Original paper

Physical Properties and Starch Structure of Ground Rice Puree

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To improve issues of workability and insufficient volume of bread containing rice gel or puree, low-adhesiveness ground rice purees were produced using a stone grinder. Hardness and adhesiveness of ground purees were much lower than those of conventional rice puree. The hardness of bread containing ground purees was significantly lower than that of bread containing rice flour at 1 and 5 d storage at 5 °C and comparable to that of wheat flour. The distribution of amylopectin chain lengths was almost identical; however, the molecular weight of amylopectin of ground purees was lower than that of rice flour and conventional rice puree. These results suggested that ground purees produce softer bread due to the smaller amylopectin molecules following shearing and digestion of amorphous regions by grinding, indicating that ground purees improve food quality and are much easier to use than conventional rice puree.

Keywords: amylopectin, bread characteristics, physical properties, rice puree, starch

Introduction

Rice is the third most abundantly produced grain following maize and wheat, and is particularly widely grown and consumed in Asia and Africa (Fairhurst and Dobermann, 2002; Awika, 2011). Japanese people eat large quantities of steamed rice, rice snacks, and rice cake, while rice noodles such as rice sticks and rice pho are eaten in China and south-east Asia. The addition of rice flour to processed foods, mainly composed of wheat flour and/or egg such as bread and Western-style cakes, is increasing, and there is a trend to replace wheat flour with rice flour, in whole or in part (Higo and Wada, 2009; Kusunose, 2009). Thus, consumption of rice is expected to expand worldwide. However, there are some barriers to the wider use of rice, including difficulties in mixing rice flour and wheat flour, starch retrogradation after cooking, and non-uniformity of cells in bread (Okunishi, 2009). The behavior of gelatinized starch during cooling and storage is generally termed as ‘starch retrogradation’. Crystallites of the chains of starch eventually begin to form, and this is accompanied by gradual increases in rigidity and phase separation between the polymer and solvent (Karim *et al.*, 2000). Starch retrogradation comprises two types:

short-term development of gel structure via amylose crystallization and long-term reordering of amylopectin (Miles *et al.*, 1985), which is a much slower process involving recrystallization of outer branches (DP = 15) of this polymer (Ring *et al.*, 1987). Moreover, a decrease in gluten concentration upon addition of rice flour can lead to insufficient bread volume. To overcome these problems, gelatinized rice starch has been used in bread instead of rice flour with some success (Okunishi, 2009, Katsuno *et al.*, 2010).

It is possible to make 100 % rice bread with a mixture of crystalline rice flour and amorphous rice flour using a shearing and heat milling machine (SHMM) (Katsuno *et al.*, 2010; Yano *et al.*, 2017). The molecular weight of amorphous rice flour is decreased upon digestion of amorphous regions of amylopectin (Murakami *et al.*, 2018). The degree of crystallinity of rice flour can be adjusted to SHMM conditions such as temperature, distance between milling mortars, and the water content of rice grains (Murakami *et al.*, 2018). Rice gel made from steamed high amylose rice containing excess water and prepared using a high-speed mixer has a unique hard texture (Shibata *et al.*, 2012), and rice gels can be effectively used to prepare

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Fig. 1. (A) Scanning electron microscope observation of bread dough before fermentation. (B) Appearance and specific volume of baked breads (average $\pm$ SE, $n=3$) using wheat flour, rice flour, rice puree and ground puree (clearance 0.1 and 0.5 mm). Values with different letters (a, b) are significantly different from one another as determined by Turkey-Kramer test at $P < 0.05$. 'Wheat flour' means common bread (not substituted). 'Rice flour', 'Rice puree' and 'Ground puree' mean rice flour-substituted, rice puree-substituted, and ground purees-substituted, respectively.

processed foods such as bread and noodles (Sugiyama, 2015).

We produced conventional rice puree by steam heating and straining in oxygen-free conditions. Using rice puree instead of rice flour can overcome the issue of insufficient bread volume (Fig. 1B). However, the workability of rice puree during food processing was not optimal because of its high adhesiveness. In the present work, therefore, low-adhesiveness ground rice puree was prepared from steamed rice using a stone grinder. Bread was prepared in which 15 % of wheat flour was substituted with conventional rice puree and ground rice purees as well as rice flour. The physical properties of purees and breads and the starch structure of ground purees were compared with those of conventional rice puree, rice flour, and wheat flour.

Materials and Methods

Materials The ultra-high-yield Japonica rice cultivar 'Akita 63' was cultivated in a paddy field in Oogata village, Akita Prefecture in 2015. Rice flour, rice puree, and ground purees were prepared from polished rice grains. The average grain size of rice flour was 72 μm , measured using an LMS-2000 instrument (Beckman Coulter, Inc. Brea, USA). The 'Super Camellia' cultivar was used as wheat flour and other breads. Salt (HAKATA SALT Co., Ltd.), salt-free butter (MEGMILK SNOW BRAND Co., Ltd.), skim milk (MEGMILK SNOW BRAND Co., Ltd.) and dry yeast (Lesaffre Yeast Corp.) were purchased commercially.

Preparation of Rice Puree and Ground Puree Samples A 4-fold volume of water was added to polished rice and steamed at 90 $^{\circ}\text{C}$ for 20 min in an oven set at 160 $^{\circ}\text{C}$. The steamed rice

Table 1. Ingredient of breads used in the present study.

Ingredient	Formulation (g)				
	Wheat flour	Rice flour	Rice puree	Ground puree (0.1) (0.5)	
Wheat flour	279	237.2	237.2	237.2	237.2
Rice flour or puree	0	39.6	199.6	199.6	199.6
White refined sugar	19	19	19	19	19
Salt	5.6	5.6	5.6	5.6	5.6
Salt-free butter	11.2	11.2	11.2	11.2	11.2
Skim milk	6.7	6.7	6.7	6.7	6.7
Dry yeast	3.1	3.1	3.1	3.1	3.1
Water	202.6	204.8	44.9	44.9	44.9

The values in parenthesis show the clearance (mm) when steamed rice was ground with a grinder.

was strained with a strainer (Soliton; Nepuree Corp., Japan) and used as 'rice puree'. The average and median grain size of rice puree was 307 μm and 88.8 μm , respectively, as measured by a Beckman Coulter LS 13 instrument. Steamed rice was ground with a stone grinder (Super Masucolider IV; Masuko Sangyo Co., Ltd., Japan) at 1,500 rpm with 0.1–0.2 mm clearance (hereafter referred to as '0.1') or 0.4–0.5 mm clearance (hereafter referred to as '0.5') and used as 'ground puree'. The average and median grain sizes of ground puree were 110 μm (0.1 mm clearance) and 245 μm (0.5 mm clearance), and 34.7 μm (0.1 mm clearance) and 49.4 μm (0.5 mm clearance), respectively. Both purees were stored in the freezer (-18°C) before use. 'Wheat flour' bread (not substituted) was made using a home bakery system (HBK-100; Mk Seiko Co., Ltd., Japan) following the manufacturer's instructions. 'Rice flour', 'rice puree' and 'ground puree' breads were made using the same method except that 15 % wheat flour (dry weight) was replaced with rice flour, rice puree and ground purees, respectively, according to Table 1. Rice puree and ground purees just after thawing were used for breads. Bread volume was measured using the rapeseed substitution method and the specific volume (mL/g) of each bread was calculated.

Observation of Bread Dough by Scanning Electron Microscopy Bread doughs of wheat flour (not substituted), rice flour (mixture of wheat flour and rice flour) and purees (mixture of wheat flour and purees) before fermentation were freeze dried with a freeze-dryer (FDU-2110; Tokyo Rikakikai Co., Ltd., Japan). Dried samples were cut with a razor and small pieces of samples (ca 8 mm³) were coated with gold using a sputtering apparatus (FINE COATER JFC-1200; JEOL Co., Ltd., Japan) and were observed by scanning electron microscopy (SEM, JEOL-5600; JEOL Co., Ltd.)

Measurement of Physical Properties of Purees and Breads Physical properties of purees and breads were measured by the

two-bite method with a texture tester (TEX-100N; Japan Instrumentation System Co., Ltd., Japan) using a cylindrical probe (diameter: 20 mm). Frozen purees were thawed with running water and physical properties were measured at room temperature. Purees were filled into a 40 mm diameter saucer. The probe was allowed to penetrate 15 mm from the surface of the sample at a speed of 50 mm/min and pull at a speed of 600 mm/min. Samples were measured in triplicate. The breads after baking were cooled for 1 h, wrapped in a plastic bag, and left for 23 h at room temperature. A one-fourth slice of the breads was used for measurement of physical properties at 1 day after preparation and the remaining breads were stored at 5°C for 1 to 5 d. The physical properties at 0, 1, 2, and 5 days after storage at 5°C (namely 1, 2, 3, and 6 d after preparation) were measured at room temperature. The probe was allowed to penetrate six points in the crumb of two breads at a speed of 600 mm/min with a compression rate of 70 % and pull at a speed of 600 mm/min. Hardness of both purees and breads, and adhesiveness of purees were defined as the first maximum stress and the first minimum stress, respectively.

Analysis of Starch Structure The distribution of amylopectin chain lengths of rice flour and purees was determined by capillary electrophoresis. Five milligrams of rice flour or 25 mg of rice puree and ground purees were solubilized by alkaline treatment, and debranching of amylopectin was performed using *Pseudomonas* isoamylase (generous gift from Hayashibara Co., Ltd. Japan) in 60 mM Na-acetate buffer (pH 4.4) as described previously (Fujita *et al.*, 2001). Debranched glucans were labeled by 1-aminopyrene-3,6,8-trisulfonic acid, and analyzed by capillary electrophoresis (P/ACE MDQ Carbohydrate System; AB Sciex Pte. Ltd., USA) as previously described (O'Shea *et al.*, 1996; Fujita *et al.*, 2001).

The molecular size distribution of whole starch was analyzed by gel filtration chromatography according to a previously published report (Utsumi *et al.*, 2003). Briefly,

Table 2. Hardness and adhesiveness of rice purees and the effect of starch retrogradation.

	Clearance (mm)	Days after thawing	Hardness (Pa)	Adhesiveness (Pa)
Rice puree	-	0	8,128 ± 215a	1.42 ± 0.03a
Ground puree	0.1		2,122 ± 56c	0.38 ± 0.01c
	0.5		4,403 ± 112b	0.70 ± 0.03bc
Rice puree	-	3	10,229 ± 506a	1.63 ± 0.07a
Ground puree	0.1		3,141 ± 140bc	0.51 ± 0.03bc
	0.5		5,136 ± 298b	0.83 ± 0.05b

Values in the same column with different letters significantly differ according to Tukey-Kramer test following ANOVA ($P < 0.05$).

20 mg of rice flour or 100 mg of puree (~80 % water content) were resuspended in distilled water by adding up to 1.6 mL and 0.4 mL of 5 M NaOH, respectively, and the samples were incubated for 1 h to gelatinize. Distilled water (4 mL) was then added, and the suspension was filtered through a 5- μ m filter membrane (Durapore Membrane Filters, SVLP01300; Merck KGaA, Ireland). A 0.5-mL sample of the filtrate was applied to a 4-column system comprising Toyopearl HW75Sx2, HW65S, and HW55S columns (22 mm diameter, 30 cm length; Tosoh Corp., Japan) pre-equilibrated with 0.1 M NaOH and 0.2 % NaCl, and progress was analyzed using a refractive index (RI) detector (RI-8020; Tosoh Corp.). The columns were incubated at 40 °C, and samples were eluted with the same solution at a flow rate of 1 mL/min.

Data Analyses The specific volume of breads and physical properties of purees and breads were subjected to one-way analysis of variance (ANOVA), followed by the Turkey-Kramer test ($p < 0.05$).

Results and Discussion

Physical Properties of Purees The rice puree and ground purees (using grinder clearances of 0.1 and 0.5 mm) were prepared and stored at -18 °C before use. The hardness and adhesiveness of the purees at the day just after thawing (0 days after thawing) and at 3 days after storage at 5 °C following thawing (3 days after thawing) were measured using a texture tester (Table 2). The hardness of purees at 3 days after thawing tended to be greater than that at 0 days due to starch retrogradation at 5 °C. The hardness of both ground purees was significantly lower than that of rice puree at 0 and 3 days after thawing, and the hardness of ground puree prepared using 0.1 mm clearance was significantly lower than that of ground puree prepared using 0.5 mm clearance immediately after thawing (0 days) (Table 2). The adhesiveness of both purees at 3 days after thawing also tended to be higher than that at 0 days, and the adhesiveness of ground purees at 0 and 3 days was significantly lower than that of rice puree (Table 2). These results suggested that grinding with a stone grinder reduced the hardness and adhesiveness of puree and inhibited starch retrogradation after storage at 5 °C for 3 days.

Properties of Breads Breads were made using wheat flour [100 %, ‘common bread’ (not substituted)] and mixtures of 85 % wheat flour and 15 % rice flour (‘rice flour-substituted bread’), rice puree (‘rice puree-substituted bread’), or ground purees (‘ground puree-substituted bread’) prepared using 0.1 and 0.5 mm clearances. The interior of the bread dough before fermentation was observed by SEM (Fig. 1A). Large and small spherically shaped starch granules were observed in the wheat flour dough, and smaller polygonally shaped starch granules were observed in rice flour dough (15 % wheat flour was substituted with rice flour). In dough containing rice puree and ground purees, wheat starch granules were coated with purees, and there were fewer gaps between starch granules than in dough made with wheat flour and rice flour (Fig. 1A), although the SEM observation of freeze-dried samples may have some artifacts.

The specific volume (mL/g) of bread made from rice puree tended to be larger than that from rice flour and comparable to that from wheat flour (Fig. 1B). The specific volume of bread containing ground puree (clearance 0.1 mm) was significantly greater than that of bread containing rice flour ($P < 0.05$ by Tukey-Kramer’s test; Fig. 1B).

Next, the hardness of these five breads at 1, 2, 3, and 6 days after preparation (0, 1, 2, and 5 days storage at 5 °C, respectively) was measured using a texture tester (Table 3). Breads containing rice flour and ground puree (0.1) were the hardest and softest among the five bread types from 1 to 6 days after preparation, respectively. The standard error was the largest for bread containing rice flour at 6 days after preparation (stored at 5 °C for 5 days), which is thought to be due to the gel-like cell wall of the crumb of this bread (data not shown). The hardness of bread containing ground purees was significantly lower than that of bread containing rice flour and comparable to that from wheat flour at 2 to 6 days after preparation (1 and 5 days storage at 5 °C, respectively) (Table 3).

Gelatinized rice in the form of steamed rice (Ohnishi, 2009, Iwashita *et al.*, 2011), rice gel (Houjyo *et al.*, 2017), and amorphous rice flour (Yano *et al.*, 2017, Murakami *et al.*, 2018) can be used to produce bread of sufficient volume,

Table 3. Hardness of baked breads measured at 1, 2, 3 and 6 days after preparation.

	Days after preparation (Days storage at 5 °C)			
	1 (0)	2 (1)	3 (2)	6 (5)
Wheat flour	13,375 ± 1,280a	29,248 ± 2,566b	41,031 ± 2,738ab	39,106 ± 2,226ab
Rice flour	12,048 ± 2,969a	46,687 ± 3,606a	47,292 ± 4,856a	72,168 ± 15,448a
Rice puree	7,443 ± 483a	27,609 ± 3,411b	33,280 ± 2,623ab	30,750 ± 1,500b
Ground puree (0.1)	5,661 ± 723a	15,757 ± 1,352b	22,006 ± 2,945b	22,235 ± 3,205b
Ground puree (0.5)	7,767 ± 894a	22,500 ± 1,320b	29,720 ± 5,218ab	29,938 ± 5,318b

Values in the same column with different letters significantly differ according to Tukey-Kramer test following ANOVA ($P < 0.05$).

Wheat flour: common bread (not substituted), Rice flour: rice flour-substituted bread, Rice puree: rice puree-substituted bread, Ground puree: ground rice puree-substituted bread.

(0.1) and (0.5) show the clearance (mm) when steamed rice was ground with a grinder.

Fig. 2. Chain-length distribution of amylopectin extracted from rice flour, rice puree and ground puree (0.1 mm and 0.5 mm clearance were used when rice puree was grinding) analyzed by capillary electrophoresis. DP, degree of polymerization. The analyses of each sample performed at least twice and both data were almost identical (data not shown).

although the mechanism of the increase in bread volume remains unknown. Bread containing rice flour exhibited incomplete rising (Fig. 1B) and incomplete gelatinization with calcination, as indicated by the gel-like crumbs observed on the underside of this bread (data not shown). We speculated that this could be improved by the addition of puree, which reduced the inter-particle distance between starch granules (Fig. 1A). This will likely lead to complete rising and gelatinization with calcination, and may form networks similar to gluten networks obtained in wheat flour bread. In the present study, the specific volume and hardness of the breads containing ground purees were comparable or superior to those of conventional rice puree. In particular, the specific volume of bread produced using 0.1 mm clearance ground puree, was significantly greater

than that of bread made from rice flour (Fig. 1B), and maintained significantly more softness for 2-6 d after preparation and storage at 5 °C for 1-5 d compared to bread containing rice flour (Table 3). This indicated that ground purees, especially that ground with 0.1 mm clearance, suppress starch retrogradation (Table 2), which assists in the retention of bread softness (Table 3). Moreover, the hardness and adhesiveness of ground purees were significantly lower than those of conventional rice puree (Table 2), indicating that the purees ground with a stone grinder should enhance workability during food processing. For example, when a large batch of dough is mixed by hand, the workability may significantly decline if the adhesiveness of the bread dough is high.

Structure of Starch in Purees The distribution of

Fig. 3. Gel-filtration chromatography of whole starch from rice flour, rice puree and ground puree (0.1 mm and 0.5 mm clearance were used when steamed rice was grinding). (A) Fractionation of whole starch extracted from rice flour. Fr. I, II and III are corresponding to the large amylopectin, small amylopectin and amylose molecules, respectively based on the $\lambda_{\max}$ value of each fraction stained I_2 -KI solution (right axis). (B) The comparison of the patterns among 4 samples. Typical patterns among at least two reproducible data were shown in the figure.

amylopectin chain lengths in rice flour, rice puree, and purees ground with 0.1 and 0.5 mm clearance was determined by capillary electrophoresis (Fig. 2). The results revealed an identical chain length distribution for all four samples, indicating that straining following steam heating or grinding with a stone grinder did not affect the distribution of amylopectin chain lengths in the extracted starch.

To investigate the structure of non-debranched starches, whole starch extracted from each sample was subjected to gel filtration chromatography (Fig. 3). Whole starch extracted from rice flour yielded three peaks (Fr. I, II and III, Fig. 3A). Based on the $\lambda_{\max}$ value of α -glucan complexes with iodine in each fraction, most, if not all, amylopectin was eluted in Fr. I and the first half of Fr. II (retention time = 125–170 min), while amylose was eluted in the second half of Fr. II and Fr. III (170–230 min). Therefore, high molecular weight amylopectin was eluted in Fr. I and smaller amylopectin molecules were eluted in the first half of Fr. II. The pattern of peaks for rice puree was similar to that for rice flour except that Fr. I and Fr.

II peaks were slightly smaller and larger, respectively (Fig. 3B). By contrast, the Fr. I peak for ground purees (both 0.1 and 0.5 mm clearance samples) was much smaller than for rice flour and rice puree, whereas Fr. II peaks of both ground puree types were much larger than those of rice flour and rice puree. Fr. I and Fr. II peaks for ground puree (0.5) were larger and smaller, respectively, than those for ground puree (0.1). The size of the Fr. III peak corresponding to amylose was similar among all four samples. Taken together, these results suggest that the molecular weight of amylopectin extracted from ground purees was smaller than that of rice flour and rice purees (Fig. 3B). This means that the amylopectin molecules of the ground purees were more degraded following grinding with a stone grinder.

The molecular weight of amylopectin in amorphous rice flour prepared with a SHMM device at high temperature with strong shearing was reported to be significantly decreased (Murakami *et al.*, 2018), although the distribution of amylopectin chain lengths in amorphous rice flour did not

significantly differ from that in crystalline rice flour, indicating that interconnecting clusters of amylopectin chains were cleaved (Murakami *et al.*, 2018). Similarly, the distribution of amylopectin chain lengths in ground purees was almost identical to that in conventional rice puree and rice flour in the present work (Fig. 2), indicating cleavage of interconnecting clusters of amylopectin chains. This could be due to the smaller amylopectin molecules resulting from shearing compared with rice flour and conventional rice puree (Fig. 3B). The suppression of starch retrogradation of ground purees (Tables 2 and 3) could be due to the smaller amylopectin molecules resulting from shearing compared with rice flour and conventional rice puree. On the other hand, degradation of amylose, which is the main cause of starch retrogradation, was not detected in this study (Fig. 3B). Thus, further work is needed to clarify the molecular structure of starch in ground purees, and to investigate relationships between starch retrogradation and structure.

Grinding with a stone grinder (clearances of 0.1 and 0.5 mm) altered the degree of amylopectin degradation (Fig. 3B), and hardness and adhesiveness (Table 1) of ground puree, suggesting that physical properties and the extent of starch retrogradation could be regulated by the degree of shearing. Moreover, purees could presumably fill gaps between starch granules of different grain size from different plant species (Fig. 1A). In summary, a soft and low-adhesiveness ground puree, especially that ground with stronger shearing such as 0.1 mm clearance, is easily mixed with different starches and materials during food processing, improving food quality and making it much easier to use than conventional rice puree. We anticipate that ground rice puree could be used to improve various foods, especially gluten-free foods.

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