The Microstructure of Polished, Milled and Air-classified Rice and Rice Bran

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The microstructure of polished rice, its milled and air classified fractions was observed under a scanning electron microscope. The cells of rice endosperm, showing long rectangular column shape, distributed radially from center to outer layer. In the cells, many compound starch granules in polygonal shape plugged up. By immersion in water, NaCl or alkaline solution, cleavage and degradation of the structure were accelerated. Milling and air-classification of polished rice were examined to get a protein rich fraction. Such a fraction (total N: 9.4%) was obtained from the flour (total N: 7.4%). The sonic treatment of rice bran in the presence of n-hexane and successive milling and air-classification gave a relatively promising result to get protein rich flour.

Rice is the most important and staple source in the Japanese dietary habit. Recent surplus production of rice increased attention to the utilization not only in grain state like cooked rice but also in flour state. The trial to fractionate and prepare protein rich flour was reported by STRINGFELLOW et al., HOUSTON et al. and MITSUDA et al. from rice grain and by BARBER et al., CONNER et al., SAUNDERS et al. and MITSUDA et al. from rice bran. A few among them employed a dry system, that is, milling and air-classification.

Microstructure of rice has been reported by MITSUDA et al., JULIANO, EVERS and JULIANO, OGAWA et al., BECHTEL and POMERANZ and WATSON et al., especially about protein bodies in aleuron and subaleurone regions of endosperms.

In this paper we report the effects of pre-treatments and conditions on the fractionation with the purpose of preparing the protein rich flour from polished rice or bran by dry system; we also observed the structure of polished rice, bran and their flours.

Materials and Methods

Effect of premoisturing on fractionation
Polished rice (cultivar Sasanishiki, short grain type, polished to 91% of its initial weight to remove the husk and bran) was immersed in water, 1.5 M NaCl solution or alkaline solution (adjusted to pH 8 with NaOH) for 30, 60 and 120 min and dried to about 30% moisture at room temperature. Premoistured rice kernels were mounted on aluminium stubs with silver cement, in order to observe the surface of polished rice and inside face fractured at the cleavage produced by immersion, and coated with carbon and then with gold. The initial rice kernels were observed similarly to premoistured ones. Electron micrographs were taken with a scanning electron microscope (SEM, Hitachi HHS-2 R) at 20 kV.

Milling and air-classification of polished rice
The polished rice (cultivar Kiyonishiki, short grain type, polished to 90%) was frozen-milled by an impact mill (Kolloplex 160Z) followed by a jet mill (Aeroplex 200 AS) 2 times and then air-classified at 5 μm by an air-classifier (Alpine Multiplex 100 MZR). Each fraction obtained by the above processes, was submitted to micro-kjeldahl analysis and SEM observation. To observe flour samples, aluminum stubs were covered with aluminum foil and milled or air-classified flours were scattered on aluminum foil after nail paint put lightly to fix the flour. The samples were coated with gold. The flow sheet of fractionation is shown in Figure 1.
Sonification, milling and air-classification of rice bran

Rice bran commercially prepared, was suspended with the same weight of n-hexane, sonicated by a sonicator (Toyoriko UD-N-50-6) at 50~55 kHz at 100 W for 20 min at room temperature and air-dried to remove n-hexane. The defatted rice bran was milled by an impact mill (Kolloplex 160 Z) and fractionated through the sieve of 73 μm. One half of the fraction which passed through 73 μm, was air-classified at 10 μm and the other half was jet-milled and then again air-classified at 10 μm. The flow sheet of fractionation is shown in Figure 2. Rice bran was mounted similarly to milled rice flours for observation with a SEM. Rice bran used in this experiment was kindly supplied by Boso Oil Co., Ltd. Brown rice was polished to 90% in stepwise manner with a commercial polisher (Satake). The polishing of first, second, third and fourth steps gave 1, 5, 2.5 and 1.5% brans respectively to the initial brown rice.

Results

Microstructure of polished rice and its changes by premoisturing

Figure 3-A shows the outer face of polished rice grain, where the clear structure cannot be observed because of the fragments attached to the surface. Figure 3-B shows inside surface carefully fractured at the cleavage, namely traverse fractured face, in which the radial distribution of rectangular cells was observed in central region. As shown in Figures 3-C and D, the long rectangular cells in which starchy granules were polygonal and compounded with the structure of a pentagonal dodecahedron, seemed to be coated with thin cell wall. Protein bodies were about 1 μm in size and located between starch granules. The protein bodies were observed more frequently in subaleurion layer near outer layer than in central region. In Figure 3-E, protein bodies are observed between starch granules.

The polished rice could absorb the maximum amount of water after 30 min and Figures 4-A, B and Figures C, D show the outer layer and traverse faces, respectively. In Figures 4-A, B, the rectangular cells crossed by polishing was clearly observed, being different from Figure 3-A with many fragments. Compound starch granules in cells in Figures 4-C, D
Fig. 3 Microstructure of polished rice grain

A: Outer face, showing the cleavage in central region. Bar represents 30 μm.
B: Traverse fractured face, showing the radial distribution of rectangular cells. Bar represents 100 μm.
C: Traverse fractured face. Bar represents 20 μm.
D: Compound starch granules in rectangular cells. Bar represents 4 μm.
E: Fractured compound starch granules in rectangular cells, showing protein bodies between starch granules. Bar represents 5 μm.
seemed to be clearly bordered than those before water immersion in Figures 3-C, D.

In the case of immersion in NaCl solution, the solution became turbid after about 60 min. Figures 5-A, B show outer layer and traverse faces of rice after 60 min, respectively. In the SEM image of Figure 5-C with higher magnification, a starch granule with pentagonal hexahedron shape was shown distinctly.

The immersion in alkaline solution made the solution turbid and yellow after only 10 to 30 min. Figures 6-A, B may suggest that immersion in alkaline solution accelerated the loosing of compound starch granules to separate into single starch granules much more than the cases of water and NaCl, and that it damaged cell walls or other microstructures.

**Milling and air-classification of polished rice**

Premoisturing accelerated the separation into single starch granules (4~5 μm in size) and those small single starch granules prevented the air-classification of protein bodies (1~4 μm). Therefore, freeze-milling at -21°C was carried out to polish rice and it was followed by milling with an impact mill and successive milling with a jet mill. Table 1 shows the yield and protein content of final fractions which were separated at 5 μm cut point, following the flow sheet in Figure 1. Figures 7-A, B, C show initial flour, coarse and fine flours after air-classification, respectively. In the course of this observation with a SEM, we found that the remaining part after each jet milling showed special structure.
Fig. 5 Microstructure of polished rice after immersion in NaCl solution for 60 min
A: Outer face. Bar represents 30 μm.
B: Traverse fractured face. Bar represents 20 μm.
C: Traverse fractured face with higher magnification, showing a starch granule with pentagonal hexahedron shape. Bar represents 5 μm.

Fig. 6 Microstructure of polished rice after immersion in alkaline solution for 60 min.
A: Outer face. Bar represents 20 μm.
B: Traverse fractured face, showing compound starch granules. Bar represents 5 μm.
Fig. 7 Microstructure of milled and air-classified rice flour

Fig. 8 Microstructure of rice bran
like potato, probably because starch granules were scraped during grinding (Figure 7-D). As shown in Table 2, the two residual fractions were much less in protein content than the initial flour.

Sonification, milling and sieving of rice bran

Figures 8-A, B, C, D show the microstructure of raw bran. The bran obtained from the first two steps-polishing contained a large part of pericarp and that from the successive two steps consisted of aleuron or subaleurone layers, in which many protein bodies were observed. Table 3 shows the effect of sonic treatment on the yield and protein content of fractionated flours, following the flow sheet of Figure 2. The result showed that the treatment clearly contributed to increase the yield of protein content of fine flours.

Discussion

Japanese have had milled rice traditionally in home industries and have used it for confectionery. They polish, premoisture with water and pound the rice grain to get fine flour. It is known from historical experience that vitreous region near outer layer is harder than central region, consequently the flour derived from outer layer is easily collected into coarse fraction, which is richer in protein content than fractionated protein rich flour depending on these empirical processes and examined the characteristics of fractionated flours. In order to prepare rice starch not only for confectionery but also for painting, photography, printing and cosmetics, modern milling factories immerse rice in alkaline solution ahead of milling. The premoisturing ex-
Experiments in present paper revealed that pre-moisturing treatments made the cement between compound starch granules loose and separated them into single starch granules. The treatment seemed to be useful to get single starch granules without damage. However, it was not effective for the protein fractionation followed by air-classification because single starch granules were easily mixed with protein bodies.

Milling and air-classification carried out in this paper increased protein content of very fine flour slightly but this method may not be economical from the viewpoint of energy consumption. It a sense, it is notable that the residue of jet-milling contained less protein, showing potato-like shape, because the shape of fine flour often contributes to functional properties, namely, this may be creaky and smooth to the tongue.

On the other hand, sonification with n-hexane, milling and air-classification of rice bran might be promising by establishing the proper conditions in the future work. Although the mixture of different characteristic bran obtained by stepwise polishing, was used in this experiment, it will be possible to collect only aleuron and subaleurone layers. These regions contain more protein bodies and less starch with fibrous fragments derived from pericarp. The number and size of protein bodies observed in fibrous fragments seem to be more suitable for milling and air-classification, if protein bodies can be dispersed by sonification.

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