# MOLECULAR SPECIES OF TRIGLYCERIDE IN RICE BRAN

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**ABSTRACT**

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Triglyceride was isolated from rice bran, and the fatty acid composition and molecular species were analyzed with gas-liquid chromatography and gas chromatography/mass spectrometry. Neutral lipid and polar lipid were present in the ratio 90.6:9.4. The main component fatty acids were, in decreasing order, linoleic, oleic and palmitic acid. Among the fatty acid components, linoleic acid was rich in the C-2 position of the glycerol radical, palmitic acid exclusively in C-1 and C-3, and oleic acid equally distributed in C-1, C-2, and C-3. When the molecular species of triglyceride was expressed in terms of combination of fatty acids (carbon number:double bond number)[1] in C-1, C-2, and C-3 positions, the major types of triglyceride in rice bran included 16:0–18:2–18:1 and/or 18:1–18:2–16:0, 16:0–18:2–18:2 and/or 18:2–18:2–16:0, and 18:1–18:1–18:1.

Many studies have been conducted in various laboratories on the general nature and fatty acid composition of rice bran oil (1,2), but triglyceride, the main component of the bran oil, has not been investigated at the molecular level. The present study was carried out to isolate triglyceride from bran oil, to determine the positional distribution of fatty acids in the molecule and to elucidate the molecular species of the triglyceride. All the experiments in this paper have been replicated at least twice.

## MATERIALS AND METHODS

### Materials

Shin-ei, the nonglutinous rice, harvested at Hokkaido Prefecture, Japan, in 1973 and 1974, was obtained from the Hokkaido Central Agricultural Experimental Station, Naganuma, Hokkaido. Brown rice 11.8 kg (3.5 kg and 8.3 kg from the 1973 and 1974 crops, respectively) was polished with the Polishing Machine (Satake Seisakusho Co., Ltd., Tokyo) to obtain 2.4 kg of rice bran, which was immediately steamed for 3 min to inhibit enzymatic activity.

### Extraction of Total Lipid

Rice bran after steaming was extracted three times with 4 vol of chloroform-methanol (2:1, v/v) and twice with 4 vol of water-saturated butanol (3) at 70° C for 2 hr with stirring in water bath. All the combined extracts were evaporated to dryness in a rotary evaporator. The residue was dissolved in chloroform-methanol (2:1, v/v) and washed with water to obtain purified total lipid (4).

### Isolation of Triglyceride

The total lipid was applied to a silicic acid column and eluted successively with chloroform for the neutral lipid fraction and with methanol for the polar lipid fraction (5). The neutral lipid fraction was applied to another silicic column to isolate triglyceride. Hydrocarbon was eluted with hexane, sterolester with

[1]Fatty acid is expressed as a symbol of number of carbon atoms: number of double bonds.

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hexane-benzene (85:15, v/v) and triglyceride with ether-hexane (5:95, v/v), respectively (6). The triglyceride which was contaminated with a small amount of free fatty acid was further purified to remove the contaminant on a silicic column with the same solvent system and identified by silica gel G thin-layer chromatography and infrared spectrophotometry. Infrared spectrum was taken on an Infrared Recording Spectrophotometer (IR-G type, Nippon Bunko Kogyo Co., Ltd., Tokyo), using KBr pellet containing 3 mg of triglyceride.

**Analysis of Total Fatty Acid Component**

After methanolysis of triglyceride with 5% HCl in methanol for 4 hr at 110° C, the fatty acid methylesters obtained were analyzed by gas-liquid chromatography. A Gas Chromatograph (Hitachi Model 063, Hitachi Seisakusho Co., Ltd., Tokyo) equipped with a hydrogen-flame ionization detector was used. A glass column (0.3 × 200 cm) was packed with 10% DEGS on 80–100 mesh of Chromosorb W. Carrier gas was $N_2$ (flow rate, 40 ml/min) and column temperature was 175° C.

**Positional Analysis of Fatty Acid Component** (7,8)

L-Lysophosphatidylphenol and free fatty acid, obtained by hydrolysis with phospholipase $A_2$ (venom of snake, *Trimeresurus fravoviridis*) of D,L-phosphatidylphenol derived from 1,2- and 2,3-diglyceride which had been prepared by treatment of triglyceride with pancreatin (Wako Junyaku Kogyo Co., Ltd., Tokyo) (9), were respectively analyzed by gas-liquid chromatography as fatty acid methylesters. Fatty acid composition at C-1 position was determined from l-lysophosphatidylphenol and that at C-2 from free fatty acid. Fatty acid composition at C-3 position was calculated by subtracting the composition at C-1 and C-2 from the total composition.

**Fractionation of Triglyceride**

Triglyceride was fractionated according to the degree of unsaturation by silicic acid/silver nitrate thin-layer chromatography (10,11,12). Silicic acid (Wako Gel B-5, Wako Junyaku Kogyo Co., Ltd., Tokyo) and silver nitrate in a ratio of 19:1 were suspended in water. This suspension was applied to a glass plate, which was developed with chloroform-methanol (98.8:1.2, v/v). Triglyceride bands were located under ultraviolet light after spraying with Rhodamine 6G in methanol. The appropriate band was scraped off the plate and extracted with chloroform to recover the subfraction of triglyceride. Determination of the relative amount of each triglyceride subfraction was carried out by comparison of the fatty acid methylesters with a known amount of arachidic methylester as an internal standard on gas-liquid chromatograms.

**Analysis of Molecular Species**

Molecular species of triglyceride subfractionated were analyzed with the gas chromatograph/mass spectrometer (Hitachi RMU-6MG type, Hitachi Seisakusho Co., Ltd., Tokyo). A glass column (100 × 0.3 cm) was packed with Diasolid ZT. The column temperature was 340° C and the carrier gas was helium (flow rate, 60 ml/min). The molecular separator and the ion source were maintained at 360° C, respectively. Ionizing voltage was 20eV and trap current 70 $\mu$A.

First, triglyceride was subjected to gas-liquid chromatography by a total ion monitor. Carbon numbers of triglyceride (sum of carbon numbers of three acyl groups in the molecule) at each peak in the chromatogram were determined by comparison with tripalmitin as standard and the relative retention times in the literature (13). Then, each triglyceride was applied to mass spectrometry to record up to m/e 900 at apex of the peak, and the spectrum depicted was interpreted by referring to the data reported in the literature (14,15,16). The fatty acid moieties were identified by the ion peaks for $[M\text{-}RCOO]^+$, $[RCO]^+$, $[RCO+74]^+$ and $[RCO+128]^+$. The fatty acid in the C-2 position was determined

**TABLE I**
**Fatty Acid Composition of Triglyceride in Rice Bran**

| Fatty Acid | Total (%) | Position C-1[a] (%) | C-2[a] (%) | C-3[a] (%) |
|---|---|---|---|---|
| 14:0 | 0.1 | 0.2 | ... | 0.1 |
| 16:0 | 20.8 | 37.1 | 1.4 | 24.0 |
| 18:0 | 1.7 | 3.4 | ... | 1.6 |
| 18:1 | 34.6 | 29.1 | 31.9 | 42.7 |
| 18:2 | 41.6 | 30.2 | 65.0 | 29.5 |
| 18:3 | 1.2 | ... | 1.7 | 1.9 |
| Saturated | 22.6 | 40.7 | 1.4 | 25.7 |
| Unsaturated | 77.4 | 59.3 | 98.6 | 74.1 |

[a]Carbon numbers of *sn*-glycerol in triglyceride.

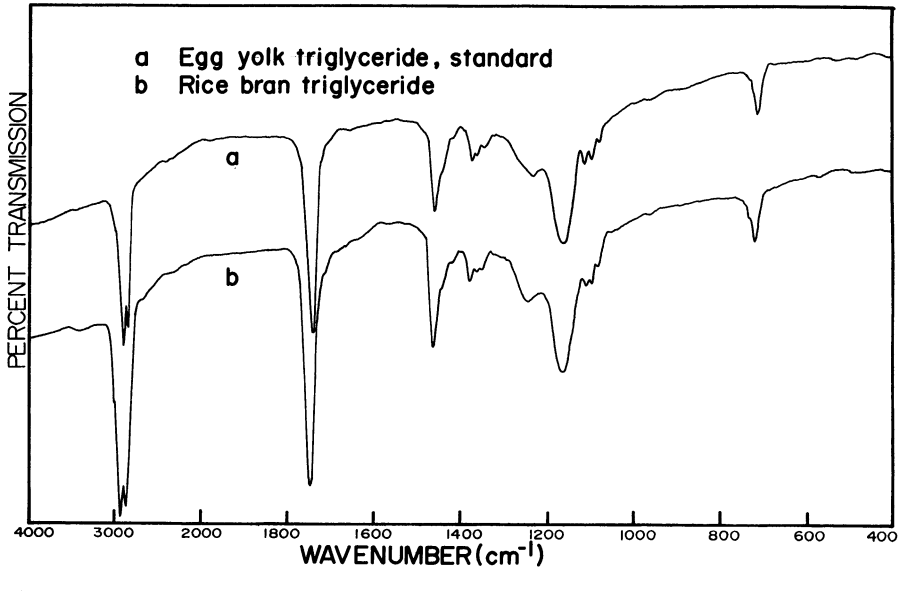


Fig. 1. Infrared spectrum of rice bran triglyceride.

from the fact that $[M\text{-}R^2COOCH_2]^+$ peak was usually much smaller than $[M\text{-}R^{1,3}COOCH_2]^+$ peaks (15,16,17). Although the fatty acids esterified at C-1 and C-3 positions in triglyceride were indistinguishable by mass spectrometry, they were deduced by taking into account the positional analysis of fatty acid component of the triglyceride.

## RESULTS

### Yields

The yield of bran from brown rice was 20.9%. The higher yield than usual was due to the excess milling required to completely remove the lipid rich bran layers from the endosperm. Lipid content of the resulting rice bran was 10.7%. The lower content than usual was probably due to some contamination of the rice

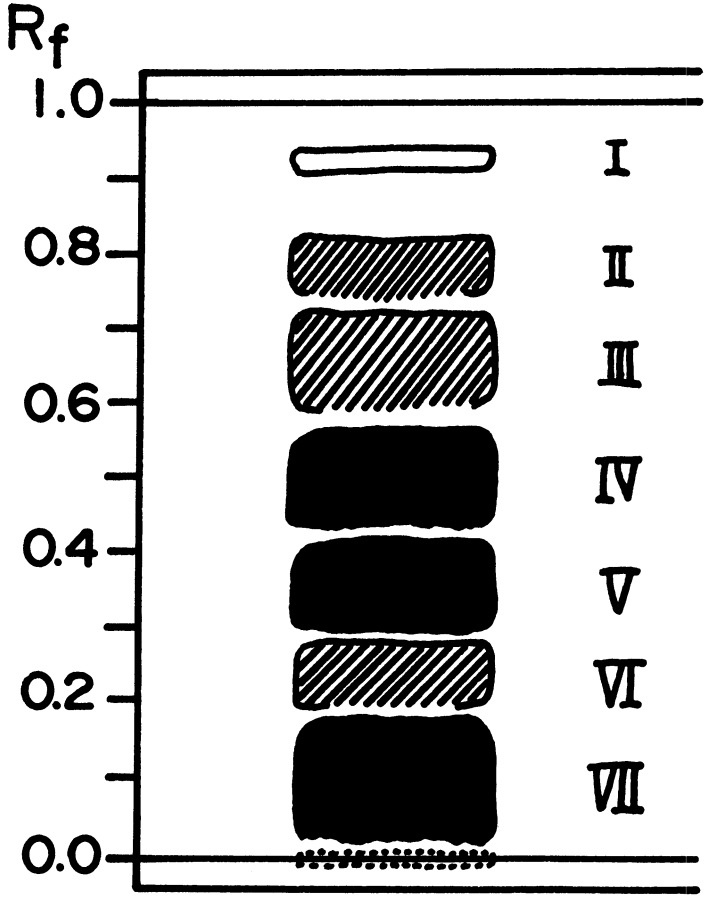


Fig. 2. Thin-layer chromatogram of triglyceride in rice bran. Glass plate was coated with silicic acid/silver nitrate (19:1), developed by 1.2% methanol in chloroform, and visualized by U. V. light after spraying with Rhodamine 6-G in methanol.

bran by particles of endosperm removed during milling. Yields of neutral and polar lipid fractionated by silicic acid column chromatography were 90.6 and 9.4%, respectively. Yield of triglyceride separated by column chromatography amounted to 72.4% of neutral lipid fraction.

**Identification of Triglyceride**

Triglyceride isolated gave a single spot on the thin-layer chromatographic plate. Infrared spectrum of triglyceride is shown in Fig. 1. This spectrum showed the characteristic absorptions due to C=0 stretching of ester (1740 $cm^{-1}$) and C-0 stretching of ester (1155 $cm^{-1}$). Absorption pattern of the spectrum was identical with that of egg yolk triglyceride.

**TABLE II**
**Fatty Acid Composition of Triglyceride Subfractions of Rice Bran**

| Subfraction Number[a] | Ratio (%) | Fatty Acid 16:0 (%) | 18:0 (%) | 18:1 (%) | 18:2 (%) |
|---|---|---|---|---|---|
| I | 0.3 | 69.3 | 16.5 | 14.2 | ... |
| II | 5.6 | 64.9 | 4.5 | 30.6 | ... |
| III | 11.8 | 29.8 | 3.2 | 67.0 | ... |
| IV | 22.6 | 34.1 | 1.8 | 43.7 | 20.4 |
| V | 22.7 | 30.8 | 2.6 | 33.1 | 33.5 |
| VI | 11.3 | 3.1 | ... | 61.1 | 35.8 |
| VII | 26.0 | 19.7 | 1.6 | 17.8 | 60.9 |

[a]Number refers to Fig. 2.

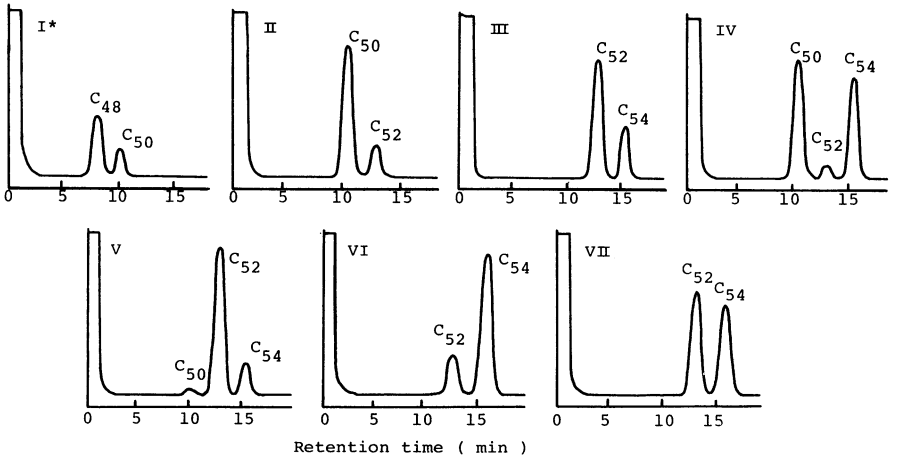


Fig. 3. Chromatogram of total ions of rice bran triglyceride subfractions. Roman numerals refer to Fig. 2.

**Fatty Acid Composition of Triglyceride**

Total and positional fatty acid compositions of triglyceride are shown in Table I. The major component fatty acids were palmitic, oleic, and linoleic acids, among which linoleic acid was the most abundant. The ratio of saturated and unsaturated acid was about 23:77. Distribution of fatty acids in triglyceride molecule was different among C-1, C-2, and C-3 positions. The unsaturated acid was richest in C-2 position, then C-3, and C-1 in decreasing order, and was unsymmetrical at times between C-3 and C-1. The general tendency in triglyceride of plant seeds has been reported to be symmetrical (18). Positional distribution of fatty acids in triglyceride of rice bran was quite similar to that of rice starch (19).

**Fatty Acid Component of Triglyceride Subfraction**

Fractionation of triglyceride by silicic acid/silver nitrate thin-layer chromatography is illustrated in Fig. 2. As shown, triglyceride was fractionated into seven bands (I~VII) on the plate. The major subfractions were IV, V, and VII, the moderate bands were II, III, and VI, and the minor one was I. Fatty acid compositions of triglyceride subfraction extracted from each band are shown in Table II. It was noted from I to VII that the ratio of saturated to unsaturated acids decreased and that stearic acid decreased whereas linoleic acid increased. Major triglyceride in subfraction II and III seemed to be dipalmitoyl oleoyl type and palmitoyl dioleoyl one, respectively.

**Carbon Number of Triglyceride Subfraction**

Figure 3 shows the chromatogram by total ions of each triglyceride subfraction. The main carbon numbers were $C_{48}$ and $C_{50}$ for I, $C_{50}$ for II, $C_{52}$ for III, $C_{50}$ and $C_{54}$ for IV, $C_{52}$ for V, $C_{54}$ for VI, $C_{52}$ and $C_{54}$ for VII, respectively.

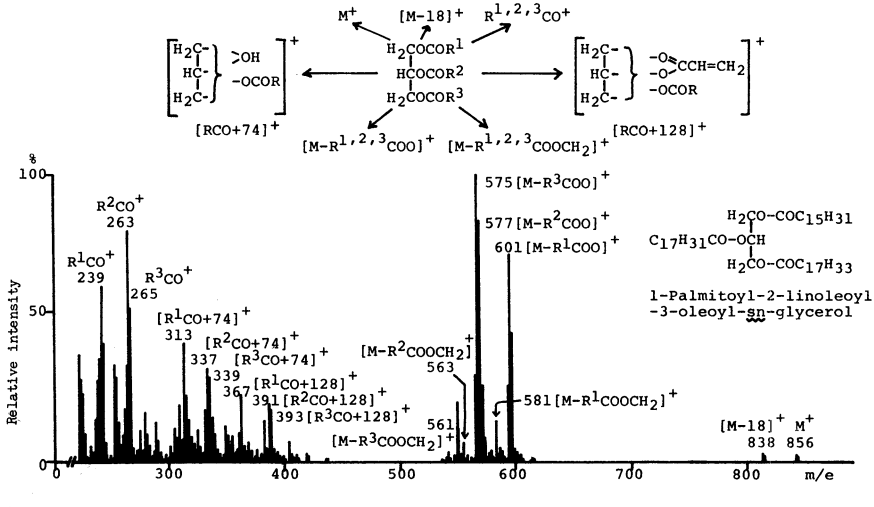


Fig. 4. Mass spectrum of rice bran triglyceride (V-$C_{52}$ in Fig. 3).

**TABLE III**
**Molecular Species of Rice Bran Triglyceride**

| Triglyceride | Ratio (%) | Major Molecular Species[b] |
|---|---|---|
| II-$C_{50}$[a] | 3.6 | 16:0–18:1–16:0 |
| II-$C_{52}$ and III-$C_{52}$ | 11.6 | 16:0–18:1–18:1, 18:1–18:1–16:0 |
| IV-$C_{50}$ and V-$C_{50}$ | 8.8 | 16:0–18:2–16:0 |
| III-$C_{54}$ and IV-$C_{54}$ | 13.8 | 18:1–18:1–18:1 |
| IV-$C_{52}$, V-$C_{52}$ and VI-$C_{52}$ | 21.6 | 16:0–18:2–18:1, 18:1–18:2–16:0 |
| V-$C_{54}$ and VI-$C_{54}$ | 11.3 | 18:1–18:2–18:1 |
| VII-$C_{52}$ | 14.0 | 16:0–18:2–18:2, 18:2–18:2–16:0 |
| VII-$C_{54}$ | 12.0 | 18:1–18:2–18:2, 18:2–18:2–18:1 |

[a]Symbols refer to Fig. 3.
[b]Expressed as sequence of component fatty acids at C-1, C-2, and C-3 of triacyl-**sn**-glycerol.

Intermixture with contiguous subfractions was noted to some extent in most of the triglyceride subfractions.

### Molecular Species of Triglyceride

An example of the mass spectrum is shown in Fig. 4 for triglyceride V-$C_{52}$ in Fig. 3. From this spectrum and Table I, it was concluded that the component fatty acids of the triglyceride V-$C_{52}$ were 16:0, 18:1, and 18:2, that C-2 position was occupied by 18:2 and that C-1 and C-3 positions contained 16:0 and 18:1 in almost equal amounts. Therefore, if the molecular species of triglyceride are expressed by combination of fatty acids in C-1, C-2, and C-3 positions, those of the triglyceride V-$C_{52}$ are postulated to consist of 16:0–18:2–18:1 and 18:1–18:2–16:0. The principal molecular species of rice bran triglyceride determined in the same manner as above are shown in Table III. The main species were 16:0–18:2–18:1 and/or 18:1–18:2–16:0, 16:0–18:2–18:2 and/or 18:2–18:2–16:0, 18:1–18:1–18:1 and so forth. According to the degree of unsaturation, the trienoic type was the most abundant, then tetraenoic, dienoic, polyenoic, and monoenoic type followed in decreasing order.

## DISCUSSION

In the present study, fatty acid distribution and molecular species of triglyceride in rice bran were characterized. Shin-ei, the sample used, was a nonglutinous variety of Japonica type. There are a great many varieties of rice (more than 1,000 varieties in Japan alone), including Japonica and Indica types, glutinous and nonglutinous, upland-cultivated and waterfield-cultivated, early-grown and late-grown, warmness-adaptive and coldness-adaptive varieties. Therefore, we can not conclude that the data presented here is common to rice oil in general. Thus, more comprehensive studies on fatty acid distribution and molecular species of rice triglyceride must be conducted. The techniques described here will be applicable for surveying the lipid profile of the diversified varieties of rice. Experiments along this line are being carried out in our laboratory.

## Literature Cited

1. AKIYA, T. Rice oil for food (in Japanese). In Shokuryo, ed and published by Food Research Institute of Department of Agriculture, p. 118, Tokyo, Japan (1965).
2. JULIANO, B. O. The rice caryopsis and its composition. Rice: Chemistry and technology, ed by D. F. Houston. p. 16, AACC: St. Paul, Minn. (1972).
3. ACKER, L., and SCHMITZ, H. J. Lipid in wheat starch. I. Review of lipids in grain starches. Die Starke 19:223 (1967).
4. FOLCH, J., LEES, M., and SLOAN-STANLEY, G. H. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497 (1957).
5. ROUSER, G., KRITCHEVSKY, G., SIMON. G., and NELSON, G. J. Quantitative analysis of brain and spinach leaf lipid employing silicic column chromatography and acetone for elution of glycolipids. Lipids 2:37 (1968).
6. BARRON, E. J., and HANAHAN, D. J., Observations on the silicic acid chromatography of the neutral lipids of rat liver, beef liver and yeast. J. Biol. Chem. 231:493 (1958).
7. BROCKERHOFF, H. Stereospecific analysis of triglycerides. J. Lipid Res. 6:10 (1965).
8. BROCKERHOFF, H. Stereospecific analysis of triglycerides: an alternative method. J. Lipid Res. 8:10 (1967).
9. ANDERSON, R. E., BOTTINO, N. R., and REISER, R. Pancreatic lipase hydrolysis as a source of diglycerides for the stereo-specific analysis of triglycerides. Lipids 2:440 (1967).
10. BARRETT, C. B., DALLAS, M. S. J., and PADLEY, F. B. The quantitative analysis of triglyceride mixture by thin-layer chromatography on silicic impregnated with silver nitrate. J. Amer. Oil Chemist's Soc. 40:580 (1963).
11. BLANK, M. L., VERDINO, B., and PRIVETT, F. B. Determination of triglyceride structure via silver nitrate-TLC. J. Amer. Oil Chemist's Soc. 42:87 (1965).
12. KAUFMAN, H. P., and WESSELS, H. Die Dünnschicht-Chromatographie auf dem fettgebiet. XIV. Die Trennung der Triglyceride durch Kombination der Adsorptions- und der Umkehrphasen-Chromatographie. Fette Seifen Anstrichm. 66:81 (1964).
13. KUKSIS, A. Gas liquid chromatography of glycerides. J. Amer. Oil Chemist's Soc. 42:269 (1965).
14. BARBER, M., MERREN, T. O., and KELLY, W. The mass spectroscopy of large molecules. I. The triglycerides of straight chain fatty acids. Tetrahedron Lett. 10:1063 (1964).
15. MURATA, T., and TAKAHASHI, S. Analysis of triglyceride mixture by gas chromatography-mas spectrometry. Anal. Chem. 45:1816 (1973).
16. HITES, R. A. Quantitative analysis of triglyceride mixtures by mass spectrometry, Ibid 42:1736 (1970).
17. LAUER, W. M., AASEN, A. J., GRAFF, G., and HOLMAN, R. T. Mass spectrometry of triglycerides: 1. Structural effects. Lipids 5:861 (1970).
18. BROCKERHOFF, H., and YURKOWSKI, M. Stereospecific analysis of several vegetable fats. J. Lipid Res. 7:62 (1966).
19. FUJINO, Y., and MIYAZAWA, T. Neutral lipids present in starch of URUCHI and MOCHI rice. Die Starke 28:414 (1976).

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