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SCANNING ELECTRON MICROSCOPE OBSERVATION OF MICROSTRUCTURAL CHANGES IN RICE GRAIN DURING STORAGE

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ABSTRACT

Changes in cytoplasmic membrane and lipid bodies in an aleurone cell are related closely to the deterioration in flavour of boiled rice. The progress of membranous damage and browning of lipid bodies was observed with a Scanning Electron Microscope (SEM). However, the differences noted in the deterioration process during drying and storage could not be attributed to thermal conditions created by the air temperature. This study will be continued in the future by improving on the treatment of specimens and observational techniques.

INTRODUCTION

Many biochemists have reported that chemical changes in lipid bodies are the cause of flavour deterioration of stored rice. Among such researchers, Yasumatsu *et al.* (1964, 1966) investigated those chemical effects on flavour, while Morita *et al.* (1972) explained systematically the mechanism of deterioration. Aibara *et al.*, (1986) and Bechtel *et al.*, (1977) reported that lipid bodies caused fusion when an aleurone cell was ruptured by an external physical force. Morita (1984) also explained that deformations and fusion occurred even under normal temperatures. On the basis of these results, Aibara (1986) recommended the introduction of a drying method under normal temperatures so that no rupture of the aleurone cells would take place. However, it is the feeling of the present authors that the change in flavour accompanying deterioration is integral in the whole grain and not every aleurone cell suffers physical rupture in the process of rice drying and storage. Therefore, deformations and fusion of lipid bodies during storage must happen spontaneously even in a non-ruptured aleurone cell; our objective was to prove this by microscopy. This presentation shows scanning electron microscope pictures depicting changes in aleurone cells of specimens dried and stored under different thermal conditions. Maintenance of rice quality during storage is influenced by storage conditions, including

fumigation and modified atmosphere treatments undertaken for control of insect pests. In this context, an understanding of the mechanisms of quality deterioration is relevant to this conference.

MATERIALS

During the harvesting period of October 1991, samples of rice (Mutsunishiki var.) at 22.8%, 24.5%, and 23.0% moisture content (m.c.) were collected on the 1st, 4th, and 8th day of the month, respectively. These samples were then dried using three methods, namely:

(1) Spreading rough rice on a room floor to dry to 14.1% m.c.
(2) Reduction to 14.2% m.c. by heating at 37°C.
(3) Moisture removal down to 15.0% by dehumidifying the drying air under near-ambient temperature conditions.

METHODS

Storage

A total of 36 glass bottles were filled each with 1 kg dried rough rice and put inside a walk-in chamber. Temperatures were set at 10, 15, and 20°C, but these settings fluctuated from 9.4-11.5°C, 13.5-15.0°C, and 18.5-23.8°C, respectively. In a 40 x 50 x 60 cm constant-air chamber, there was little fluctuation of the set temperature of 30°C. After approximately one month in storage, all of the samples were husked so as to accelerate the rate of deterioration.

Preliminary arrangements for SEM observations.

Lateral sections of brown rice 1 mm thick were produced carefully and mounted on a brass stub. A preliminary test on the possibility of chemically fixing the samples was done using a small bottle saturated with osmium oxide gas. The cytoplasmic membrane dissolved due to the modifying effect of phospholipids and only protein bodies remained. Therefore, this procedure was abandoned.

Protein bodies were less distinct around the semi-transparent, non-sputtered cytoplasmic membrane (Fig. 1), hence it was very difficult to notice any microstructural changes of lipid bodies. As such, gold sputtering was done to protect the charge-up and avoid heat damage to the specimens. After trying various thicknesses of film coating, 50 nm was chosen to avoid electron beam and vacuum damage during SEM observations. A model JFC-1100 ion sputter equipment with a 1.33×10^{-4} kPa vacuum gauge made by Japan Electron Optics Laboratory Co. (JEOL) was used for these experiments.

SEM used for observation and its adjustments

A model JSM-T200 made by JEOL was used with a resolving power of 7 nm, 25 kV accelerating voltage and maximum magnification of 10^5. Photographs of CRT reflection outputs were taken using Polaroid instant pack film T665.

RESULTS

The inside of cells, ruptured when sectioned under normal temperatures, could be seen in one or two layers of aleurone cells arrayed circumferentially on the sectioned face. At the beginning, parts of lipid bodies became clearly visible at a magnification ranging from $1x10^3$ - $2x10^3$. Once a proper spot was selected, the magnification was increased to $1x10^4$ and a photograph was taken after careful observation.

Observations soon after the beginning of storage.

Figs. 2A, 2B, and 2C show the appearance of lipid bodies dispersed within the cytoplasmic membrane in an aleurone cell. With a sputtered film thickness of 50 nm, the specimens could only be described as artifacts. Nonetheless, the spherical lipid bodies dispersed on the relatively smooth membranous surface were clearly visible and their sizes differed from one location to another. Naturally, they might exist inside an opaque membrane. Again, the appearance of the obscure membrane initially invisible on a non-sputtered surface became clear and relatively smooth. The fissures on the membraneous surface resulted from electron beam damage. No differences were detected on the samples due to the three methods of drying adopted for the experiment.

Observations after six months of storage

Two notable changes had occurred: (1) folds and ruptured parts on the membrane increased, revealing more protein bodies, and (2) the lipid bodies had become brown and partially visible. From observations made every month, specimens stored at 20 and 30°C showed evidence of rancidity after two months. After five months, those samples stored at 10°C also started showing signs of rancidity. Browning was quite noticeable on samples observed after six months in storage (Figs. 3A, 3B, 3C, and 3D). Membrane changes advanced earlier than browning, this being detected after two and half months. Had the sputtered film been thin enough, browning could have been apparent much earlier.

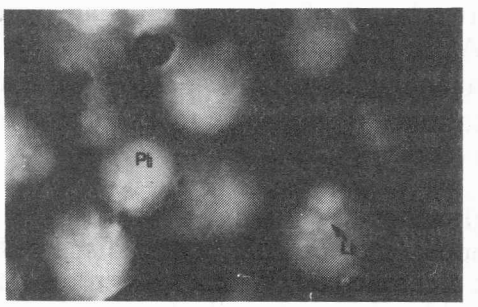

Fig. 1: View of a non–sputtered cytoplasmic membrane.

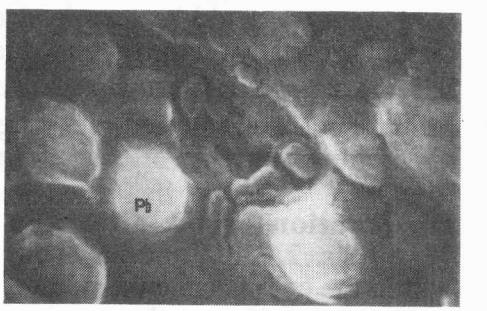

Fig. 2A: At 10°C

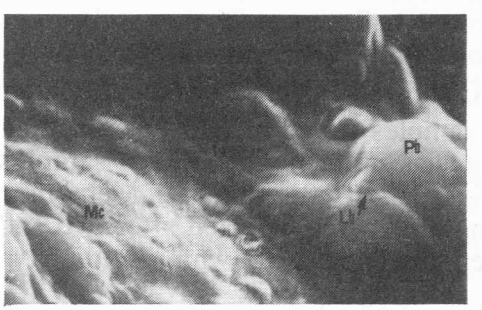

Fig. 2B: At 20°C

Fig. 2C: At 30°C

Fig. 2: View of sputtered cytoplasmic membrane of heat-dried rice (at 37°C) soon after the beginning of storage; (A) at 10°C, (B) at 20°C, (C) at 30°C.

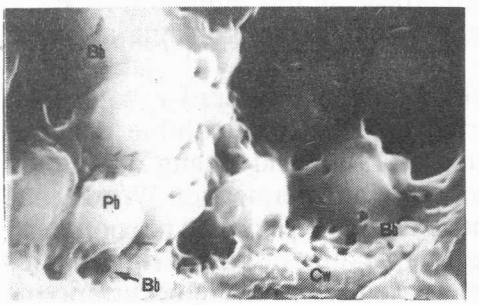

Fig. 3A: At 10°C

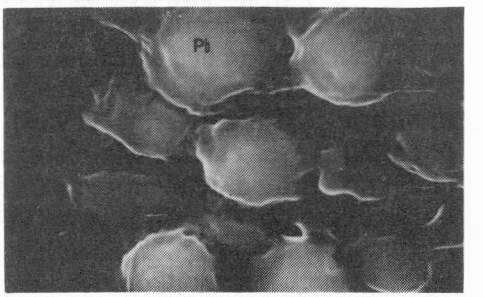

Fig. 3B: At 15°C

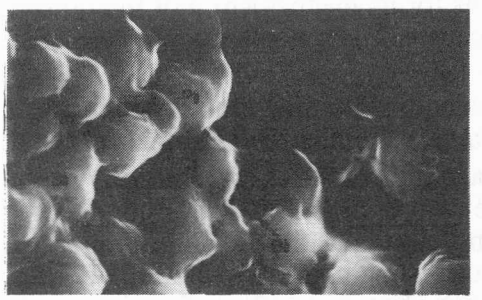

Fig. 3C: At 20°C

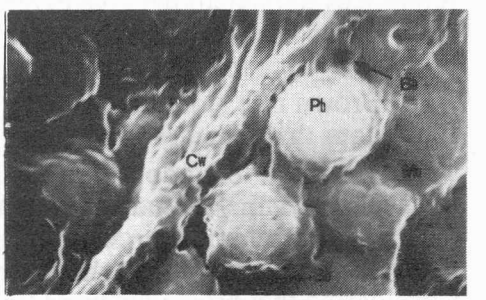

Fig. 3D: At 30°C

Fig. 3: View of sputtered cytoplasmic membrane of heat-dried rice (at 37°C) after 6 months of storage; (A) at 10°C, (B) at 15°C, (C) at 20°C, (D) at 30°C.

Adjustments:
Magnification: 10^4 times
(white bar: 1μm)
Accelerative voltage: 25 kV
Working distance 20 mm

Symbols:
Pb: Protein body
Lb: Lipid body
Mc: Cytoplasmic membrane
Bb: Browned body
Cw: Cell wall

DISCUSSION AND CONCLUSIONS

Observation of the sectioned piece using SEM without applying any artificial treatment revealed evidence of electron beam damage to the cytoplasmic membrane. As such, gold sputtering was necessary to avoid these damages and to enhance clarity of reflections. Although increased sputtering thickness protected the membrane from external physical forces, the specimens became no more than artifacts that differ greatly from known natural appearance. As sputtering thickness increased, stability and naturalness of the membrane became reciprocal. In this situation, no other alternative was available except to reach a conclusion from the apparent differences before and after storage.

Concerning the lipid bodies visible on the sputtered cytoplasmic membrane, Takano *et al.* (1989) reported that the lipid soluble enzyme, phospholipase, caused the changes. Aibara (1986) and Zhang and Bekki (1991) presented detailed facts on fusion, ooze, and permeation. Work done by Aguilera (1990) and Huang (1984) have also supported the phenomenon of fusion whenever there is cell rupture. Our finding of brown lipid bodies other than changes cited in the above references signifies the occurrence of chemical changes. Sakurai and Kurata (1966) explained that browning appears easily due to the reaction of unsaturated carbonyl compounds produced by autoxidation of lipids. Nakabayashi *et al.* (1967) described it as carbonyl compounds and peroxides produced by lipids reacting with protein. Similarly, Moritaka *et al.* (1972) reported that deterioration involves an SH-radical. According to Karel (1975), lipid oxidation and non-enzymatic browning reached a peak at a water activity of about 0.6. Usually the lipid is able to stain with various soluble pigments or by aldehyde reaction and the references quoted above give expressions for its mechanism. For the present case, it was hard to judge the extent because of a monochrome film, in spite of the colour changing to brown.

Brown rice stored at 30°C in glass bottles became rancid, producing a foul odour that increased in intensity after six months. The samples stored at 10°C produced less odour. Higher storage temperatures and intensity of the unusual smell may be indicators of the extent of rancidity. It was conjectured that catalytic autoxidation was proceeding. Again, as the moisture evaporated naturally from brown rice, they started clumping together within the bottles. Considering that brown coloured lipid bodies became evident after six months on samples kept at 10°C when odour alone could not detect rancidity, the conclusion is that chemical changes may occur in the lipid bodies and influence the flavour. Yasumatsu *et al.* (1966), Aibara *et al.* (1986), and Yoshizawa *et al.* (1980) reported that chemical changes causing deterioration in flavour resulted from neutral lipids hydrolyzed under catalysis of lipase and increase in free fatty acids.

This present work is significant in presenting the microstructural features and demonstrating the accompanying changes. The results could

provide useful information for the improvement of appearance and storage of rice (rough, brown or milled rice). If treatment of specimens is improved and SEM with a higher resolving power is used in future, it will be possible to find a close relationship between flavour and microstructural changes on stored rice.

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