THE EFFECT OF STORAGE TIME ON THE COMPOSITIONAL PATTERNS OF RICE FATTY ACIDS[1]

TEN-CHING LEE, WEI T. WU, AND VIRGINIA R. WILLIAMS

ABSTRACT

As part of a study on the aging of rice, an investigation has been made of the effect of storage at room temperature on the compositional patterns of rice fatty acids. One large sample of freshly harvested Belle Patna rice was used for the entire experiment and was sampled every 2 weeks for a period of 6 months. The rice was shelled, milled, and ground to 20-mesh on the day of sampling and was immediately extracted with chloroform: methanol. The lipid extract was separated into four fractions by silicic acid chromatography. Three of these fractions were methylated by transesterification, and the resultant methyl ester mixtures analyzed by gas chromatography. Significant variation occurred from one sampling period to the next, but significant correlation between percentage composition and time was found in only two cases: the oleic acid and the linoleic acid of the phospholipid fraction. The oleic acid increased and the linoleic acid decreased with storage time. The same trend was observed in the fraction containing free fatty acids and mono- and diglycerides, although the correlation coefficients were slightly below that corresponding to the 5% probability level.

Freshly harvested rice progressively improves in cooking and processing characteristics during the first 3 or 4 months of storage. Although this phenomenon, known as "aging," is a familiar one in the rice industry, the precise chemical and/or physical changes in the rice responsible for the effect are unknown.

Preliminary results obtained in our laboratory with a number of domestic varieties suggested that there might be changes in fatty acid composition during aging (1). Accordingly, we have made an intensive investigation of a uniform sample of a domestic variety, Belle Patna, as reported below.

Lipids of the rice endosperm have received limited attention, a few studies having appeared prior to the development of the gas chromatographic technique (2,3,4). Two recent reports of importance are (a) a general compositional study of Asian varieties analyzed at the International Rice Research Institute at Los Baños, the Philippines (5), and (b) an investigation of the influence of variety, soil and climate, and storage temperature on the fatty acid composition of two Japanese rice varieties (6).

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Materials and Methods

Rice (*Oryza sativa*) of the Belle Patna variety was obtained from the Rice Experiment Station, Crowley, Louisiana, in July 1963. Unisil, an activated acid-washed silicic acid of 100- to 200-mesh particle size was obtained from Clarkson Chemical Co., Inc., Williamsport, Pa. Practical grade 2,2-dimethoxypropane was obtained from Eastman Organic Chemicals, Rochester, N.Y. Chromport was supplied by Micro-Tek Instruments, Inc., Baton Rouge, La. Gas-chrom Z was obtained from Applied Science Laboratories, Inc., State College, Pa.

Preparation, Pretreatment, and Extraction of Rice Sample. The rice sample had been harvested during the second week of July 1963, and dried in a commercial rice dryer. As soon as the rough rice was received at Louisiana State University, it was stored in a burlap sack which was placed in a closed aluminum container. The container was then kept in the laboratory at room temperature, which averaged 23.2°C. The first sampling was made on July 23, 1963, and thereafter, every 2 weeks for a 24-week period. The shelling, milling, and polishing of rough rice samples were conducted on the day of sampling on standard laboratory-scale McGill equipment, and the milled white rice was then ground to 20-mesh in a Wiley laboratory mill. Immediately after grinding, duplicate 20-g. rice samples were extracted at room temperature with five to six small portions of chloroform:methanol (2:1 v./v.) to a final volume of 12 ml. of solvent per g. of sample. Each addition of solvent was stirred to suspend the rice and was subsequently collected by filtration before the next addition of solvent. Separation of the miscible chloroform:methanol extract into two immiscible layers, with subsequent partitioning of the lipids into the chloroform layer, was achieved by the addition of 0.2*M* magnesium chloride in the proportion of 1 volume of magnesium chloride solution per 5 volumes of lipid extract. Partitioning was allowed to proceed for 24 hr. The lipid-free upper phase was discarded and the lower-phase solvent removed on a rotary evaporator at 40°C. The lipid residue was taken up in petroleum ether for fractionation.

Fractionation of Lipids on Silicic Acid. The petroleum ether extracts were fractionated into cholesteryl esters (I), triglycerides (II), free cholesterol, free fatty acid, and mono- and diglycerides (III), and phospholipids (IV) according to the method of Lis *et al.* (7). The fractions were eluted with 1% ethyl ether in petroleum ether (I), 4% ethyl ether in petroleum ether (II), 50% ethyl ether in petroleum ether (III), and absolute methanol (IV). The various solvents were removed under reduced pressure and the weight of lipid recorded.

Preparation of Methyl Esters. Fractions II, III, and IV were methylated by the method of Mason and Waller (8). Fraction I was not analyzed, because it did not contain a significant part of the total fatty acids of the rice.

Gas Chromatographic Analysis. Analysis of the fatty acid methyl esters was achieved by gas-lipid chromatography on a Microtek GC-2500 gas chromatograph with hydrogen flame detector, operated isothermally at 188° with a helium flow rate of 120 cc. per min. Separation of esters was achieved on a stainless-steel column 8 ft. by ¼ in., packed with 20% diethylene-glycol succinate polyester containing 2% phosphoric acid adsorbed on 80- to 100-mesh Chromport as the solid support. Identification of the unknown methyl esters was based on their retention times as compared with authentic standards. Quantitation was effected by computing peak area with a planimeter. Calibration was effected by calculating the response of each methyl ester component in the synthetic mixture. Figure 1 is a typical chromatogram of the methyl esters found in fraction II (triglycerides).

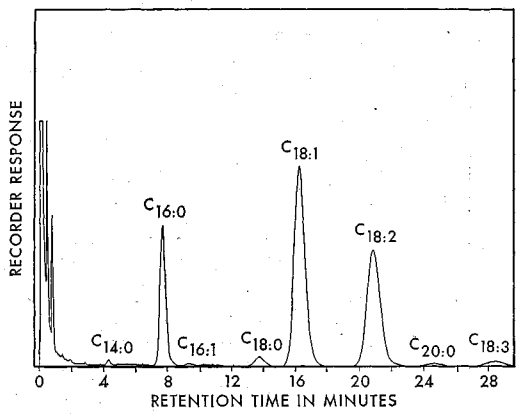

Fig. 1. A typical chromatogram of the methyl esters found in fraction II (triglycerides) prepared from rice lipids.

Results and Discussion

The average distribution of total lipids among the four fractions obtained by silicic acid chromatography was: I (cholesteryl esters), 2.3%; II (triglycerides), 64.7%; III (free cholesterol, free fatty acid, and mono- and diglycerides), 20.4%; and IV (phospholipids), 12.6%.

Palmitic acid, oleic acid, and linoleic acid were the major common components. Myristic, palmitoleic, stearic, arachidic, and linolenic acids were found in small amounts. Trace amounts of myris-

TABLE I

AVERAGE[a] FATTY ACID COMPOSITION OF RICE LIPIDS FROM MILLED BELLE PATNA VARIETY

FRACTION	FATTY ACID COMPOSITION[b]			
	16:0	18:0	18:1	18:2
	%	%	%	%
II. (Triglycerides)	14.5	1.70	39.7	44.1
III. (Free fatty acids, mono- and diglycerides)	12.5	1.50	31.4	55.6
IV. (Phospholipids)	20.0	...	36.2	43.8

[a] Averages of duplicate samples for 12 biweekly sampling periods.
[b] In the subheadings, the number to the left of the colon denotes the length of the carbon chain, and the number to the right of the colon represents the number of double bonds in the fatty acid.

toleic acid were often encountered in fractions II and III. In addition, a peak, tentatively identified as $C_{16:2}$ (hexadecadienoic acid), was found in fraction IV but never in the others. A few samples were also analyzed by Sr^{90} ionization on the Barber-Colman Model 10 equipped with a 9-ft. glass capillary column packed with 12% diethylene glycol succinate on 120- to 140-mesh Gas-chrom Z. In these samples, fatty acids corresponding to 21:0, 20:3, and 24:0 were detected in small amounts (0.6–1.4% range).

The average distribution patterns for the major components of fractions II, III, and IV are shown in Table I. These results are in good agreement with our preliminary data (9) and with the recent report of Yasumatsu and Moritaka (6), but are considerably at variance with the results published by Lugay and Juliano (5). The differences arise from the choice of extracting solvent, Lugay and Juliano having employed petroleum ether whereas this laboratory and the Osaka group used chloroform:methanol. Dr. Juliano kindly supplied us with

TABLE II

COMPARISON OF FATTY ACID DISTRIBUTION PATTERNS OF SAPONIFIABLE LIPIDS FROM TWO MILLED RICE VARIETIES EXTRACTED FOR GAS CHROMATOGRAPHIC ANALYSIS WITH DIFFERENT SOLVENTS

VARIETY	EXTRACTION SOLVENT	FATTY ACID COMPOSITION[a]							
		14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Peta[b]	Petroleum ether	0.6	25.1	0.5	1.4	42.6	28.3	0.9	0.6
	Chloroform: methanol	0.8	23.3	0.7	1.7	31.1	36.2	1.3	0.5
Malagkit[b] Sungsong Puti	Petroleum ether	0.5	28.7	0.4	2.4	46.1	21.3	0.4	0.4
	Chloroform: methanol	1.2	28.7	0.4	1.9	27.6	35.8	0.9	0.4

[a] In the subheadings, the number to the left of the colon denotes the length of the carbon chain, and the number to the right of the colon represents the number of double bonds in the fatty acid.
[b] Sample kindly supplied by Benvenido Juliano. Data for petroleum ether extracts are quoted from Lugay and Juliano (5).

two of the samples (same lots) analyzed by his laboratory, and we proceeded to analyze them according to our method. The results for saponifiable lipids are given in Table II, emphasizing the extent to which the choice of extractant influences the observed pattern of lipid distribution. The most striking difference occurred in the oleic: linoleic acid ratios. Petroleum ether extraction yielded samples in which the ratio exceeded unity, whereas chloroform:methanol gave samples having oleic:linoleic acid ratios less than unity. It is well recognized that petroleum ether extracts less lipid, particularly polar lipid, than does chloroform:methanol.

Biweekly percentage composition data for the three principal component fatty acids of fractions II, III, and IV are given in Table III. Inspection of the table shows that considerable variation was found in the composition from one sampling period to the next. Agreement between duplicates was excellent, therefore the source of variation was either (a) lack of uniformity in the rough rice or (b) slight differences in degree of milling from one sampling period to the next. The latter difficulty could have been avoided by conducting the study on a large uniform sample of milled rice; however, the effects of air-oxidation would then have to be considered in the interpretation of results. A difference in degree of milling would influence results, since bran lipids are more highly unsaturated than endosperm lipids, and bran layers are so much more lipid-rich than endosperm. The data shown in Table III were subjected to statistical analysis, as presented in Table IV. Analysis of variance for each major fatty acid component has been carried out. The significance of variation is represented by F-values which are obtained from the F-test. The F-value required

TABLE III

FATTY ACID COMPOSITION OF BIWEEKLY SAMPLES OF RICE LIPIDS
(Each figure represents the average of duplicate samples)

SAMPLE PERIOD	PALMITIC ACID			OLEIC ACID			LINOLEIC ACID		
	Fr. II	Fr. III	Fr. IV	Fr. II	Fr. III	Fr. IV	Fr. II	Fr. III	Fr. IV
	%	%	%	%	%	%	%	%	%
1	14.3	13.9	18.9	38.5	31.7	36.5	45.1	54.5	44.6
2	15.4	14.3	24.3	39.5	30.6	31.4	43.5	55.1	44.4
3	15.7	13.0	20.7	39.9	30.3	32.3	42.4	56.8	47.0
4	13.9	11.7	18.6	37.9	28.3	34.7	46.6	58.7	46.7
5	15.1	12.7	21.4	39.2	31.5	34.2	44.0	53.9	44.4
6	15.1	12.3	18.0	41.5	30.4	39.0	41.8	56.0	43.0
7	14.2	11.6	20.4	39.1	29.6	37.2	45.1	57.3	42.4
8	13.8	11.2	18.2	39.4	29.2	36.2	45.0	58.0	45.6
9	13.5	11.1	20.4	40.8	36.5	39.6	44.1	52.5	40.0
10	15.1	12.9	22.4	40.6	30.3	36.0	42.5	56.9	41.6
11	14.3	13.9	18.8	39.3	35.7	38.0	45.0	50.4	43.3
12	13.3	12.6	18.1	40.5	36.0	38.9	44.6	51.5	43.1

TABLE IV

F-VALUES, CORRELATION COEFFICIENTS, AND REGRESSION COEFFICIENT FOR FRACTIONATED RICE LIPIDS

FRACTION	FATTY ACID COMPONENT	F-VALUE	CORRELATION COEFFICIENT	REGRESSION COEFFICIENT
II	Palmitic acid	2.72*	−0.504	
	Oleic acid	5.14*	0.463	
	Linoleic acid	6.83*	0.0163	
III	Palmitic acid	4.77*	−0.0969	
	Oleic acid	22.19*	0.570	
	Linoleic acid	22.05*	−0.439	
IV	Palmitic acid	4.98*	0.296	
	Oleic acid	3.74*	0.677*	0.488
	Linoleic acid	4.61*	−0.586*	−0.329
5% Probability		2.72	±0.576	

for 5% probability was 2.72. Fractions displaying significant variation are indicated by asterisks. The fact that all components possessed significant F-values means simply that the variation among biweekly samples was significant as compared with variation between replicates. Therefore the data were analyzed for the existence of trends by determining correlation coefficients. The correlation coefficients which predict the correlation between the two variables, percentage composition *vs.* time, are shown in the fourth column. The value required for the 5% level was ± 0.576. The data indicated that the percentage of oleic acid tended to increase significantly and the percentage of linoleic acid tended to decrease significantly in fraction IV with respect to age of sample. Regression coefficients from the scatter diagrams for these two components are shown in Table IV. The same trend is indicated in the correlation coefficients for oleic acid and linoleic acid in fraction III, although the results cannot be described as statistically significant. The basic cause of the compositional change is unknown at this time, just as it is presently impossible to say whether the change has any bearing on the aging phenomenon. The increased degree of saturation of extracted lipids may represent a true compositional change in the endosperm or may, to propose an alternate hypothesis, reflect a change in the degree of extractability of the more polar lipids. At present it represents one of the few known changes that accompany the aging process of rice.

Our results are supported by a recent report of Yasumatsu and Moritaka (6), who compared the fatty acid content of several lipid fractions prepared from polished rice stored for 6 months at two temperatures, 9°C. and room temperature. Samples were analyzed only at the beginning and end of the experiment. The fatty acid composition of the free fatty acid fraction was essentially the same in the sample

stored at 9°C. as it was in the fresh rice at the beginning of the experiment. The sample stored at room temperature, however, differed remarkably from these two controls in that it was higher in oleic acid and lower in linoleic acid, paralleling our observations with fraction IV.

Hogan (10) has measured the effect of age on changes in properties such as hydration, pasting characteristics, cohesiveness, etc., but failed to detect any significant change in gross composition, i.e., starch, protein, and sugar, over a 6-month period. He also found a definite pattern of change in the native amylase content as well as in the amylase susceptibility. Houston and co-workers (11,12,13,14) reported that total acids, organic acids, and free fatty acids of the endosperm increased during the storage of both rough rice and brown rice. Changes in the limiting viscosity of the amylose and amylopectin fractions have been studied by Primo *et al.* (15,16,17), in addition to attempts to correlate cooking quality and palatability with protein type and content. All of these suggestive results emphasize the need for basic research on the aging phenomenon, since it is probably a complex interaction of lipid, starch, and protein.

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