0.25 in., temp 203C, 20% DEGS on C-22 firebrick. 4 x-attenuation.

Although the I.V. of the experimental samples were lower, their fatty acid composition agrees with those of other investigators (Table III). The GLC data of Morita and Higashiya (15) on a Japanese commercial bran oil sample, however, indicate only seven acids. The reported absence of linolenic acid in the two samples of Philippine bran oil samples of Cruz, et al. (5,6) was based on a negative hexabromide test. This hexabromide method was later shown not to be reliable for the detection of small amounts of this acid, and rice bran oil has only ca. 1% linolenic acid (11).

Recently, Wu and Williams (18) reported the fatty acid composition range of the lipids of 50 samples of U.S. milled rice, as determined by GLC, to be 0.6–1.8% myristic, 20–30% palmitic, 22–34% oleic, and 40–50% linoleic acids. Our data differ in that nine acids were detected with relatively higher contents of palmitic and oleic acids, but much lower contents of linoleic and myristic acids. They did not detect stearic acid. Their extracting solvent was 85% methanol, instead of petroleum ether.

From the mean composition of the brown rice fractions (Table I), and their lipids (Table II), the mean

TABLE II

Iodine Value and Fatty Acid Composition of Lipids of Rice Bran and Milled Rice

Variety	I.V. (Wijs)	Fatty acid [a]							
		14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Rice bran lipids									
Peta	88.8	0.3	20.5	0.2	1.1	41.9	34.1	1.1	0.7
Malagkit Sungsong Puti	85.4	0.4	17.8	0.2	1.4	44.2	34.5	0.9	0.7
Taichung 65	97.3	0.2	16.9	0.1	1.8	40.8	38.9	0.9	0.3
Taichung Glutinous 46	90.4	0.5	18.0	0.2	1.4	37.1	40.7	1.4	0.7
Mean	90.5	0.3	18.3	0.2	1.4	41.0	37.1	1.1	0.6
Milled rice lipids									
Peta	73.1	0.6	25.1	0.5	1.4	42.6	28.3	0.9	0.6
Malagkit Sungsong Puti	69.2	0.5	28.7	0.4	2.4	46.1	21.3	0.4	0.4
Taichung 65	48.5	0.6	37.4	0.4	3.8	45.2	12.0	0.3	0.3
Taichung Glutinous 46	57.2	0.8	44.1	0.4	3.3	39.4	10.6	0.8	0.5
Mean	62.0	0.6	33.8	0.4	2.7	43.3	18.0	0.6	0.4
Sources of variation [b]									
Brown rice fraction (F)	*	*	*	**	*	n.s.	*	*	n.s.
Variety (V)	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.
Interaction (FxV)	**	*	**	n.s.	*	**	**	n.s.	n.s.

[a] Wt % of total acids. Mean of 3 GLC replications. Trace 12:0 present.
[b] n.s. = not significant; * = significant; and ** = highly significant.

TABLE III

Iodine Value and Fatty Acid Composition of Rice Bran Lipids and Commercial Oils as Reported by Various Investigators

Sample origin	Iodine value	Fatty acid [a]							
		14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Philippines (Table I) [b]	90.5	0.4	18.3	0.2	1.4	41.0	37.0	1.1	0.6
United States (crude oil) [c]	103.4	0.4	17.9	0.2	1.3	41.2	37.7	1.1	0.1
United States (edible oil) [c]	105.3	0.5	18.3	0.4	1.5	45.0	33.5	0.5	0.3
Japan (15) [d]	.....	0.55 [e]	20.99	....	1.63	38.14	37.79	0.90	....
Philippines (5) [d]	99.3	0.2	18.7	....	1.9	49.3	29.9	....	0.7
Philippines (6) [d]	99.5	0.1	18.3	....	2.8	49.0	29.8	....	0.5
Various sources (7)	99-108	0.4–1.0	13–18	....	1–3	40–50	29–42	0–1	....

[a] Wt % of total acids.
[b] Mean of 4 varieties. Trace 12:0 present.
[c] Mean of 3 determinations. Trace 12:0 present.
[d] Recalculated to exclude unsaponifiable matter.
[e] Including 12:0.

fatty acid composition of the brown rice lipids of the four Asian rice samples may be calculated as 0.4% myristic, 20.4% palmitic, 0.2% palmitoleic, 1.6% stearic, 41.3% oleic, 34.5% linoleic, 1.0% linolenic, 0.6% arachidic, and trace lauric. This compares well with data based on U.S. whole rice lipids: 13% palmitic, 2% stearic, 4% other saturated acids, 42% oleic, 38% linoleic, and 1% other unsaturated acids calculated as percentage of total acids (8).

ACKNOWLEDGMENTS

Crude rice bran oil obtained through R. R. Mickus, Director of Research, Rice Growers Association of California, Sacramento; edible oil, from Harriet Nawry, Comet Rice Mills, Houston, Texas. Moisture and lipids contents by Remedios Santiago.

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[Received September 3, 1963—Accepted November 18, 1963]

Effect of Oxygen and Other Factors in Selenium Catalyzed Isomerization of Unsaturated Fatty Acid Esters[1,2]

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Abstract

In the *trans*-isomerization of ethyl linoleate using 1% selenium as catalyst under nitrogen, 200C was found to be the optimal temperature. Conjugation and polymerization were concurrent with the formation of the *trans* esters. In the isomerization of ethyl oleate with selenium, the double bond did not migrate to any appreciable extent.

Isomerization studies were performed on olive, safflower and linseed oils and ethyl esters of oleic, linoleic and linolenic acids. Time to reach maximal *trans* isomer content was longest with linolenate esters; glycerides reacted more rapidly than ethyl esters, and with lesser polymerization.

Oxygen was found to be an important participant in the *trans*-isomerization reaction. Its exclusion resulted in sharply diminished reaction rates. Benzoyl peroxide and hydrogen peroxide accelerated while an antioxidant (BHT) retarded the reaction.

[1] Journal Paper No. 2046, Purdue University Agricultural Experiment Station.
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Introduction

HEATING OF AN unsaturated fatty acid with selenium is known to produce changes in configuration; at equilibrium the product has definite proportions of *cis* and *trans* isomers. The rates of isomerization under nitrogen have been determined by various workers; in many of these studies, older methods of estimation of *trans* isomers were used and often led to conflicting results. The present experiments were performed to determine the optimal conditions for the preparation of *trans* fatty acids.

Bertram (1), in 1938, converted oleic acid to elaidic acid by heating with 0.5% selenium at 150C for 28 hr; results showed that the reaction was trimolecular. Kass and co-workers (2,3) have studied isomerization of methyl linoleate and mixed methyl esters of linseed oil. During the preparation of linolelaidate and linolenelaidate with 1% selenium under nitrogen, they found that 6 and 17 hr, respectively, were needed to attain maximum *trans* ester formation. Hilditch and Jasperson (4) from their studies concluded that oleate and linoleate are similar in the amount of time required for complete isomerization. In more recent years Fitzpatrick and Orchin (5) have studied oleic acid isomerization with selenium. They reported that