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Umemoto, Takayuki

Tsugawa, Hyoe

Tange, Munetoshi

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# SOME FEATURES IN LIPID− AND FATTY ACID COMPOSITIONS OF THE BROWN RICES STORED AT LOW TEMPERATURE FOR A LONG TIME

Hironobu TSUCHIDA*, Takayuki UMEMOTO**, Hyoe TSUGAWA**, Mitsuo SAKATA***, Susumu MIZUNO***and Munetoshi TANGE**

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**Abstract**

Lipid- and fatty acid compositions of the long time-stored and freshly harvested brown rices (some varieties of Japonica) were examined to elucidate some features of deterioration of their qualities during storage at low temperature for a long time.

A feature of lipid-compositional pattern in the brown rices stored at 10°C for a long time was the decrease in contents of neutral lipid-, phospholipid- and non-saponified lipid fractions, and the increase in contents of glycolipid- and free fatty acid fractions.

Some features of compositional patterns of major fatty acids of the lipid fractions obtained from the long time-stored brown rices were explained as follows; 1) the increase in content of palmitic acid in every lipid fractions and the decrease in content of linoeic plus linolenic acids in the lipid fractions except phospholipid fraction. 2) the increase in oleic acid content of glycolipid- and free fatty acid fractions and the decrease in its content of phospholipid fraction. 3) the increase of two unknown components (U-1 and U-2) and the decrease of an unknown component (U-3) among three unknown components detected only in glycolipid fraction.

## Introduction

A sensory quality of rice deteriorates gradually during its storage. A characteristic of the deteriorated rice is mainly represented by production of off-flavor and deterioration of sensory texture. It is generally recognized that the phenomena would originate from lipid deterioration of the stored rice. Studies on the lipid deterioration or the production of off-flavor during storage of rice under various conditions have been carried out by several investigators.[1~6] At present, most of rices produced in Japan are stored as brown rice under reasonable conditions (at low temperature of 10°C to 15°C, holding the relative humidity of 70 to 80%). However, a feature of lipid deterioration during long time-storage under this condition has not been examined so far in detail.

In this paper, distributions of neutral lipid-, glycolipid-, phospholipid- and free fatty acid fractions and the compositional patterns of major fatty acids in their fractions are examined on the long time-stored and the freshly harvested brown rices to elucidate some features of deterioration of their qualities.

* Center for Development of Cooperative Research
** Laboratory of Crop Science
*** Laboratory of Food Preservation Technology

## Materials and Methods

*Materials.* Four varieties of Japonica brown rices (*Oryza sativa L. cv.* Ishikari, Shiokari, Sasanishiki and Nipponbare) were used in this experiment. The rice samples were harvested in autumn 1972 and 1985 in four localities Hokkaido, Miyagi, Okayama and Kumamoto). Immediately after being received at Kobe University, rice samples were stored in stoppred plastic bottles, which placed in a closed alminium container. The container was then kept in the laboratory holding a temperature of 10°C and a relative humidity of about 70% until rice samples were analyzed. Brown rices "Ishikari" and "Shiokari" were kindly supplied by the Hokkaido Food's Office, "Sasanishiki" by the Miyagi Food's Office, and "Nipponbare" by the Okayama- and Kumamoto Food's Offices.

*Extraction of total lipid.* The pulverized brown rice (100 g) was extracted with 4 vol. of chloroform-methanol (2 : 1 v/v) and five times with 4 vol. of water-saturated butanol at 25°C for 3 hr..[7] Non-lipid fraction of the extract was removed by Folch's method.[8] The non-lipid freed extract (chloroform solution) was dried over anhydrous sodium sulfate, evaporated to dryness under reduced pressure and sufficiently dried over phosphorus pentaoxide in a desiccator in a vacuo till constant weight.

*Fractionation of total lipid.* Total lipid obtained above was fractionated to neutral lipid-, glycolipid- and phospholipid fractions by silica gel (Mallinckrot's product for chromatography) chromatography using chloroform, acetone and methanol as eluents.[9] Non-saponified lipid fraction of total lipid was determined by micro method.[10] Free fatty acid fraction of total lipid was obtained by Mattik's extraction method.[11]

*Analysis of fatty acid composition.* Each lipid fraction (ca 10 mg) was dissolved in 1 mℓ of 5% hydrogen chloride-methanol and heated at 100°C for 3 hr. in a sealed tube. After cooling, 0.5 mℓ of deionized water was added to the reaction mixture and the produced methyl esters of fatty acids were extracted four times with 1 mℓ of petroleum ether. The combined extract was washed with 3 mℓ of deionized water and dried over anhydrous sodium sulfate. Methylation of free fatty acid fraction was accomplished by the freshly prepared diazomethan. The methyl ester solutions were analyzed by gas liquid chromagraphy using a fused silica capillary column (silicon OV-1 Shimazu CBP 1–S 25–50) and FID as detector. The chromatograph was a Shimazu GC–9A type gas chromatograph combined with Shimazu C–R 3 integrator, and was operated according to the following conditions: column temperature 200–300°C, program rate 2°C/min., injection and detector temperatures 310°C, and flow rate of carrier gas (nitrogen) 50mℓ/min. The peaks on gas chromatograms were confirmed by gas chromatography-mass spectrometry (GC–MS). GC–MS analysis was performed with a Hitachi Model RMU–6MG gas chromatograph-mass spectrometer using an all glass jet separator as the GC–MS analysis was performed with a Hitachi Model RMU–6MG gas chromatograph-mass was operated under the following conditions: ionizing electron energy of 20 eV, ion accelerating voltage of 3.2kV, total emission of 100uA, and ion source temperature of 180°C.

## Results and Discussion

*Some features of lipid composition of the long time-stored brown rice.* Recently, Shin *et al.*[6] reported that the total lipid contents of brown rice did not decreased at 5°C during 12 months storage, but contents of neutral lipid-, glycolipid- and phospolipid fractions significantly decreased and alone content of free fatty acid fraction intensively increased. The results shown in Table 1 suggested that the total lipid content in brown rice hardly gave significant difference between the fresh and the 13 years-stored brown rices or among their varieties. However,

difference in content of each lipid fraction between the long time-stored and the fresh brown rices gave the very different interpretation from the results on changes in their lipid fractions during 12 months storage made by Shin *et al.*[6]: contents of glycolipid- and free fatty acid fractions have increased significantly with the decrease in contents of neutral lipid-, phospholipid- and non-saponified lipid fractions as shown in Table 1. Yasumatsu *et al.*[3] and Morita[12] already published that free fatty acid content greatly increased during storage of rice. The results obtained on changes in lipid components during storage of rice grain by Matsuda and Hirayama[5] showed that lysolecithin and lysophosphatidyl ethanolamine of polar lipid components increased significantly. However, there was hitherto no report on the increase of glycolipid fraction of rice during storage at low temperature for a long time. As to molecular species of rice glycolipid fraction, five glyceroglycolipids, eight glycosylceramides and six glycosylsterols have been isolated and identified by Fujino *et al.*[13] Molecular species of glycolipid fraction increasing during storage of rice for a long time remains to be elucidated in future.

*Compositional patterns of fatty acids of total lipid fractions of the fresh and the long time-stored brown rices.* The compositional pattern of fatty acids of total lipid fraction in brown rice has been examined in relation to rice quality, the daily mean temperature during rice-ripening and the fatty acid composition in rice-total lipid,[14] difference in fatty acid composition among rice varieties[15] and changes in their compositional patterns during storage.[1~6] The fatty acid compositions of the fresh brown rices grown in Japan were examined by Taira et al.[14] The results are suggested as follows: myristic acid (0.2–0.4%), palmitic acid (16.5–18.1%), palmitoleic acid (0.2–0.5%), stearic acid (1.6–2.3%), oleic acid (36.6–43.0%), linoleic acid (35.7–39.2%) and linolenic acid (1.4–2.1%). The contents of fatty acids of the fresh rices in Table 2 were similar to the above values respectively, except myristic and palmitoleic acids. As to the fatty acid compositions in the fresh rices of different varieties, there was highly negative correlation between contents of oleic and linoleic acids as described by Taira *et al.*[15] In the different varieties of the long time-stored brown rices, however, there was no correlation between their contents. The

Table 1 Distributions of neutral lipid-, glycolipid- and phospholipid-fractions obtained from the 13 years-stored and the fresh brown rices.

| Rice-producing district<br>Storage period<br>(Variety) | Total lipid<br>% of rice | Lipid fraction (%)/total lipid | | | | |
|---|---|---|---|---|---|---|
| | | NL-fraction | GL-fraction | PL-fraction | FFA | NSL |
| Hokkaido | | | | | | |
| 13 years (Ishikari) | 2.93 | 79.2 (73.2) | 10.8 | 10.0 | 4.5 | 1.5 |
| fresh (Shiokari) | 3.00 | 82.4 (78.4) | 3.5 | 14.1 | 2.2 | 1.8 |
| Miyagi | | | | | | |
| 13 years (Sasanishiki) | 3.13 | 84.1 (79.4) | 7.9 | 8.0 | 4.3 | 0.4 |
| fresh (Sasanishiki) | 3.44 | 87.6 (84.4) | 3.0 | 9.4 | 1.9 | 1.3 |
| Okayama | | | | | | |
| 13 years (Nipponbare) | 3.16 | 79.7 (74.8) | 10.4 | 9.9 | 4.5 | 0.4 |
| fresh (Nipponbare) | 3.36 | 81.1 (77.4) | 6.5 | 12.4 | 2.1 | 1.6 |
| Kumamoto | | | | | | |
| 13 years (Nipponbare) | 3.19 | 81.2 (76.1) | 7.2 | 9.6 | 4.4 | 0.7 |
| fresh (Nipponbare) | 2.90 | 87.4 (83.5) | 2.3 | 12.3 | 2.0 | 1.9 |

NL: neutral lipid, GL: glycolipid, PL: phospholipid, FFA: free fatty acid, NSL: non-saponified lipid Parenthesized value = NL-fraction – (FFA + NSL)

Table 2 Compositional ratios of major fatty acids of the total lipids obtained from the 13 years-stored and the fresh brown rices.

| Rice-producing district<br>Storage period<br>(Variety) | Fatty acid composition (%) | | | | | |
|---|---|---|---|---|---|---|
| | $C_{14:0}$ | $C_{16:0}$ | $C_{16:1}$ | $C_{18:0}$ | $C_{18:1}$ | $C_{18:2}+C_{18:3}$ |
| Hokkaido | | | | | | |
| 13 years (Ishikari) | 0.67 | 24.40 | 0.20 | 1.73 | 39.35 | 31.55 |
| fresh (Shiokari) | 0.71 | 20.38 | 0.19 | 1.68 | 37.66 | 37.74 |
| Miyagi | | | | | | |
| 13 years (Sasanishiki) | 0.75 | 25.91 | 0.21 | 1.62 | 39.39 | 30.30 |
| fresh (Sasanishiki) | 0.63 | 22.27 | 0.20 | 1.60 | 40.65 | 33.74 |
| Okayama | | | | | | |
| 13 years (Nipponbare) | 0.80 | 25.93 | 0.16 | 1.75 | 36.74 | 33.14 |
| fresh (Nipponbare) | 0.76 | 21.54 | 0.21 | 1.59 | 38.21 | 36.41 |
| Kumamoto | | | | | | |
| 13 years (Nipponbare) | 0.76 | 23.34 | 0.22 | 1.66 | 37.73 | 34.35 |
| fresh (Nipponbare) | 0.70 | 20.92 | 0.20 | 1.50 | 35.39 | 40.07 |

increase of palmitic acid content and the decrease in content of linoleic plus linolenic acids were demonstrated as a feature of the compositional pattern of fatty acids in total lipid obtained from the long time-stored brown rices.

*A feature of the compositional pattern of fatty acids of neutral lipid- and free fatty acid fractions obtained from the long time-stored brown rices.* The decrease of neutral lipid content and the increase of free fatty acid content are probably significant at the primary stage of lipid-deterioration of brown rice during storage. Morita[12] reported on the difference between the rice stored at 4°C for 6 months and the fresh rice as follows: the increase of contents of oleic, linoleic and linolenic acids of free fatty acid fraction during 6 months. In the brown rices stored at 10°C for 13 years, the decrease in contents of palmitic, stearic and oleic acids of free fatty acid fraction was characteric as seen in Table 3. However, the increase in oleic acid content of neutral lipid fraction with the long time-storage was not recognized as shown in Table 4. According to Matsuda and Hirayama,[5] lipase activity of the brown rice highly decreased to the low activity value of 1/4 of the initial value during 2 years storage. This suggests

Table 3 Compositional ratios of major free fatty acids of the 13 years-stored and the fresh brown rices.

| Rice-producing district<br>Storage period<br>(Variety) | Fatty acid composition (%)/free fatty acid | | | | | |
|---|---|---|---|---|---|---|
| | $C_{14:0}$ | $C_{16:0}$ | $C_{16:1}$ | $C_{18:0}$ | $C_{18:1}$ | $C_{18:2}+C_{18:3}$ |
| Hokkaido | | | | | | |
| 13 years (Ishikari) | 1.05 | 25.81 | 0.27 | 1.76 | 25.84 | 35.85 |
| fresh (Shiokari) | 1.21 | 22.40 | 0.28 | 1.49 | 22.87 | 46.53 |
| Miyagi | | | | | | |
| 13 years (Sasanishiki) | 1.27 | 31.48 | 0.34 | 1.68 | 26.86 | 33.98 |
| fresh (Sasanishiki) | 1.28 | 23.65 | 0.29 | 1.47 | 26.04 | 41.97 |
| Okayama | | | | | | |
| 13 years (Nipponbare) | 1.34 | 28.53 | 0.30 | 1.75 | 24.31 | 37.02 |
| fresh (Nipponbare) | 1.74 | 24.90 | 0.27 | 1.23 | 23.16 | 43.20 |
| Kumamoto | | | | | | |
| 13 years (Nipponbare) | 1.40 | 29.85 | 0.36 | 1.54 | 25.51 | 34.59 |
| fresh (Nipponbare) | 1.32 | 23.30 | 0.26 | 1.32 | 21.00 | 48.00 |

Table 4 Compositional ratios of major fatty acids of the neutral lipid fractions obtained from the 13 years-stored and the fresh brown rices

| Rice-producing district<br>Storage period (Variety) | Fatty acid composition (%) | | | | | |
|---|---|---|---|---|---|---|
| | $C_{14:0}$ | $C_{16:0}$ | $C_{16:1}$ | $C_{18:0}$ | $C_{18:1}$ | $C_{18:2}+C_{18:3}$ |
| Hokkaido | | | | | | |
| 13 years (Ishikari) | 0.53 | 24.54 | 0.25 | 1.64 | 39.58 | 31.14 |
| fresh (Shiokari) | 0.57 | 21.45 | 0.27 | 1.58 | 37.17 | 37.68 |
| Miyagi | | | | | | |
| 13 years (Sasanishiki) | 0.59 | 25.85 | 0.29 | 1.57 | 39.70 | 30.64 |
| fresh (Sasanishiki) | 0.48 | 20.39 | 0.26 | 1.56 | 39.84 | 36.18 |
| Okayama | | | | | | |
| 13 years (Nipponbare) | 0.64 | 27.30 | 0.27 | 1.59 | 36.04 | 32.18 |
| fresh (Nipponbare) | 0.49 | 19.48 | 0.25 | 1.55 | 38.81 | 38.11 |
| Kumamoto | | | | | | |
| 13 years (Nipponbare) | 0.62 | 25.77 | 0.28 | 1.54 | 36.98 | 33.16 |
| fresh (Nipponbare) | 0.46 | 20.13 | 0.23 | 1.43 | 35.65 | 40.89 |

a possibility that there is a correlation between fatty acid composition of the neutral lipid fraction and the compositional pattern of free fatty acids only at the primary stage during storage of brown rice. In longer time-stored brown rices, it is assumed that there is a relationship between the increase of oleic acid content of free fatty acid fraction and the decrease of oleic acid content of phospholipid fraction (Table 6).

*A feature of the compositional pattern of fatty acids of glycolipid fraction obtained from the long time-stored brown rices.* Gas chromatographic patterns of fatty acid methyl esters obtained from glycolipid- and neutral lipid fractions are shown in Fig. 1 and Fig. 2. In the glycolipid fraction, some unknown peaks were detected in shorter retention time than that of methyl myristate. Unknown peaks 1 (U–1), 2 (U–2) and 3 (U–3) were not detected in the other lipid fractions.

The content ratios of major fatty acids of the glycolipid fraction obtained from the 13 years-stored and the fresh brown rices are shown in Table 5. In the 13 years-stored brown rices, contents of U–1, U–2 and oleic acid greatly increased. On the contrary, contents of U–3 and linoleic plus linolenic acids decreased. As to the

Table 5 Compositional ratios of major fatty acids of the glycolipid fractions obtained from the 13 years-stored and the fresh brown rices.

| Rice-producing district<br>Storage period (Variety) | Fatty acid composition (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | U-1 | U-2 | U-3 | $C_{14:0}$ | $C_{16:0}$ | $C_{16:1}$ | $C_{18:0}$ | $C_{18:1}$ | $C_{18:2}+C_{18:3}$ |
| Hokkaido | | | | | | | | | |
| 13 years (Ishikari) | 5.98 | 13.85 | 4.71 | 1.01 | 25.40 | 0.26 | 1.79 | 17.13 | 13.16 |
| fresh (Shiokari) | — | 0.57 | 9.98 | 1.98 | 31.41 | 0.57 | 2.05 | 13.51 | 20.98 |
| Miyagi | | | | | | | | | |
| 13 years (Sasanishiki) | 3.87 | 10.88 | 2.78 | 2.65 | 27.24 | 0.68 | 1.87 | 21.32 | 17.19 |
| fresh (Sasanishiki) | — | 1.29 | 9.48 | 1.64 | 26.25 | 0.58 | 1.87 | 16.86 | 21.92 |
| Okayama | | | | | | | | | |
| 13 years (Nipponbare) | 3.97 | 11.07 | 5.18 | 1.27 | 29.26 | 0.31 | 1.86 | 17.54 | 15.07 |
| fresh (Nipponbare) | — | 0.48 | 8.46 | 1.90 | 27.61 | 0.59 | 2.05 | 15.27 | 22.48 |
| Kumamoto | | | | | | | | | |
| 13 years (Nipponbare) | 3.80 | 11.56 | 5.24 | 1.11 | 26.78 | 0.40 | 2.00 | 17.69 | 14.95 |
| fresh (Nipponbare) | — | 0.35 | 8.06 | 1.72 | 28.50 | 0.58 | 2.07 | 17.23 | 24.85 |

U-1: unknown fatty acid 1, U-2: unknown fatty acid 2, U-3: unknown fatty acid 3

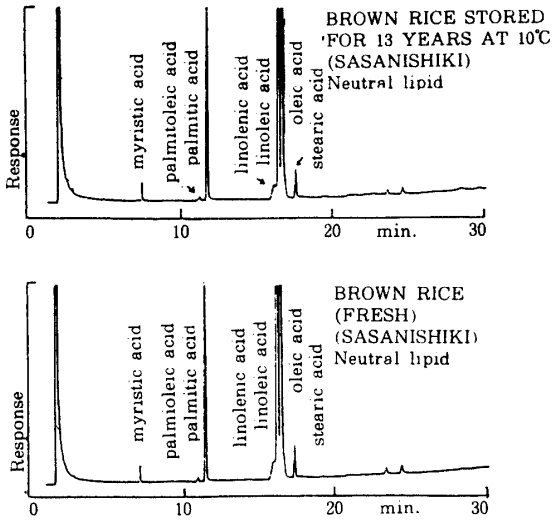


Fig. 1 Gas chromatograms of fatty acids in neutral lipid fraction obtained from the brown rices.

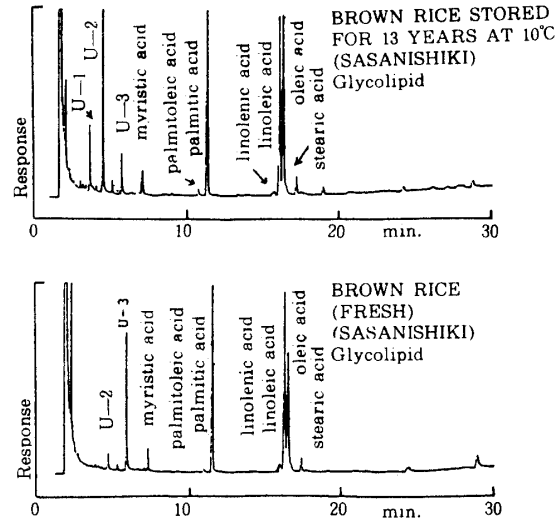


Fig. 2 Gas chromatograms of fatty acids in glycolipid fraction obtained from the brown rices.

increase of oleic acid content and the decrease of linoleic acid content in the glycolipid fraction during storage of brown rice, Shin *et al.*[6] already reported, but there was no report on the increase of U–1 and U–2 and the decrease of U–3 during their storage at low temperature. Mass spectra of their unknown components are shown in Fig. 3. Their components could not be identified by mass spectra alone, and it is expected that their compounds would be novel components in rice glycolipid fraction.

*A feature of fatty acid composition of phospholipid fraction obtained from the long time-stored brown rices.* The major fatty acid compositions of phospholipid fraction obtained from the 13 years-stored and the fresh brown rices are shown in Table 6. In the fresh rices, content ratios of fatty acids of the phospholipid fraction were in ascending order of palmitic, linoleic plus linolenic, oleic, myristic and stearic acids. Their content ratios were hardly different among the varieties of the fresh rices. Main molecular species of glycerophospholipids in rice, rice lecithin (1–palmitoyl–2–linoleoyl–3–cholinephosphoryl–sn–glycerol) and rice cephalin (1–palmitoyl–2–oleoyl–3–aminoethylphosphoryl–sn–glycerol), were already isolated by Fujino et al.[13] That palmitic acid was most abundant in the phospholipid fraction

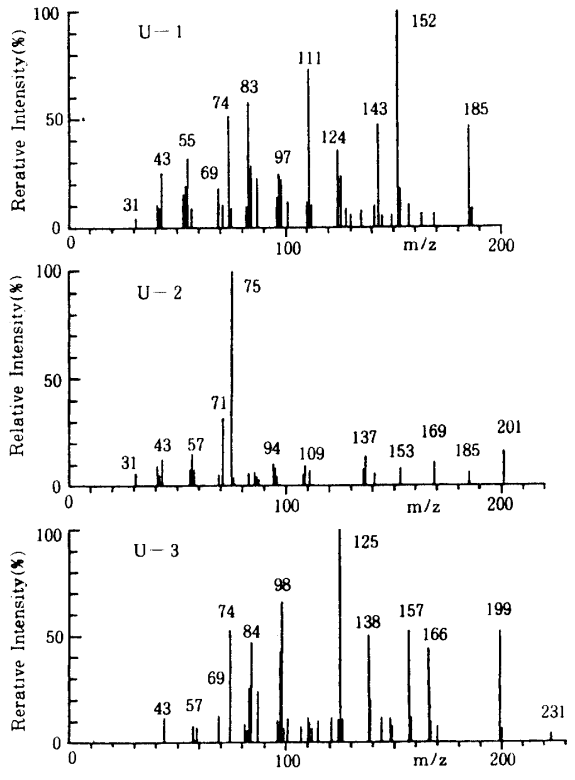


Fig. 3 Mass spectra of unknown peaks U–1, U–2 and U–3 detected in methanolyzate of the glycolipid fraction obtained from brown rice (Japonica).

Table 6 Compositional ratios of major fatty acids of the phospholipid fractions obtained from the 13 years-stored and the fresh brown rices.

| Rice-producing district<br>Storage period (Variety) | Fatty acid compositin (%) | | | | | |
|---|---|---|---|---|---|---|
| | $C_{14:0}$ | $C_{16:0}$ | $C_{16:1}$ | $C_{18:0}$ | $C_{18:1}$ | $C_{18:2}+C_{18:3}$ |
| Hokkaido | | | | | | |
| 13 years (Ishikari) | 4.09 | 47.97 | 0.24 | 1.51 | 11.51 | 32.93 |
| fresh (Shiokari) | 4.51 | 41.79 | 0.23 | 1.16 | 18.04 | 33.08 |
| Miyagi | | | | | | |
| 13 years (Sasanishiki) | 4.17 | 48.07 | 0.25 | 1.23 | 13.56 | 31.15 |
| fresh (Sasanishiki) | 4.00 | 40.05 | 0.27 | 0.81 | 19.80 | 31.00 |
| Okayama | | | | | | |
| 13 years (Nipponbare) | 4.61 | 49.61 | 0.21 | 1.32 | 10.80 | 32.33 |
| fresh (Nipponbare) | 4.10 | 41.51 | 0.24 | 1.11 | 20.00 | 32.05 |
| Kumamoto | | | | | | |
| 13 years (Nipponbare) | 4.82 | 49.39 | 0.22 | 1.27 | 11.63 | 31.50 |
| fresh (Nipponbare) | 4.63 | 42.46 | 0.25 | 1.16 | 18.87 | 31.30 |

could be estimated from the structures of their rice glycerophospholipids. Comparison of the 13 years-stored brown rices with the fresh brown rices showed the tendency slightly different from the change patterns in fatty acid compositions of the other lipid fraction during storage for a long time: in the 13 years-stored brown rices, the decrease of oleic and linoleic plus linolenic acids, and the increase of palmitic and stearic acids were recognized in Table 6. The results were considerably different from the change pattern in fatty acid composition of phospholipid fraction in brown rice during the shorter time-storage described by Shin *et al.*[6].

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# 長期低温貯蔵玄米の脂質及び脂肪酸組成の特徴

土田廣信・梅本貴之・津川兵衛
坂田光生・水野　進・丹下宗俊

## 要　約

従来，わが国の政府保管米の貯蔵は玄米貯蔵法が採用されてきた。常温貯蔵では米の品質劣化及び虫害による損失が著しいため，現在では低温貯蔵が広く採用されている。この貯蔵法によるとかなり長期間品質を保持できるといわれている。

本報では，このような低温長期貯蔵米の品質特性を明らかにするため，水稲4品種の玄米を10℃で13年間貯蔵し，これら長期貯蔵玄米の脂質及び脂肪酸の劣化特性について検討を行なった。

その結果，長期低温貯蔵玄米の脂質の特性として，常温貯蔵による古米化現象で認められるのと同様，中性脂質，リン脂質の減少，ならびに遊離脂肪酸の増加現象が認められた。しかし，短期常温貯蔵の際には見られない現象として，糖脂質含量が増加するという新知見を得た。また，中性脂質，糖脂質及びリン脂質及び遊離脂肪酸画分中の主要脂肪酸組成比を調べたところ，長期低温貯蔵玄米について，次の三の特徴のあることが判った。

1）パルミチン酸含量は，いずれの脂質画分においても増加するが，リノール酸及びリノレィン酸含量は，リン脂質を除くすべての画分で逆に減少した。

2）オレイン酸含量は糖脂質画分では増加するが，それに反しリン脂質画分では減少を示した。

3）糖脂質画分のメタノリシス生成物としと新たに確認された未知成分U－1，U－2及びU－3のうち，U－1及びU－2は著しく増加，逆にU－3は減少する傾向が認められた。