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Effects of Milling Ratio on Properties of Endosperm Starches and Rice Flours from Milled Japanese Rice Grains

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Abstract: Flour and endosperm starch prepared from rice grains of three Japanese rice cultivars milled from 90 to 30% of their original weight were subjected to physicochemical/structural analysis in order to examine the relationship between milling ratio and physicochemical/starch structural properties. The peak viscosity of the rice flours was found to increase with decreasing milling ratio, while that of the purified starches did not change significantly. The gelatinization peak temperature of the rice flours was found to decrease while enthalpy changes increased with decreasing milling ratio. The gelatinization peak temperature of the purified starches was not found to change significantly with different milling ratios. Differences in structural properties of purified starches were examined by gel-filtration chromatography of isoamylase debranched starch. The FI (percentage of amylose) and FIIb/FIIa (ratio of short-to-long chain amylopectin) were found to be constant for all three cultivars for milling ratios ranging from brown rice to 30%.

Key words: milling, milled rice grain, pasting properties, gelatinization properties, starch structure

Brown rice is highly milled for use in making sake, in contrast to use as a staple food. The ratio (percentage by weight) of milled rice to original brown rice is used as a technical index in sake brewing, which is called the "milling ratio". Milling ratios usually range from 75 to 35%. The average milling ratio was 66% in the 2005 sake brewing season. The main purpose of milling is to remove proteins, lipids and inorganic substances such as potassium and phosphate, which are abundant in the germ and surface fraction of rice grains. An excess of these constituents negatively affects sake quality.[1)] Although the quality of sake increases when low milling ratios are used, the cost of sake production also increases because the cost of rice is significant in the entire process. Consequently, there is great interest in producing high quality of sake using rice grains having a high milling ratio.

As the milling ratio decreases, the starch content gradually increases while the content of protein, crude fat and inorganic substances (ash) decreases.[1,2)] With respect to protein composition, the amount of protein body I (PBI) and protein body II (PBII) decreases with decreasing milling ratio, while the ratio of PBI/PBII does not change.[3)] Concerning the composition of crude fat, the ratio of saturated to unsaturated fatty acids increases.[4)] Pasting temperature as measured by amylography was reported to decrease with decreasing milling ratio.[5)] While differences in granule size, and physicochemical and structural properties in starch found in the outer layer (92–75%) *versus* the central core (below 75%) of 92% milled rice grains have been reported,[6,7)] similar information for rice grains having a milling ratio of less than 75% (appropriate for use in sake brewing) is not currently available.

It has been proposed that the amylose/amylopectin ratio among rice cultivars affects the sake brewing process because a low amylose content has been reported to result in high enzyme digestibility of steamed rice grains.[8,9)] Amylopectin chain length distribution and retrogradation properties have also been observed to affect enzyme digestibility of steamed rice grains.[9–11)]

In the present study, the physicochemical and structural properties of endosperm starches and rice flours prepared from milled Japanese rice grains of various milling ratios were examined in order to clarify the relationship between milling ratio and starch properties of milled rice grains.

MATERIALS AND METHODS

Materials. Three Japanese short-grain varieties were harvested during the 2004 season in Japan. *Yamadanishiki* (grown at Hyogo) and *Gohyakumangoku* (grown at Niigata) are "sake-type" rices, and *Nipponbare* (grown at Saitama) is a "cooking-type" rice.

Brown rice was milled from 90 to 70% of its original weight using a milling machine (TM-05; Satake Co., Ltd., Higashi-Hiroshima, Japan) at a rate of 1000 rpm. It was then milled from 60 to 30% of its original weight using a different milling machine (HS01, CHIYODA Co. Ltd., Hiroshima, Japan) at a rate of 1700 rpm. Rice flour was prepared from the milled rice grains using an automated crusher (AC1A; Satake). Starch was isolated from the rice grain flours by the alkali method.[12)]

One thousand kernel weight. One thousand kernel weight of brown and milled rice grains was measured by

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the official methods of analysis of the National Tax Administration Agency.[13)]

Analytical Methods. Starch content was determined by a Megazyme Total Starch Assay Kit (Megazyme, Bray, Ireland, AACC Method 76-13, ICC Method No. 168). Protein content (N×5.95) was determined according to the Dumas method using an automated elemental analyzer (vario EL III; Hanau, Germany). Gel filtration HPLC of isoamylase-debranched starch was performed as described elsewhere.[9)]

Physical Analysis. Pasting properties of starches and rice flours were analyzed using a Rapid Visco Analyzer (RVA) (RVA-3D; Newport Scientific, Australia). Each sample suspension (9%, w/w, dry weight basis; 28 g total weight) was equilibrated at 50°C for 1 min, heated at a rate of 5°C/min to 95°C, maintained at that temperature for 5 min, and then cooled to 50°C at a rate of 5°C/min, and then maintained at that temperature for 6 min. A constant rotating paddle (160 rpm) was used.

The gelatinization temperature of starches and rice flours was determined by differential scanning calorimetry (DSC) (Micro DSC III; Setram, France). Samples (200 mg each) were weighed in sample pans, mixed with distilled water (500 mg), and sealed. Sample suspensions were then heated at a rate of 1°C/min from 20°C to 120 °C. Distilled water (595 mg) was used as a reference standard. Enthalpy change (ΔH), gelatinization onset temperature (T_o) and peak temperature (T_p) were computed automatically.

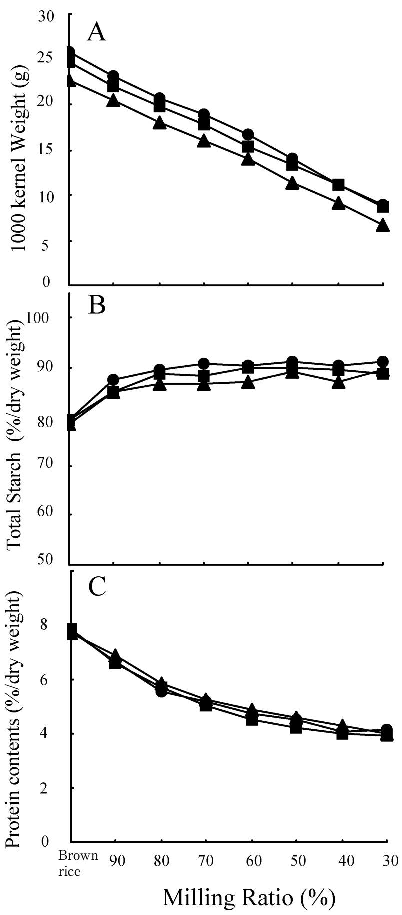

Fig. 1. Changes in 1000 kernel weight (A), total starch (B[1]) and protein content (C[2]) of rice grains as a function of milling ratio.

Milling ratio (%) is the ratio by weight of milled rice to the original brown rice. ─●─, *Yamadanishiki*; ─■─, *Gohyakumangoku*; ─▲─, *Nipponbare*. [1]Values are mean of duplicate samples. [2]Values are mean of triplicate samples.

RESULTS

Protein content.

Three Japanese brown rice cultivars were milled from 90 to 30% milling ratios at 10% intervals and assayed for 1000 kernel weight, total starch and protein content (Fig. 1). One thousand kernel weight decreased linearly with the decreasing milling ratio (Fig. 1A). Total starch increased gradually with the decreasing milling ratio until the ratio of about 70%, after which it did not change much (Fig. 1B). Total starch content increased with milling from 78–79% (brown rice) to 89–91% (30% milling rice). Changes in protein content were very similar among the three cultivars. Protein content decreased gradually with decreasing milling ratio until a ratio of about 50%, after which it decreased slightly, in good agreement with a previous study.[1)] Protein content decreased from 7.7–7.8% for brown rice to about 3.9–4.2% at a 30% milling ratio (Fig. 1C).

Pasting properties.

Brown and milled rice grains were floured, and then the endosperm starch was isolated and purified. Figure 2 shows the pasting properties of rice flours and purified endosperm starch from unmilled and milled brown rice as measured by RVA. For the rice flours, peak viscosity, setback, and pasting temperature of the brown rice samples were 139–176 RVU, 84–96 RVU, and 68–72°C, respectively (Fig. 2A). The peak viscosity of the rice flours in-

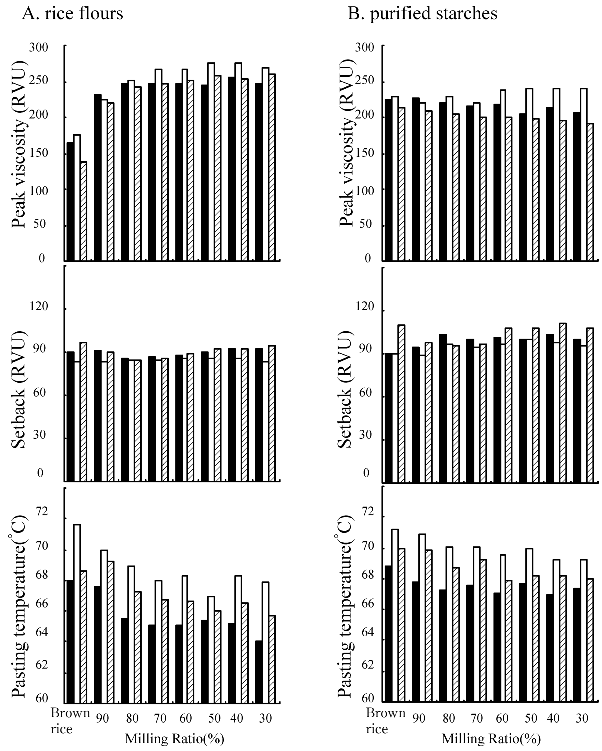

Fig. 2. Rapid Visco Analyzer (RVA) pasting characteristics of rice flour and purified starch from milled rice grains.

■, *Yamadanisiki*; □, *Gohyakumangoku*; ▨, *Nipponbare*. Values are means of duplicate samples. RVU, Rapid Visco Analyzer unit.

creased, but pasting temperature decreased with decreasing milling ratio to 70%, below which these parameters did not change significantly. On the other hand, setback was not found to change with decreasing milling ratios (Fig. 2A). The peak viscosity, setback and pasting temperature of 30% milled rices were 247–269 RVU, 84–95 RVU and 64–68°C, respectively. With respect to the purified starches, fewer differences in peak viscosity and pasting temperature were observed as a function of milling ratio than among the rice flours (Fig. 2B). In the milling ratios from 80 to 30%, the rice flours showed higher peak viscosity than the starches purified from them. At the same milling ratio, both the rice flours and starches of the *Gohyakumangoku* cultivar exhibited higher peak viscosity values within milling ratios of 80–30% (rice flour, 252–275 RVU; starch, 219–240 RVU), as compared to values for the *Yamadanishiki* cultivar (rice flour, 246–254 RVU; starch, 203–221 RVU) or the *Nipponbare* cultivar (rice flour, 243–259 RVU; starch, 191–203 RVU).

Gelatinization properties.

Gelatinization properties of rice flours and purified endosperm starches were measured by DSC (Fig. 3). For the rice flours, the onset temperature (T_o), peak temperature (T_p), and enthalpy change ($\varDelta H$) values for the brown rice samples were 59.9–64.2°C, 67.1–70.7°C and 7.5–9.4 J/g, respectively (Fig. 3A). As the milling ratio decreased, the T_p decreased until the milling ratio reached 70% (T_p, 65.6–69.0°C at a 70% milling ratio), below which it did not change significantly (T_p, 65.2–68.7°C at a 30% milling ratio). On the other hand, $\varDelta H$ values for the rice flours gradually increased from 7.5–9.4 J/g for the unmilled brown rice to 11.1–11.8 J/g for the 30% milling ratio samples. For the purified starches, the T_o, T_p and $\varDelta H$ values did not differ for the different milling ratios as much as for the rice flours. Both T_o and T_p values for the purified starches were lower than those for the rice flours, while the $\varDelta H$ values of the purified starches were

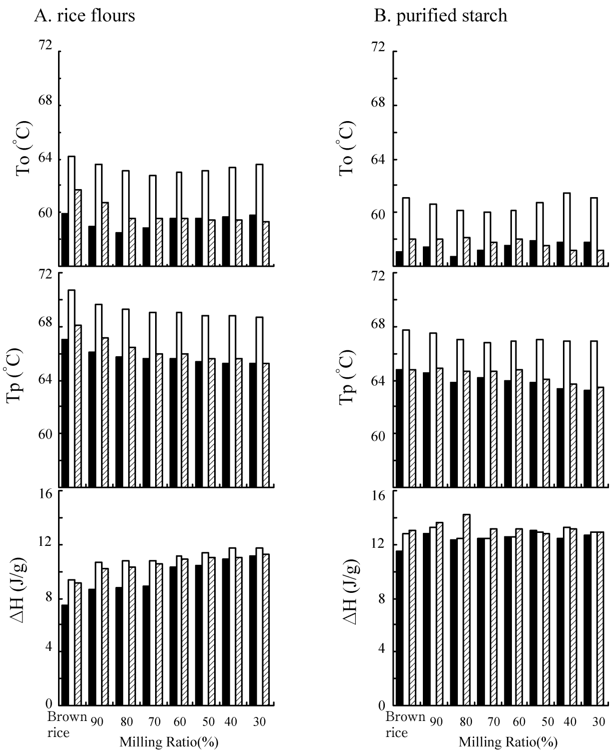

Fig. 3. Differential scanning calorimetry (DSC) gelatinization temperature of rice flours and purified starch from milled rice grains.

■, *Yamadanisiki*; □, *Gohyakumangoku*; ▨, *Nipponbare*. Values are means of duplicate samples. T_o, onset temperature (°C); T_p, peak temperature (°C); $\varDelta H$, enthalpy change (J/g).

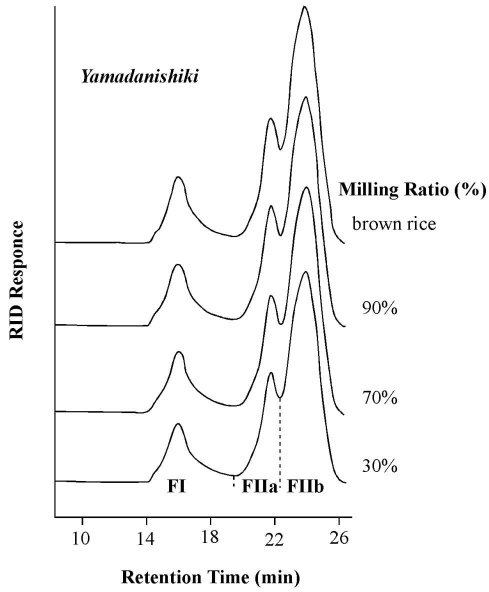

Fig. 4. Representative chain length distribution of isoamylase debranched starch from milled rice grains as determined by gel-filtration chromatography.

Table 1. Proportion of chains in debranched starch by gel-filtration chromatography.

Milling ratio	FI (%)	FIIa (%)	FIIb (%)	FIIb/FIIa
Yamadanishiki				
Brown rice	16.6±0.8	21.4±0.3	61.9±0.5	2.89±0.02
90%	16.6±0.9	21.4±0.3	62.0±0.6	2.90±0.02
80%	16.7±0.7	21.3±0.3	62.0±0.7	2.90±0.06
70%	16.8±0.4	21.2±0.2	62.0±0.4	2.93±0.03
60%	17.3±0.2	21.2±0.1	61.5±0.1	2.90±0.01
50%	17.3±0.1	21.1±0.2	61.6±0.1	2.91±0.03
40%	17.2±0.0	21.1±0.2	61.7±0.2	2.92±0.04
30%	16.9±0.4	21.1±0.2	62.1±0.4	2.95±0.04
Gohyakumangoku				
Brown rice	15.5±1.0	23.0±0.4	61.5±1.1	2.67±0.08
90%	15.4±1.0	23.2±0.3	61.5±1.0	2.66±0.06
80%	15.3±0.9	23.1±0.2	61.6±1.0	2.67±0.07
70%	15.3±0.7	23.2±0.1	61.5±0.7	2.66±0.04
60%	15.8±0.5	23.2±0.2	61.0±0.6	2.63±0.05
50%	15.5±0.9	23.1±0.1	61.3±0.9	2.65±0.05
40%	15.3±1.1	23.3±0.2	61.4±1.3	2.63±0.08
30%	15.2±0.7	23.3±0.2	61.5±0.5	2.64±0.02
Nipponbare				
Brown rice	17.3±0.2	20.9±0.0	61.8±0.2	2.95±0.00
90%	17.0±0.2	20.9±0.0	62.1±0.2	2.98±0.00
80%	17.3±0.4	20.7±0.1	61.9±0.4	2.99±0.01
70%	17.0±0.5	20.7±0.1	62.3±0.4	3.02±0.02
60%	17.6±0.3	20.8±0.1	61.6±0.2	2.97±0.01
50%	17.5±0.4	20.6±0.1	61.9±0.3	3.00±0.02
40%	17.4±1.3	20.6±0.4	62.0±0.9	3.01±0.01
30%	17.4±0.5	20.6±0.4	62.0±0.6	3.00±0.07

Values are means of triplicate measurements±SD. Proportion of chains in debranched starch was measured by gel-filtration chromatography. Fraction I = long linear chains from amylose. Fraction IIa (FIIa) and IIb (FIIb) = long-length and short+intermediate-length chains from amylopectin, respectively.

higher than those of the rice flours (Figs. 3A, B). At the same milling ratio, the *Gohyakumangoku* cultivar had higher T_p values (rice flour, 68.7–70.7°C; starch, 66.8–67.7°C), than those for *Yamadanishiki* (rice flour, 65.2–67.1°C; starch, 63.3–64.7°C) or *Nipponbare* (rice flour, 65.3–68.2°C; starch, 63.5–64.8°C).

Structural analysis.

Representative gel-filtration chromatograms of isoamylase debranched starch isolated from milled grains with different milling ratios are shown in Fig. 4. Chromatogram peaks were very similar for the unmilled brown rice samples and for the 90–30% milling ratio samples. The proportions of FI (percentage of amylose), FIIa (percentage of long-length chains from amylopectin) and FIIb (percentage of short+intermediate-length chains from amylopectin) were calculated from the respective areas of the debranched starches (Table 1). The FI and FIIb/FIIa (ratio of short-to-long chain amylopectin) values did not differ for the various milling ratio samples among these three cultivars. The *Gohyakumangoku* cultivar had lower FI and FIIb/FIIa values (means=15.4% and 2.65, respectively) than the *Yamadanishiki* (means=16.9% and 2.91, respectively) or *Nipponbare* cultivars (means=17.3% and 2.99, respectively).

DISCUSSION

In the present study, we examined the relationship between milling ratio and physicochemical and structural properties of milled rice grains. As the milling ratio decreased, the peak viscosity of rice flour was observed to increase. T_p values decreased for samples having milling ratios of 90–70%, but not in the range of 70–30% (Figs. 2A, 3A). He *et al*.[7] reported that starch granules in the outer layer were smaller than in the central core, and that outer layer starch exhibited a higher peak gelatinization temperature. Our results are in agreement with their report.[7] However, we found that amylose content and starch structure in the milled rice grains were fairly constant at all milling ratios (Table 1), which predicts few changes in physicochemical properties, because these parameters are a function of amylose content and starch structure.[9–11,14–16] The results obtained from analysis of the rice flour samples (Figs. 2A, 3A) would not have been predicted from the amylose content and starch structure data (Table 1). The unexpected differences observed among rice flour samples in the 90–70% milling ratio range may be due to changes in the amounts of starch, protein or other constituents as a function of decreasing milling ratio. With respect to rice flour, RVA peak viscosity values were low for samples of brown rice through 80% milling ratios (Fig. 2A). In this range, starch content was found to be low, but levels of protein and other constituents were high as shown in Fig. 1. The peak viscosity values of purified starches did not change with decreasing milling ratio (Fig. 2B). Therefore, it is possible that the low starch content may account for the low peak viscosity. Furthermore, because high levels of α-amylase are found in the outer layer of the rice kernel,[17] the low peak viscosity might be due to α-amylase digestion of the endosperm starch. In the milling ratios from 80 to 30%, the peak viscosity values of rice flours were higher than those of purified starches (Fig. 2). From the starch content data (Fig. 1B), it is expected that the rice flour exhibit lower peak viscosity values than the purified starch. Singh *et al*.[18] reported that the comparison of pasting characteristics between the flour and pure isolated starch whose starch concentrations were equalized showed higher viscosity in the flour than in the starch of non-waxy Japonica rice. Our results are in agreement with their report. The unexpected higher peak viscosity values of rice flour may be due to the presence of a fat, negligible quantity of protein and other constituents, as Singh *et al*.[18] discussed previously.

The gelatinization temperature (T_p) values for the rice flour samples were higher than for the purified starches (Fig. 3A). This is in good agreement with a previous report.[19] In the rice flour, starch swelling might be limited by the endosperm cell walls or by protein or other constituents. Prevention of swelling of the starch by these factors might result in a higher gelatinization temperature. The enthalpy change (ΔH) values for the rice flour samples were low in the range of brown rice to a 70% milling ratio (Fig. 3A) as were the peak viscosity values for the rice flour samples in the same range (Fig. 2A). Furthermore, the purified starches had higher ΔH values than the rice flours (Figs. 3A, B). These results may be caused by the lower starch content of the rice flours relative to the purified starches (Fig. 1B).

Among the cultivars, *Gohyakumangoku* had the highest peak viscosity values for both rice flour and purified starches at 80–30% milling ratios (Fig. 2). The high peak viscosity for *Gohyakumangoku* is likely due to its low amylose content relative to the other cultivars (Table 1). The rice flours and purified starches derived from the *Gohyakumangoku* cultivar had a high gelatinization temperature (Fig. 3), which might be caused by their lower FIIb/FIIa values (Table 1). These observations are consistent with previous reports.[11,16]

FI and FIIb/FIIa values for the 70% milled rice samples closely correlate with starch retrogradation and enzyme digestibility of the steamed rice grains.[9,11] The observed FI and FIIb/FIIa values were relatively constant within the same cultivar from brown rice to a 30% milling ratio, although some differences were found among the three cultivars (Table 1). This indicates that even if the milling ratio is different, the starch structural properties may not affect starch retrogradation or possibly enzyme digestibility of steamed rice grains of the same cultivar.

From the present study, one can conclude that a milling ratio from brown rice to 30% does not change starch structure, but a milling ratio from brown rice to 70% causes changes in physicochemical properties. The reason for the change in physicochemical properties may be the change in starch and/or protein content. In the range below a 70% milling ratio that is generally used for sake brewing, changes in physicochemical properties were modest, although protein content was found to gradually decrease.

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精米歩合が日本米の胚乳デンプンおよび米粉特性に及ぼす影響

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精米歩合と物理化学的およびデンプン構造特性との関連性を調べるために，清酒醸造に使用される日本の3品種の米を，玄米から精米歩合90–30%に搗精し，米粉と精製デンプンを調製した．ラピッド・ビスコ・アナライザーで測定した粘度特性においては，米粉で精米歩合が低くなるに伴い，最高粘度が増加したが，精製デンプンでは顕著な変化はみられなかった．示差走査熱量計による糊化特性の解析では，精米歩合が低くなるに伴い，米粉では糊化ピーク温度が低下し吸熱エンタルピー変化量は増加したが，精製デンプンではこれらのパラメータの変化は小さかった．イソアミラーゼで枝切りしたデンプンのゲルろ過HPLCを行い，デンプンの組成・構造を解析した．FI (アミロースの割合)，FIIb/FIIa (アミロペクチンの短鎖/長鎖比) は，品種間に差異がみられたものの，同一品種では精米歩合90–30%で差異はみられなかった．