A Method to Remove the Outer Layer of Rice Endosperm Without Damaging Starch Granules

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ABSTRACT

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A new milling method was contrived to prepare intact starch granules from the outer layer of rice grains. The grains were first soaked in 0–100% ethanol and then wet milled. As the ethanol concentration increased, the solvent content of soaked grains decreased, and the hardness of the grains increased; both showed an inflection point at an ethanol concentration of 60%. It was proved satisfactory to soak and mill rice grains in 60–70% ethanol, based on the milling degree, milling breakage, and the properties of the starch granules prepared. This method is useful as a laboratory technique.

The heterogeneous characteristics of rice kernel from the outer layer to the central core have been shown for both chemical composition and histological structure (Choudhury and Juliano 1980, Hoshikawa 1968). Hoshikawa (1968) observed that starch granules in the outer layer increased in size more slowly and were smaller than those in the central core, and he thought that the outer layer starch might be more important for the eating quality of rice. Barber (1972) found that the changes during storage were much greater in the outer than in the inner layers. Up to now, however, most knowledge of rice quality has been based on data averaged from the entire kernel. Reports on the properties of starch from the outer and inner portions of rice grain are few.

The difficulty in studying the properties of different layers of rice kernel lies in removing the outer layer uniformly with minimal change in its properties and keeping the central core integral. Various abrasive and friction type mills have been used for this purpose in the laboratory, but a suitable one could not be found because dry milling increases the temperature of rice (Yanase and Tani 1969) and damages the starch granules, as proved by the present experiment. Solvent extraction milling of rice has been practiced commercially (Lynn 1966). However, we found that only the bran layer was softened, and hardness of starchy endosperm was essentially unchanged in rice oil and hexane, so undamaged starch granules were not obtained from the outer layer of starchy endosperm by following the solvent extraction milling procedure.

To overcome the inconvenience, we contrived a new method, so-called "wet milling," to separate the outer layer from the central core without damaging the starch granules. The result was satisfactory and is reported here.

MATERIALS AND METHODS

Rice Sample

The brown rice of a nonwaxy *japonica* (cultivar Koganemasari) was used in this experiment. Rice grains were stored under low temperature (5° C) after harvesting in 1985. In order to increase the moisture content to more than about 15%, grains were packed in paper bags and kept below 5° C, relative humidity 92%, for a certain period. When needed, rice grains were also dried in a desiccator over silica gel. A sample was screened first with a sieve then selected by hand. Only the perfect rice grains were used in the test.

Experimental Apparatus

We intended to study the properties of starch granules from different layers of rice grain, but found that the starch granules in the flour removed with a common abrasive mill were severely damaged. The same result was also shown by a friction mill. We considered that the dry type mills were not suitable for such a study

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because the rice grains were hard and resistance in the mill was strong. Rice grains can be softened with water, but the water-soaked grains were very fragile and the milling breakage was high. After a series of experiments, ethanol was found to be a desirable solvent because it can make a solution of any concentration with water, and the hardness of rice grains can be adjusted by soaking the grains in solution of a given concentration. The other advantage of ethanol is that it can inhibit enzyme activity. To mill the softened grains, the milling resistance must be gentle, at the same time, an adequate circulation of grains is needed to ensure the same milling degree in all the grains. This was achieved using the apparatus shown in Figure 1. The apparatus was composed of three parts: a stirrer motor (Tokyo Rikakikai Co. Ltd.), rotation of which can be controlled in the range of 50–1,200 rpm; an abrasive disk, 10 cm o.d., no. 120 granularity (Resibon Co. Ltd.); a 2-L beaker, 19.5 cm tall, 13 cm i.d. The container can be cooled by a surrounding water or ice bath. In this simple apparatus, the rotation of the abrasive disk, the amount of rice grain, the ratio of rice to solvent, and the temperature can be controlled as needed.

Milling Method

Distilled ethanol was diluted to a series of concentration solutions with water. Selected brown rice was soaked in solvents in the ratio of 1:6, w/v, for 24 hr at about 15–20° C. Generally, the amount of soaked grains added to the container of the apparatus was 300 ml (250 g of brown rice), and the total volume was made up to 500 ml with solvent. The disk rotation was 600 rpm. We call this method, in which rice grains are first soaked then milled in solvent, "wet milling," in contrast to common dry milling that mills the nonsoaked rice grains.

After the first milling fraction of 8% of the brown rice had been discarded, rice flour of the outer layer (from the milling fraction 9–20%) was collected. Starch granules were prepared by washing first with sodium dodecylbenzene sulfonate (SDBS), then with water. The starch was air-dried and powdered in a mortar with a pestle and passed through a 100-mesh sieve.

To investigate the effect of ethanol during wet milling on the properties of starch granules, brown rice was milled to 85% milling degree with a Satake test mill. One lot of the white rice was soaked in water, and the other in 70% ethanol for 24 hr; then each lot was broken with a Waring Blendor, and stirred for 4 hr using the wet-milling apparatus. The treated grains were finally homogenized and passed through a 200-mesh sieve. Starch granules were prepared by the SDBS method.

Analytical Methods

After every hour of wet milling, the solvent was renewed, and 3–5 g of milled rice was sampled three times to check the milling degree and milling breakage. Milling degree was calculated from the wet weights of the milled rice and the soaked brown rice after the solvent at the surface was blotted away with tissue paper. Milling breakage was calculated from weights of the broken pieces and the total sampled. Solvent content was obtained by drying the samples in a 110° C oven for 24 hr.

The hardness of rice grain was determined on the hardness meter made by Kiya Seisakusho Ltd. One grain was put on the test stage, then pressure was applied. When the first split occurred on the grain, a reading was taken as the breaking hardness, and when the

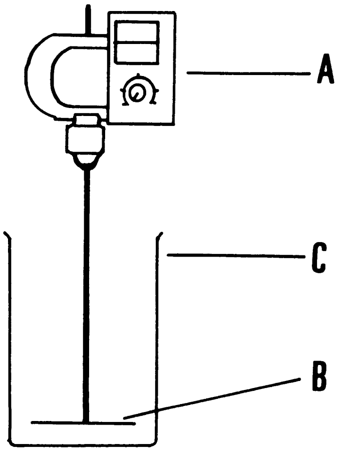


Fig. 1. Laboratory apparatus for wet milling of rice. Stirrer motor (**A**), abrasive disk (**B**), and container (**C**).

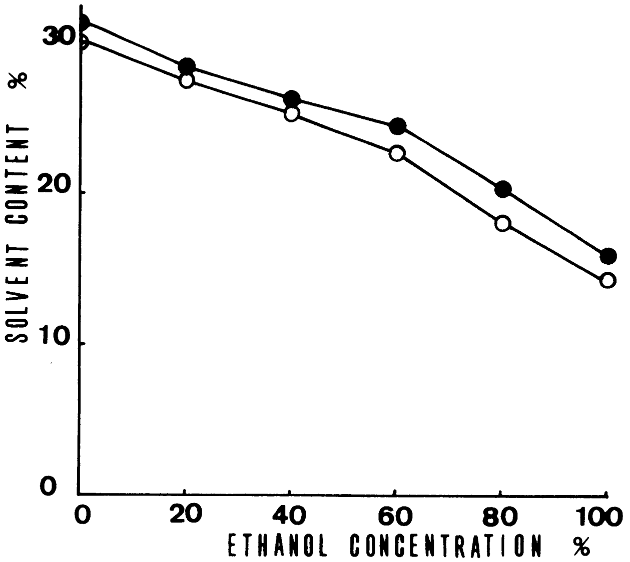


Fig. 2. Relationship between solvent content in rice and soaking ethanol concentration. ● = brown rice, ○ = milled rice of 80% milling degree. Rice grains were soaked in solvent (1:6, w/v) for 24 hr. Data are averaged values of three tests.

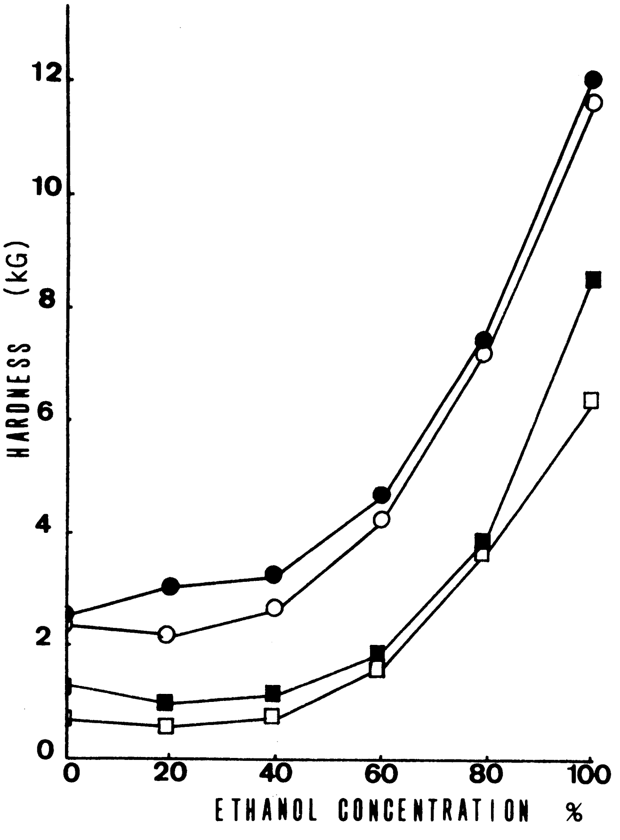


Fig. 3. Hardness of rice grains soaked in solvents with various ethanol concentrations for 24 hr. ● = crushing hardness of brown rice, ○ = crushing hardness of milled rice, ■ = breaking hardness of brown rice, □ = breaking hardness of milled rice. Data represent means of triplicates each with 30 measures.

grain crushed or deformed, it was read as the crushing hardness.

The X-ray diffraction test was conducted on a Rigakudenki X-ray diffractometer RAD II. The operating conditions were as described elsewhere (He and Suzuki 1987).

The amylograph test was conducted on a standard model Brabender amylograph instrument. Starch (39.6 g dry weight) was slurried in water, and the total weight was made to 450 g. Halick and Kelly's temperature program (Halick and Kelly 1959) was followed with some modifications. The slurry was stirred for 5 min at 30° C, then heated to 93° C at a rate of 1.5° C per min, maintained at that temperature for 8 min, and cooled at the same rate to 50° C. The amylograph cup was rotated at 75 rpm.

RESULTS AND DISCUSSION

Solvent Content and Hardness of Grains

The nonsoaked brown rice that contained 12.7% water showed breaking hardness 7.2 kg/grain (range 6.0–9.0, C.V. 15.5) and crushing hardness 9.0 kg/grain (range 7.5–11.0, C.V. 15.5). When the rice grains were soaked in water for 24 hr, the water content increased to more than 30% and the hardnesses decreased remarkably. The solvent content of grains decreased as the ethanol concentration increased (Fig. 2). When the concentration of ethanol went above 60%, the decrease in the solvent content of grains became more rapid. In this experiment, it was interesting to note that the relationship between solvent content of rice grains and the ethanol concentration in solvent was not linear, but showed an inflection at the ethanol concentration of 60%. The change in solvent content in milled rice of about 80% milling degree was similar to that in brown rice except the former contained 1–2% less solvent than the latter. A similar result was also obtained in brown waxy rice. However, waxy rice absorbed more solvent than nonwaxy rice in the same condition (data not shown).

When the grains were soaked in 20 and 40% ethanol, the hardness varied little from that of grains soaked in water (Fig. 3). When ethanol concentration exceeded 60%, hardness increased rapidly. This might be attributed mainly to the solvent content of the grain, for the correlation coefficient was −0.969 ($P = 0.01$) between the breaking hardness and solvent content, and −0.987 ($P = 0.01$) between the crushing hardness and solvent content. The milled rice showed a lower value of hardness and might be more likely to break in milling than the brown rice. Once the relationship between the hardness of rice grains and the soaking ethanol concentration was recognized, it became possible to obtain grains with suitable hardness by controlling the ethanol concentration in the soaking solution.

The solvent content and hardness of soaked rice grains also varied with the condition during soaking. Table I shows the data

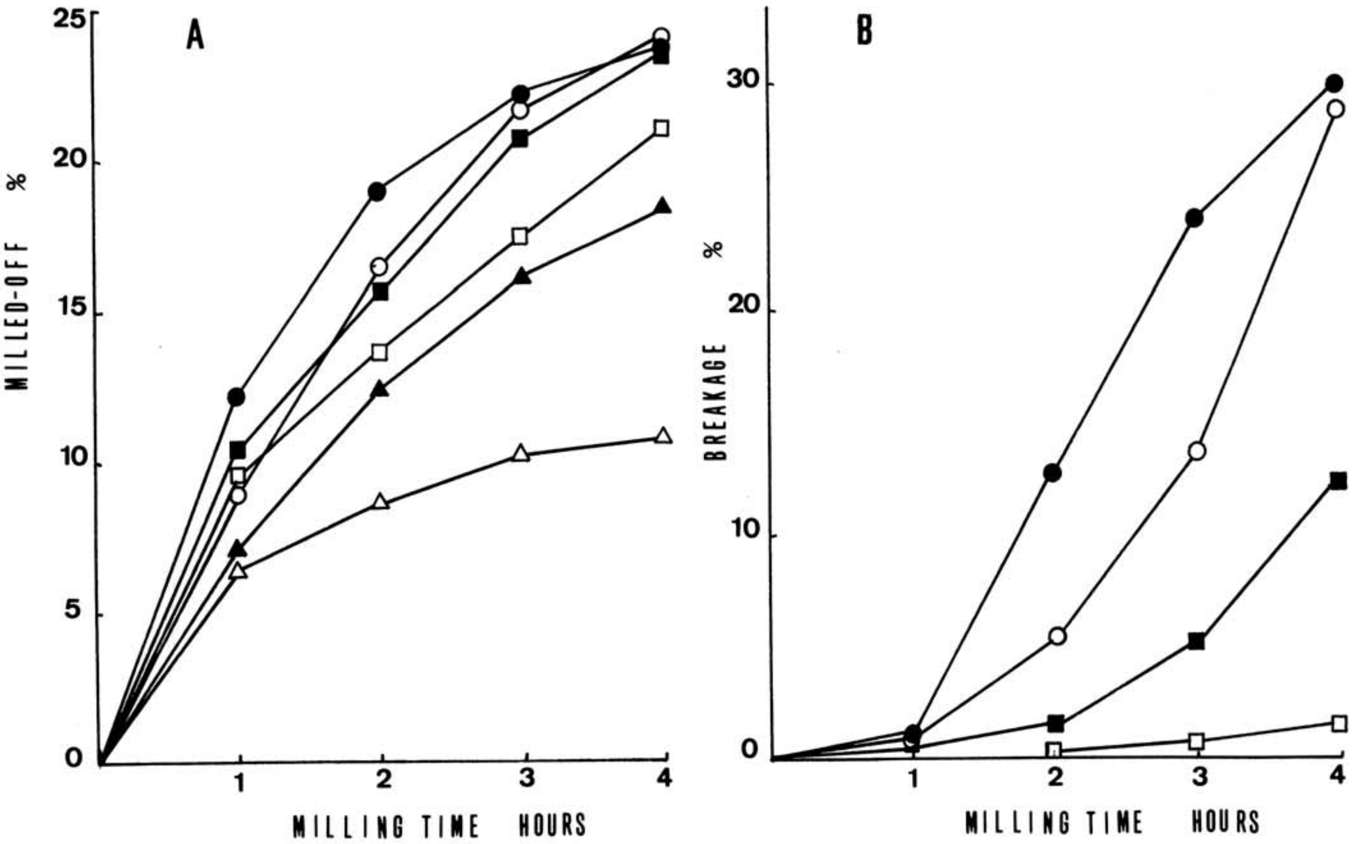


Fig. 4. A, Advance of milling degree by wet milling. **B,** Variation of milling breakage by wet milling. Rice grains were first soaked then milled in ● water, ○ 20% ethanol, ■ 40% ethanol, □ 60% ethanol, ▲ 80% ethanol, or △ 100% ethanol at room temperature. Starting moisture content of brown rice was 15.8%. Data represent means of triplicate mill tests.

TABLE I
Solvent Content and Hardness of Rice Grains Soaked Under Different Conditions

	Low-Moisture Grains (8.6%)				High-Moisture Grains (16.6%)			
Parameter	**28°C/60%**[a]	**28°C/80%**	**10°C/60%**	**10°C/80%**	**28°C/60%**	**28°C/80%**	**10°C/60%**	**10°C/80%**
Solvent content, %	25.4 ± 0.06	23.0 ± 0.01	22.0 ± 0.10	19.2 ± 0.03	25.3 ± 0.01	23.5 ± 0.34	22.3 ± 0.15	20.4 ± 0.13
Breaking hardness, kg	1.34 ± 0.23	1.98 ± 0.18	4.32 ± 1.59	6.84 ± 1.77	1.56 ± 0.35	2.21 ± 0.41	3.46 ± 0.51	4.09 ± 0.52
Crushing hardness, kg	3.38 ± 0.45	4.03 ± 0.57	7.27 ± 2.35	10.4 ± 2.26	3.39 ± 0.55	4.30 ± 0.94	6.65 ± 0.92	7.88 ± 1.06

[a] Column headings given as soaking temperature/ethanol %.

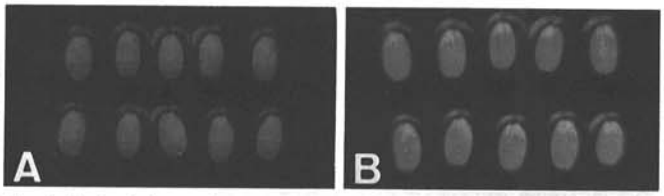


Fig. 5. Soaked rice grains of different moisture contents. **A,** There were many cracks on the surface of grains in which starting moisture content was low (8.6%); **B,** but not on those in which the starting moisture content was high (16.6%). Grains were soaked in 60% ethanol at 28° C for 24 hr.

TABLE II
Size of Rice Grains Before and After Wet Milling (mm)[a]

Sample	Length	Width	Thickness
Brown rice	5.15 ± 0.13	3.02 ± 0.06	2.07 ± 0.06
Milled rice[b]	4.52 ± 0.22	2.62 ± 0.10	1.79 ± 0.07
Difference	0.63	0.40	0.28
%	12.2	13.2	13.5

[a] Data are averages of 60 measures.

[b] Wet-milled in 60% ethanol for 5 hr; milling degree, 75%.

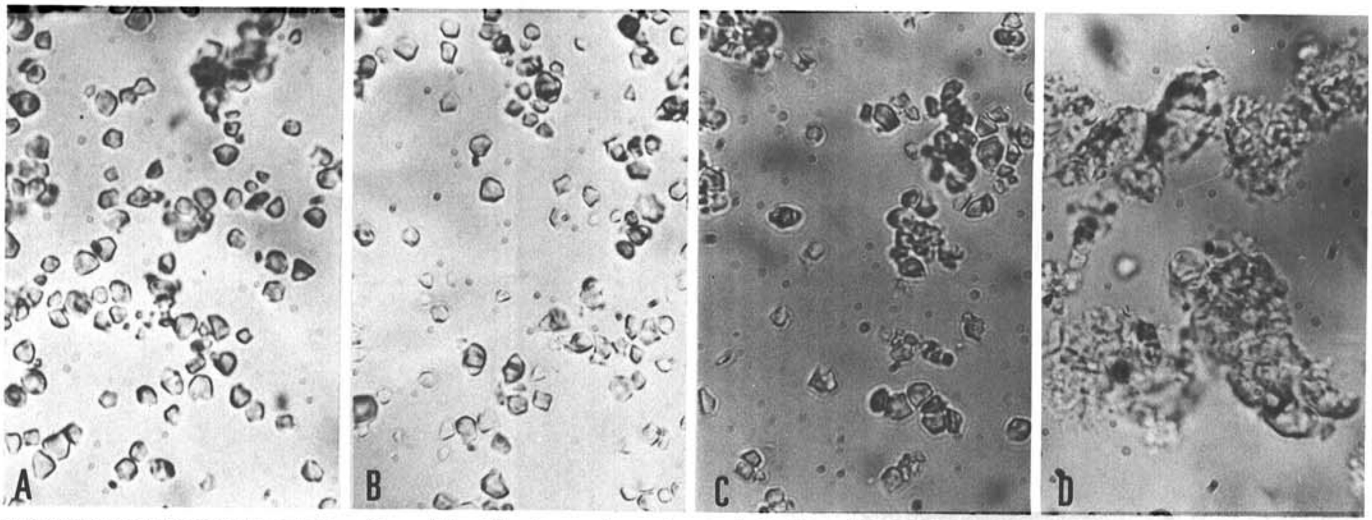


Fig. 6. Microphotograph of the outer layer flour of rice. Starch granules can be seen in the flour milled-off in **A,** water; **B,** 60%, and **C,** 80% ethanol, but not that in **D,** by a dry-type mill.

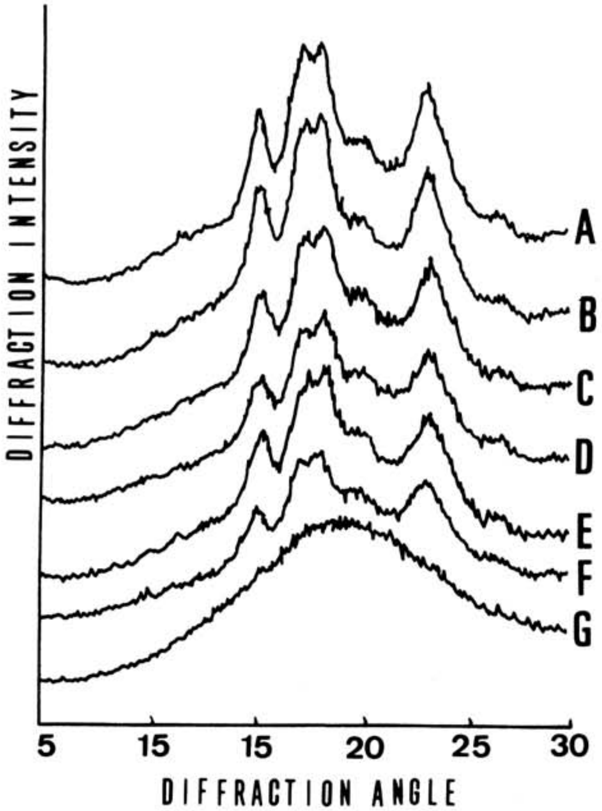


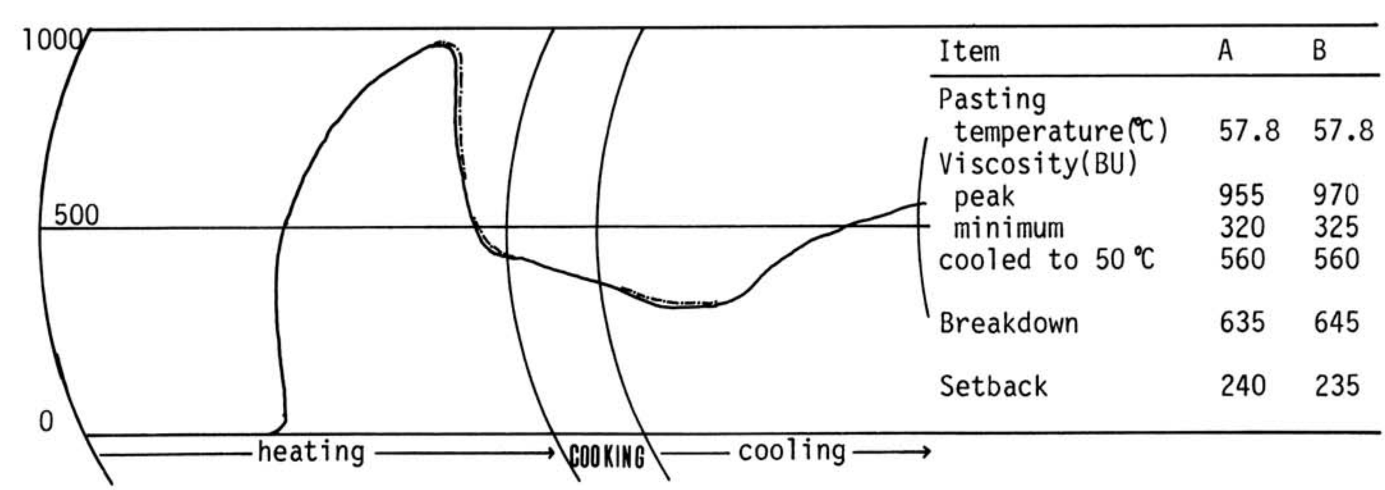
Fig. 7. X-ray diffraction patterns of rice starches and flours. The patterns of starches prepared from flour milled-off in water (**A**) and 70% ethanol (**B**) were almost the same, and those of rice flour milled in water (**C**), 60% ethanol (**D**), and 70% ethanol (**E**) are similar, but the diffraction peaks are lower when milled in 80% ethanol (**F**), and no peak appears in rice flour milled by a dry-type mill (**G**).

obtained from the rice grains that had been dried over silica gel or humidified, then soaked in 60 or 80% ethanol under high (28° C) or low (10° C) temperatures. The effect of brown rice initial moisture content on the solvent content of soaked grains was not significant. From the averages, the soaked grains of the low-moisture sample seemed to be harder than those of the high-moisture one. This was true when the grains were soaked under low temperature, but reversed under high temperature. The temperature during soaking exerted a significant effect on the solvent content and hardness of the soaked grains. Rice grains soaked at low temperature absorbed solvent about three percentage points less than those soaked at high temperature, so they were much harder. Furthermore, the difference in solvent content and hardness between the grains soaked in 60% ethanol and those in 80% ethanol was enlarged under the low temperature.

Milling Degree and Breakage

The milling degree by wet milling in various concentrations of ethanol is shown in Figure 4. It seems that the milling degree was related to the hardness of grains. It proceeded fastest for the water-soaked rice in the first 2 hr, although there was no obvious difference among the rice grains soaked in water containing up to 40% ethanol after 4 hr of milling. The milling degree slowed with the ethanol concentration, thus corresponding well with grain hardness. Wet milling the rice to the milling degree of 80% took 3 hr 45 min in 60% ethanol. The milling speed was slow compared with that of a Satake test mill, which needs only a few minutes. However, the gentle milling is necessary to maintain the integrity of the central core and the properties of the milled starch.

Rice grains became very fragile after being soaked in water. About 33% of the grains were broken after 4 hr of milling. The milling breakage was improved by soaking rice grains in ethanol solution, and the effect became better as the ethanol concentration increased. Milling for 4 hr gave a milling degree of 77% for grains soaked in 60% ethanol, and the breakage was only 3.6%, which is considered to be satisfactory. When ethanol concentration



Item	A	B
Pasting temperature(℃)	57.8	57.8
Viscosity(BU)		
peak	955	970
minimum	320	325
cooled to 50 ℃	560	560
Breakdown	635	645
Setback	240	235

Fig. 8. Amylograms of rice starch milled in water and 70% ethanol. Starch weight (dry), 39.6 g; total weight, 450 g. The pretreatment and operation are described under Materials and Methods. The solid line represents starch milled in 70% ethanol; the dashed line represents starch milled in water; pasting temperature = temperature when viscosity is at 20 BU; final viscosity = viscosity when paste was cooled to 50° C; breakdown = peak viscosity − minimum viscosity; setback = final viscosity − minimum viscosity.

increased to 80%, the breakage was lower (less than 0.5%). In this experiment, 60% appeared to be the minimum concentration of ethanol sufficient to control the breakage at a low level.

Milling breakage also depended on other factors, among which the initial moisture content of rice appeared to be the most important. Wet milling brown rice grains of the same variety that contained lower moisture (12.7%), in 60 and 80% ethanol resulted in breakage of 22.9 and 37.1% in 4 hr, respectively. The explanation for such a difference is shown in Figure 5. After being soaked in ethanol solution, there were many cracks on the surface of the grains of low moisture content, but none on that of the high-moisture grain. The low-moisture rice grains adsorb moisture rapidly when they are suddenly exposed to a moisture-adsorbing environment, and fissures or cracks result because of the stresses within the kernel (Kunze and Choudhury 1972, Kunze and Wratten 1985). A similar phenomenon was observed for white rice of low moisture content soaked in water (Yanase and Ohtsubo 1986).

Effect of Wet Milling

The length, width, and thickness of the wet-milled rice were measured to confirm whether all sides of the grain were milled to a similar degree. Results are shown in Table II. After being wet milled in 60% ethanol solution for 5 hr, the length, width, and thickness of the grain decreased by 12.2, 13.2, and 13.5%, respectively, suggesting that the outer layer of the grain was uniformly removed by wet milling.

As a milling method in the laboratory, it is desirable to minimize any change in property of the portion removed by milling. The dry-type mills seemed unsuitable because the starch granules were damaged severely, as demonstrated by microscopic observation and X-ray diffraction pattern (Figs. 6D and 7G). Under the microscope, intact starch granules were barely observed in the outer layer flour milled by a dry type mill; most of what was seen was cell wall and starch gel. An X-ray diffraction test showed that the diffraction pattern of the dry-milled flour was like that of the gelatinized flour. Results proved that there was no intact starch granule in the dry milled flour. In practice, starch granules could not be prepared from the flour following a routine procedure.

When grains were soaked in solvent, and the outer layer of endosperm was removed with the wet type mill, the starch granules remained intact in water and in 60% ethanol (Fig. 6). As to those milled in 80% ethanol, some of the starch granules seemed to be expanded and turned to gel in water, indicating the granules were damaged to certain extent. X-ray diffraction tests supported this consideration. The diffraction patterns of outer layer flours wet milled in water, 60% ethanol, or 70% ethanol were in fact the same; however, the diffraction intensity of that milled in 80% ethanol was obviously low, suggesting that the crystalline portion in starch granules was injured by wet milling in 80% ethanol.

Starch granules that were prepared from the outer layer flour wet milled in water, 60% ethanol, or 70% ethanol were also tested for X-ray diffraction (Fig. 7). There was no sign from the diffraction patterns that the starch granules were damaged when the rice grains were wet milled in ethanol up to 70% concentration. These results suggested that starch granules in outer layer flour remained intact when taken from rice grains soaked in solvent with ethanol concentration below 70% and crushing hardness below 6.5 kg/grain. Alcohols have been used in experiments of starch study by many researchers. Ethanol was proved to have no effect on the gelatinization property of starch when its concentration in slurry was below 10% (Takahashi et al 1978). In the wet-milling process, however, rice grains were immersed in the solvent for more than 24 hr, so it was necessary to verify if there was any change in pasting property induced by ethanol in this condition. Starch granules were prepared from rice grains that had been milled in water or in 70% ethanol. Their amylograms are of the same nature as shown in Figure 8. Therefore, the pasting property of rice starch appeared not to be affected by prolonged 70% ethanol treatment. Based on the results above, the new milling method is considered to be applicable for laboratory study of the properties of starch and other components in different layers of rice grain.

In this study, we found the relationship among the soaking ethanol concentration, solvent content, and hardness of the soaked rice grains. We also contrived a new method to mill the soaked grains and successfully obtained the intact starch granules from the milled-off fraction. In another paper, we will report the properties of starches from different layers in rice endosperm.

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