Original paper

Prevention of Abrupt Increases in Postprandial Blood Glucose Levels by Rice Bread Made with the Novel Rice Cultivar “Konayukinomai”

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The amylopectin long-chain rice “Konayukinomai” (MAI) is high in resistant starch (RS). In this study, we confirmed that fine rice flour for bread making could be obtained using MAI, and compared the results to the high-amylose cultivar “Koshinokaori” (KOR) and the conventional cultivar “Koshihikari” (HKR). MAI bread contained larger amounts of RS and slowly digestible starch (SDS) than that of HKR or KOR, and the RS content of MAI was easily changed by retrogradation and re-gelatinization of the starch. In a clinical trial with healthy volunteers, MAI and KOR breads could prevent abrupt increases in blood glucose and showed low glycemic index (GI) values compared to HKR bread. From the above results, we propose that SDS and RS prevent increases in blood glucose, and consider MAI to be a promising rice cultivar as a nutraceutical food material for the prevention of lifestyle-related diseases.

Keywords: starch digestibility, glycemic index, bread, long-chain rice

Introduction

Rice is a major staple food throughout the world, especially Asia, and is usually eaten as boiled rice. Moreover, rice has been widely used as a raw material for various foods such as sweets and beverages, including alcoholic drinks. However, the diversification of the diet has led to a decrease in rice consumption in Japan. This societal situation prompted us to develop novel processing technologies for rice materials that could be used to produce a variety of rice-based Western-style foods beyond the traditional Japanese foodstuff. As a result, our laboratory established two different milling technologies for rice, the so-called “milling method with enzyme pretreatment” (Shishido and Egawa, 1992) and the “two-step milling method” (Arisaka *et al.*, 1992). These novel technologies produce fine rice flours, which can be substituted for wheat flour in the production of foods such as breads, noodles and sponge cakes (i). Processed foods made with

fine rice flour would also be applicable as gluten-free foods for people with wheat allergy.

Since rice starch is generally highly digestible, foods made with fine rice flour are easily digested in the gastrointestinal tract and induce abrupt increases in postprandial blood glucose. On the other hand, in 2014 it was reported that more than 400 million people worldwide suffer from diabetes (ii). Diabetes is a lifestyle-related disease that can cause many serious complications including cardiovascular disease, and dietary control of blood glucose levels plays a key role in managing this disease. Therefore, for use in rice-based foods, the rice cultivars have been bred from the viewpoint of reducing carbohydrate bioavailability. Starch is composed of amylose and amylopectin. The digestibility of starch is related to its molecular structure, and increases in the amylose content are known to increase its digestion-resistant properties. Indeed, it was reported that the endosperm of high-amylose rice

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cultivars contains more resistant starch (RS) than that of commercially available conventional rice cultivars (Zhu *et al.*, 2011). RS is a type of dietary fiber, and is defined as the fraction of dietary starch that escapes digestion in the small intestine of healthy humans (Englyst *et al.*, 1987). In addition, the intake of high-amylose rice attenuated postprandial blood glucose and the insulin response compared to a conventional rice cultivar (Ohtsubo *et al.*, 2010; Zenel and Stewart, 2015). Saito *et al.* (2014) also reported that the ingestion of rice-based breads made with high-amylose cultivars induced a lower glycemic response compared to those made with conventional rice cultivars.

Another class of rice with digestion-resistant properties is the amylose extender (*ae*) mutant. The *ae* mutants lack the starch branching enzyme IIb (BEIIb) gene, which lowers the amount of short-chain glucans and induces more long-chain glucans in the amylopectin (Nishi *et al.*, 2001). Nakamura *et al.* (2011) reported that rice cultivars with long-chain amylopectin contain more RS than the high-amylose rice cultivar “*Hoshiyutaka*”. It was also reported that the cooked rice of an amylopectin long-chain cultivar showed low digestibility (Arai *et al.*, 2010) and attenuated postprandial blood glucose levels in humans (Arai *et al.*, 2011), using the representative cultivar “*Ayunohikari*”. Unfortunately, the preceding cultivars with long-chain amylopectin have not been widely used because of poor cultivation yields (Miura *et al.*, 2007). For that reason, the novel rice cultivar “*Konayukinomai*” was developed. This amylopectin long-chain cultivar has a cultivation yield comparable to that of the most popular conventional cultivar in Japan, *i.e.*, “*Koshihikari*”, indicating the potential of “*Konayukinomai*” as a raw material for fine rice flour.

In this study, we evaluated the processing suitability of “*Konayukinomai*” for the production of fine rice flour and rice-based bread in comparison with the conventional rice cultivar “*Koshihikari*” and the high-amylose cultivar “*Koshinokaori*”. In addition, the effect of “*Konayukinomai*” when eaten as rice-based bread on the glycemic response in humans was examined using the glycemic index (GI). The GI was proposed by Jenkins *et al.* (1981) and is accepted as a good indicator of the physiological responses to the ingestion of carbohydrates from meals or foods. Low-GI foods can prevent abrupt increases in blood glucose. From this study, we confirmed that rice cultivars with long-chain amylopectin can improve the glycemic response, and we proposed that the novel cultivar “*Konayukinomai*” is promising as a raw material for fine rice flour and its processed foods.

Materials and Methods

Materials Two rice cultivars “*Konayukinomai*” (MAI) and “*Koshinokaori*” (KOR) were cultivated at Hokuriku Research Center in the Central Agricultural Research Center, National Agriculture and Food Research Organization (NARO) in Japan. The cultivar “*Koshihikari*” (HKR) was purchased from a local rice store. Vital wheat gluten was purchased from Niigata Seifun Co.,

Ltd (Niigata, Japan). Dried yeast, sugar and salt were purchased from local markets.

Preparation of rice flours Brown rice was polished to yield endosperm of 87 – 90% by weight with a polishing machine (VP-32; Yamamoto Co., Ltd., Yamagata, Japan) and soaked in water at 20°C for 1 h. After removing the excess water with a spin-drier, the polished rice was milled using an air-flow type pulverizer (BM-15; Nisshin Engineering Co., Ltd., Tokyo, Japan) at 8,500 rpm and a flow speed of 5 kg/hr. Then, the rice flour was dried using a fluidized bed dryer (20F; NAGATO DENKI MFC Co., Ltd., Osaka, Japan) to obtain a moisture content of 12.0 – 13.5%.

Measurement of rice flour properties Amylose content was measured by the iodine colorimetric method (Juliano, 1971), using potato amylose type III and waxy rice starch as standards of amylose and amylopectin, respectively. Starch damage degree was determined using a starch damage kit (Megazyme, Wicklow, Ireland) according to the manufacturer’s instructions. Particle size and distribution of rice flour were measured with a laser diffraction-scattering analyzer (LMS-2000; Seishin Enterprise Co., Ltd, Tokyo, Japan).

Preparation of rice flour breads On a dry weight basis, 85 g rice flour was mixed with 15 g vital wheat gluten, 2.3 g salt, 0.6 g dried yeast, 0.3 g caster sugar and an adequate amount of water. The additive amount of water was determined as the amount required to give a viscosity of 500 BU (Brabender Units) in Farinograph measurement (Brabender, Inc., Duisburg, Germany). All of the materials were mixed with a vertical mixer (SK25C; SK Mixer Co., Ltd, Saitama, Japan) for preparing dough, mixing at a slow-speed for 3 min and subsequent kneading at medium-speed for 10 – 15 min. After the dough was extended with a rolling pin to obtain a thickness of 5 mm, it was rounded and kneaded by the hands to remove air bubbles. The molded dough was fermented at 30°C and 80% humidity for *ca.* 80 min and, after spraying with water, oven-baked at 230°C for 25 min. The baked breads were preserved at 20°C after being wrapped in a plastic bag. The loaf volume of the bread was measured by the rapeseed replacement method.

Nutritional composition of rice flour breads The nutritional composition of the rice flour breads was analyzed. Water content was calculated as a measure of weight loss after drying at 135°C for 2 h. Protein content was measured by the macro-Kjeldahl method (nitrogen-to-protein conversion factor: 5.95). Lipid content was measured by Soxhlet extraction using diethyl ether as the solvent. Ash content was calculated from the difference in the mass of the sample before and after incineration in a furnace at 550°C. Dietary fiber content was determined using a dietary fiber measurement kit (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) according to the manufacturer’s manual. Carbohydrate content was calculated by subtracting the weights of protein, lipid, ash and dietary fiber contents from the total weight.

Analysis of resistant starch (RS), slow digestible starch (SDS)

and rapidly digestible starch (RDS) The rice flour breads were freeze-dried, pulverized and analyzed with respect to total starch (TS), RS, SDS and RDS, using a Resistant Starch Assay Kit (Megazyme). The enzymatic reaction with α -amylase and amyloglucosidase was carried out for 2 or 6 h to classify the starch by digestibility (Nakamura *et al.*, 2015). TS content was regarded as the measured quantity of starch without enzymatic reaction. Contents of RS, SDS and RDS were calculated as follows:

RS = [starch not digested by enzymatic reaction for 6 h]

SDS = [starch not digested by enzymatic reaction for 2 h] – RS

RDS = TS – [starch not digested by enzymatic reaction for 2 h]

Clinical trial The clinical trial fulfilled the study criteria of the Japan Glycemic Index Study Group Protocol and was conducted by Niigata Bio-Research Park Inc. (Niigata, Japan) in accordance with the Ethical Guidelines of the Declaration of Helsinki. The trial was registered with UMIN Clinical Trials Registry (UMIN000016318). The protocol was reviewed and approved by the Food-human Studies Review Committee in Niigata City Bio Research Center (Niigata, Japan). After obtaining informed consent from healthy volunteers, subjects were enrolled in the trial under the following conditions: glucose tolerance, normal (fasting blood glucose level, 70 – 110 mg/dl; blood glucose level at 2 h after injection, < 140 mg/dl); body mass index (BMI), 30 kg/m² or less; hemoglobin A1c (HbA1c), 6.5% or less. The glucose tolerance tests were performed with TRELAN®-50G (A Y Pharma Co., Ltd., Tokyo, Japan) at 1 week before the trial, and the results from physical examination, hematology and biochemical tests were recorded.

The test breads were made with the rice flours of MAI, KOR and HKR one day before ingestion. The breads were cut to a thickness of 20 mm and subdivided into pieces containing 50 g of carbohydrate per intake. After 12-h fasting, all subjects were directed to eat a piece of test bread and the indicated amount of water within 10 min, in a single-blind crossover manner with a washout period of 3 days. The water content of the bread piece was taken into consideration when calculating the intake of 250 mL of water. A glucose solution (250 mL of water containing 50 g of glucose) was also ingested as a standard meal.

Blood glucose analysis and calculation of GI value Blood samples were collected from the fingertips at 0 (fasting), 15, 30, 45, 60, 90 and 120 min after the beginning of the ingestion, using a puncture device One-Touch Pen® (Johnson & Johnson Ltd., Tokyo, Japan). Blood glucose level was measured with a self-testing blood glucose meter (One Touch Ultra View®; Johnson & Johnson Ltd.).

Glycemic variation was determined by subtracting the fasting level from the postprandial level of blood glucose. The 0 – 120 min incremental area under the curve (IAUC) was calculated from the glycemic variation curve based on the trapezoidal rule, ignoring the area beneath the fasting concentration. GI value was calculated as a percent of IAUC from the test bread relative to that from the standard meal.

Statistical analysis Data from the starch components and the clinical trial were analyzed using ANOVA and Tukey's test with Excel Statics 2010 (Social Survey Research Information Co., Ltd., Tokyo, Japan). ANOVA was used to compare the starch components, the glucose variation and GI values among the three groups. *P* values less than 0.05 were considered statistically significant throughout the study.

Results and Discussion

Applicability of MAI to bread making Our research group previously determined the following recommended properties of rice flour for bread making: 80% of the amount of the flour is smaller than 75 μ m in diameter, and the degree of starch damage is less than 6%. Therefore, we examined the milling properties of MAI, wet polished using the air-flow pulverizer, in comparison to those of KOR and HKR. Table 1 shows that the properties of MAI flour satisfied the above-mentioned criteria and were comparable to the flour from HKR, which is a rice cultivar known to be applicable for bread making. Besides, the amylose content of MAI was $24.6 \pm 0.7\%$, which is also within the suitable range for bread making (from 15% to 25%) (Ishida and Morohashi, 2012). These findings indicate that MAI could be used to produce rice flour for bread making, using the current milling process applied to rice cultivars such as HKR.

Next, we made rice breads with MAI flour and compared the quality with that of KOR and HKR breads. The specific volume of MAI bread was 3.0 cm³/g, which was smaller than that of KOR and HKR breads (KOR, 3.3 cm³/g; HKR, 3.7 cm³/g), indicating that MAI bread did not rise to the same level. It was observed that while MAI bread rose to the same extent as KOR and HKR breads during baking, the MAI bread immediately shrank, unlike the breads made with the other two cultivars. These results imply that the mechanical strength of the gluten network was insufficient to maintain the height of the MAI bread. In bread making, the amount of added water was 86 g, 71 g and 77 g for the breads made with MAI, KOR and HKR, respectively, per 100 g dry weight of mixed flour, which was determined to give a viscosity of 500 BU in Farinograph measurement (Yamauchi *et al.*, 2004). Therefore, the MAI flour absorbed more water compared with the KOR and HKR

Table 1. Properties of rice flour

	amylose content (%)	starch damage degree(%)	particle size (μ m)
MAI	24.6 ± 0.7	5.1 ± 0.0	63.9 ± 1.9
KOR	33.5 ± 0.6	2.2 ± 0.1	42.0 ± 0.7
HKR	16.2 ± 0.7	3.7 ± 0.2	57.2 ± 1.5

Amylose content and starch damage degree are calculated in terms of dry weight basis. Particle size shows diameter which 80 % the amount of the flour was smaller than this. Each value represents the average $\pm$ standard deviation (amylose content and starch damage, n=3; particle size, n=6).

Table 2. Starch composition of one-day rice breads

	(%)				
	RS	SDS	RDS	SDS+RS	TS
MAI	3.2 ± 0.1 a	8.5 ± 0.3 a	51.9 ± 0.7 a	11.7 ± 0.3 a	63.5 ± 0.7 a
KOR	1.2 ± 0.1 b	6.2 ± 0.2 b	60.5 ± 1.7 b	7.4 ± 0.2 b	67.9 ± 1.8 b
HKR	0.1 ± 0.0 c	2.3 ± 0.0 c	66.4 ± 1.3 c	2.4 ± 0.0 c	68.9 ± 1.3 c

RS, SDS, RDS and TS are calculated in terms of dry weight basis. Each value represents the average ± standard deviation (n=4). Means followed by the same letter are not significantly different at $p < 0.05$.

flours. In other words, the water contributing to the formation of the gluten network decreased during MAI bread production; thus, more water should be added to improve the specific volume of MAI bread. A viscosity of less than 500 BU would be a better index for the water added in the case of MAI flour.

Overall, MAI is applicable for the production of rice flour for bread making, similar to other cultivars such as HKR; however, the procedure for bread making should be optimized for MAI flour in the near future.

Starch composition of rice breads and change in RS content Englyst *et al.* (1992) classified starch according to starch digestibility as: RS, SDS and RDS. Table 2 shows the starch composition of 1 day-old rice breads. HKR bread contained trace amounts of RS, and the RS content of MAI bread was greater than that of KOR bread. Nakamura and Ohtsubo (2015) reported that the RS contents of boiled rice were 0.30% and 4.26% for HKR and MAI, respectively. These data agreed with our results, implying that the bread making process would influence the RS content to the same extent as boiling. Table 2 also shows that MAI bread contained the greatest amount of SDS and had the lowest digestibility among the three cultivars (i.e., SDS plus RS). The European Food Safety Authority (EFSA, 2011) has proposed that high SDS meals should be consumed in order to maintain normal blood glucose levels; thus, MAI would be a beneficial rice material for nutraceutical foods.

The RS contents of MAI and KOR breads increased during storage, and the increase in MAI breads was greater than that in KOR breads (Fig. 1). On the other hand, when 3 day-old breads were oven-toasted, the RS content was reduced from 3.4% to 2.7% in MAI bread, but remained unchanged in KOR bread (data not shown). Nakamura *et al.* (2013) previously proposed that MAI very easily undergoes starch retrogradation, based on the pasting properties measured by Rapid Visco Analyzer. In addition, Hung *et al.* (2005) reported that the rapid retrogradation of starch contributes to RS formation. Therefore, in MAI bread, the increase in RS content during storage would be due to starch retrogradation, while the decline in RS by toasting would be attributable to re-gelatinization. It was reported that high amylose rice cultivars exhibit minimal re-gelatinization because of the heat stability of the retrograded amylose (Birt *et al.*, 2013). Hence, differences in RS content by toasting between MAI and KOR breads could be

Fig. 1. Changes in RS content in the breads during storage at room temperature RS is calculated in terms of dry weight basis. Vertical bar shows standard deviation of each value (n = 4). MAI: *Konayukinomai*, KOR: *Koshinokaori*, HKR: *Koshihikari*

explained by differences in the amylose content of these two cultivars (Table 1).

Nutritional composition of rice breads The nutritional composition of the rice breads is shown in Table 3. According to the GI measurement protocol defined by Jenkins *et al.* (1981), the caloric intake from rice breads containing 50 g carbohydrate was 254 kcal, 249 kcal and 244 kcal for MAI, KOR and HKR breads, respectively. All of the rice breads contained only a slight amount of dietary fiber, indicating that the dietary fiber did not contribute to the effects of these rice breads on changes in postprandial glucose levels in our clinical trial, as judged from the recommended practical intake level (Yamada *et al.*, 2013).

Clinical trial Among the 15 research participants, nine satisfied the following conditions outlined in the Japan Glycemic Index Study Group Protocol: age, from 22 to 47 years; BMI, from 18.2 to 26.2 kg/m²; fasting blood glucose level, from 82 to 103 mg/dl; HbA1c, from 4.8% to 5.5%. Fasting blood glucose level immediately before the intake of MAI, KOR and HKR breads was 80.6 ± 7.0, 83.8 ± 10.7 and 84.1 ± 8.2 mg/dl, respectively, and a significant difference among the three groups was not observed.

Table 3. Nutrition component of rice breads used in clinical trial.

	MAI	KOR	HKR
Weight (g)	110.6	96.6	98.6
Water (g)	46.1	33.0	36.3
Protein (g)	11.8	11.4	10.3
Lipid (g)	0.3	0.2	0.2
Ash (g)	1.5	1.4	1.4
Dietary fiber(g)	0.9	0.6	0.4
Carbohydrate(g)	50.0	50.0	50.0
Energy(kcal)	254	249	244

Weight is calculated by the test meal per serving. Water, protein, lipid, ash and dietary fiber show the content in the test meal.

Figure 2 shows the averaged glycemic variation pattern after the intake of the rice breads or the standard meal. Seven subjects showed these typical monomodal patterns, with a peak at 30 or 45 min after the ingestion of the standard meal. On the other hand, 2 subjects showed a bimodal pattern with the intake of the standard meal. The reason for these unusual patterns is not known; however, these patterns resulted in large variability in the data. As shown in Fig. 2, in the HKR group, the glycemic variation reached a peak value (75.1 ± 17.8 mg/dl) at 45 min. On the other hand, increases in glycemic variation in the MAI and KOR groups were significantly lower than that in the HKR group at 45 min and 60 min, and the pattern of the glycemic variation was similar between the MAI and KOR groups.

GI values of the breads were 82 ± 36 for MAI, 87 ± 44 for KOR, 113 ± 51 for HKR. The GI value of HKR bread was comparable to that of glucose. MAI and KOR breads had lower GI values than that of HKR, although not significantly so, probably due to the large variation in the glycemic response among subjects. These results together with the findings shown in Fig. 2 suggest that increases in blood glucose are repressed when MAI and KOR breads are consumed compared to conventional rice breads such as HKR. KOR breads, which contained smaller amounts of RS than MAI breads (Table 2), produced a similar effect on blood glucose levels as MAI breads, further supporting the importance of SDS and RS in preventing increases in blood glucose (Lehmann and Robin, 2007). However, the total amount of RS and SDS was significantly higher in MAI bread than in KOR bread (Table 2), although the effects on glycemic variation were similar between MAI and KOR breads. On the other hand, we found that the total amount of mono- and di-saccharides was 1.03% and 0.22% in MAI and KOR flours, respectively. Because low molecular weight saccharides can be easily metabolized, in the intake of MAI breads, the favorable effects of RS and SDS would be partly counteracted by these saccharides. Yamada *et al.* (2005) examined the inhibitory effect of the intake of bread containing 6 g of RS (prepared from tapioca) on increases in postprandial blood glucose in 20 subjects. They observed that postprandial glucose levels were significantly reduced by the ingestion of RS-containing bread in a borderline

Fig. 2. Glycemic variation after ingestion of rice breads.

Glycemic variation is calculated by subtracting the fasting blood glucose level from postprandial blood glucose level of each time, and is shown as the averaged value ($n = 9$). Vertical bars show standard deviations. Different letters mean significant difference among three test meals as shown by Tukey's tests ($p < 0.05$). Symbols: $\diamond$, MAI; $\blacksquare$, KOR; $\bullet$, HKR; $\blacktriangle$, standard meal

group (blood glucose, ≥ 111 mg/dl) but not in a normal group (blood glucose, ≤ 110 mg/dl). On the other hand, Hallström *et al.* (2011) produced high RS bread by using a high amylose wheat genotype, and the intake of this bread (containing 7.7 g RS) induced a significantly lower postprandial glucose response in healthy subjects. These inconsistent results indicate that further clinical studies are required to confirm the beneficial effects of RS and SDS on the postprandial glucose response. However, in comprehensive consideration of the current knowledge, foods with higher contents of RS and SDS are thought to improve metabolic responses to the intake of carbohydrates in the prevention of lifestyle-related diseases.

In this study, we examined the processing suitability and

nutraceutical effects of MAI as a rice cultivar with long-chain amylopectin. We propose that MAI is applicable to the production of rice flour for bread making, and that rice bread made with MAI prevents abrupt increases in postprandial blood glucose levels, probably due to the large amounts of contained RS and SDS. The improvement of glucose responses with the intake of MAI bread was comparable to that of KOR, which is a high amylose rice cultivar, indicating the usefulness of these novel rice cultivars.

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