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Characteristics of Pregelatinized *Ae* Mutant Rice Flours Prepared by Boiling after Preroasting

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ABSTRACT: As *ae* mutant rice, such as EM10, lacks the starch branching enzyme IIb, its amylopectin contains more long-chain glucans than that of ordinary Indica and Japonica rice grains. Although boiled grains of *ae* rice cultivars are too hard and nonsticky for table rice, they are promising in terms of biofunctionality, such as prevention of diabetes. The present paper investigates the characterization of a novel group of four *ae* mutant rice cultivars (EM72, EM145, EM174, and EM189). They were subjected to the evaluation for their main chemical components, physical properties, and enzyme activities at different grain conditions (raw milled rice, roasted rice, boiled rice, and rice boiled after preroasting). These mutant rice grains are characterized by high apparent amylose, high protein and high glucose contents, high pasting temperature, high α-amylase activities, high resistant starch, and low degree of gelatinization. A novel method was developed to maintain the high resistant starch contents of gelatinized rice grains. Rice boild after preroasting showed a higher ratio of resistant starch and a lower amount of glucose than ordinary boiled rice. It became possible to produce high-quality and biofunctional pregelatinized rice flours by boiling with frozen fruits, such as tomatoes, after rice grains had been preroasted. These *ae* mutants were found to be suitable materials for rice/fruit or rice/vegetable products to serve as palatable, low-glucose, and high resistant starch rice products.

KEYWORDS: *ae* mutant, resistant starch, rice boiled after preroasting, pasting properties

INTRODUCTION

As diabetes is one of the lifestyle diseases, disease prevention is very important in addition to the development of curing technology. Low glycemic index (GI) food inhibits the rapid increase of blood glucose or insulin secretion after consumption of a meal. Several researchers reported on the high-resistant-starch rice[1,2] or high-amylose and high-dietary-fiber rice[3] developed by the physical or the chemical mutation.

Indigestible dextrin (ID)[4] has been confirmed by both animal and clinical studies to have physiological functions such as intestinal regularity, moderation of postprandial blood glucose level, lowering of serum lipid, and reduction of body fat.[5]

Ae mutants were developed by Satoh[6] by the chemical mutation (MNU treatment) at Kyushu University in Japan. *Ae* mutants lack the starch branching enzyme (BE) IIb, which lowers the amount of short-chain glucans (a degree of polymerization < 17) and induces elevation of the gelatinization temperature. The *ae* mutation caused a dramatic reduction in the activity of BE IIb, and the activity of soluble starch synthase SS I in the *ae* mutation was significantly lower than in the wild type, suggesting that the mutation had a pleiotropic effect on SS I activity. The activity of SS I in the *ae* mutant EM10 was markedly decreased as compared with that of the wild type.[6]

The glycemic effect of foods depends on a number of factors such as the amylose and amylopectin structures.[5] The amylopectin of *ae* mutants have more long-chain glucans, making the texture of rice grains very hard and nonsticky after boiling and giving unacceptable palatability as boiled rice.[7] Therefore, gelatinization temperatures of *ae* mutant rice starches are very high, which lead to resistance to digestion as Kubo et al. reported.[8] Particularly EM10 is promising as a material for such low-GI foods[9,10] as breads or noodles to prevent diabetes, because it contains a substantial amount of resistant starch even after boiling.[7] Furthermore, the batter from EM10 rice flour retained less oil during frying than that from wheat flour and other types of rice flour;[11] however, in the case of ground rice flours, resistant starch has decreased sharply and glucose has increased, which caused an increase of the GI.[12]

Ae mutants of high-amylose rice are characteristically less sticky. Thus, the suitable utilizations of *ae* mutants were presumed to be materials with high strength, such as rice paper, rice noodle, sizing, and biodegradable plastic films.[13,14]

The starch industries purify and refine starches from seeds, roots, or tubers, by wet grinding, washing, sieving, and drying. Typical modified starches for technical applications are cationic starches, hydroxyethyl starch, and carboxymethylated starches.[15]

Rice flour is a form of flour made from finely milled rice. Rice flour is classified into raw type and pregelatinized type, which was prepared by heating (steaming or boiling) rice grains followed by drying and pulverizing in Japan.[15]

To improve the palatability of *ae* mutant rice, rice grains were cooked with fruits or vegetables, such as strawberry or tomato, because many consumers prefer these *ae* mutant cooked rice products according to our preliminary survey at the confectionary store in Niigata University. Development of novel rice products in terms of palatability and biofunctionality is necessary and meaningful.

In the present paper, we investigated the characterization of the new *ae* mutant rice cultivars and searched for a novel method

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to maintain the high resistant starch content even after boiling or cooking. Although these *ae* mutants are not suitable for table rice because of its low palatability, it would be suitable as a material for biofunctional foods, such as low-GI foods.

■ MATERIALS AND METHODS

Materials. Superhard rice (EM10, EM145, EM72, EM189, EM174) and high-amylose rice (Hoshiyutaka) were cultivated in the experimental field of Kyushu University in 2009. High-quality premium rice (Koshihikari) was cultivated in the experimental field of Hokuriku Reseach Center of the Central Agricultural Research Center, Joetsu, in 2009.

Preparation of Polished White Rice Samples. Brown rice was polished by an experimental friction-type rice milling machine (Yamamotoseisakusyo, Co. Ltd., Tendoh, Japan) to the milling yield (= yield of polishing) of 90−91%. Rice flour of white rice was prepared by a cyclone mill (SFC-S1, Udy, Fort Collins, CO) with a screen of 1 mm diameter pores.

Boiling of White Rice Grains. The white rice grains (10 g) were added with 10 g (×1 times, w/w), 16 g (×1.6 times, w/w), and 20 g (×2.0 times, w/w) of distilled water in an aluminum pudding cup. After soaking for 1 h, the samples were boiled in an electric rice cooker (National SR-SW182). Cooking, including boiling at 100 °C for about 15 min, took about 40 min.

Method To Boil after Preroasting Rice. Various kinds of milled rice (100 g), such as Koshihikari, Hoshiyutaka, EM10, EM145, EM72, EM189, and EM174, were roasted for 4 min at 740 W by an electric induction heater (Panasonic K2-PH 31, IH) respectively, followed by soaking in 200 mL of water for 60 min. Thereafter, rice was boiled by an electric rice cooker (Sharp KS-HA5-W) for 40 min. After boiling, the boiled rice grains were dried under a relative humidity of 50% and a temperature of 10 °C in an artificial environment room (Sanyo Medicasystems, Co. Ltd., Tokyo, Japan) for 2 days. Thereafter, rice flour was prepared by a cyclone mill (SFC-S1, Udy) with a screen of 1 mm diameter pores.

Co-cooking with Fruits or Vegetables. *Ae* mutants showed high glucose content; therefore *ae* mutants were browned easily. *Ae* mutants are not suitable for table rice because of their low palatability.[14] We tried to produce high-quality and biofunctional pregelatinized rice flour by boiling with frozen fruits (6% w/w dry base), such as blueberry, tomato, strawberry, or sweet summer orange, after the rice grains had been preroasted. Resistant starch content was measured by the enzyme kit.

Measurement of Moisture Content of Rice Flours. The moisture content of the milled rice grains was measured by an oven-drying method, drying 2 g of flours for 1 h at 135 °C. Pregelatinized starch (roasted rice, boiled rice, rice boiled after preroasting) were stored in the freezer at −80 °C, followed by lyophilizing by freeze-dryer (FD-1, Eyela, Tokyo, Japan), and rice flour was prepared by a cyclone mill (SFC-S1, Udy) with a screen of 1 mm diameter pores.

Amylose Content. The amylose content of the raw milled rice was estimated by the iodine colorimetric method of Juliano.[16] Potato amylose (type III, Sigma Chemical Co., St. Louis, MO) and waxy rice starch (removed fat and proteins from waxy rice) were used as standard amylose and standard amylopectin for amylose determination.

Protein Content. Nitrogen was determined by a nitrogen analyzer (Leco FP-528, LECO, USA) based on the combustion method (modified Dumas method). The protein content was obtained from nitrogen by multiplication with a nitrogen protein conversion factor of 5.95.

α-Amylase Activity. α-Amylase activities of raw milled rice and roasted rice were determined by the enzyme kit (Megazyme International Ireland, Ltd.). For α-amylase activity measurement, rice flour (1.5 g) was extracted with 1 mL of extraction buffer, pH 5.4, at 40 °C for 20 min and thereafter centrifuged for 10 min at 1000*g*. Extraction solution (0.1 mL) and substrate (0.1 mL) were preincubated at 40 °C for 5 min. Thereafter, each sample solution was incubated at 40 °C for exactly 20 min, followed by the addition of stopping reagent (1.5 mL). The absorbance was measured at 400 nm.

α-Glucosidase Activity. α-Glucosidase activity of raw milled rice was determined by the kit (Kikkoman Biochemicals Corp). To rice flour (0.2 g) was added 1 mL of 0.01 M acetate buffer solution (pH 5.0 including 0.5% NaCl), and the mixture was extracted at 5 °C for 16 h and then centrifuged for 5 min at 3000*g*. PNPG substrate solution (2.0 mL) was prewarmed at 37 °C for 5 min, and then 0.1 mL of extraction solution was mixed and kept at 37 °C for 10 min, followed by the addition of 1.0 mL of stopping solution (Na_2CO_3) and stirred vigorously. The absorbance of sample solution was measured at 400 nm.

Xylanase Activity. Xylanase activity of raw milled rice was determined by the enzyme kit (Megazyme International Ireland, Ltd.). For xylanase activity measurement, rice flour (0.5 g) was extracted with 5 mL of 0.1 M MES buffer including SDS (1% w/v, pH 6.0) at room temperature for 20 min and then centrifuged for 10 min at 1500*g*. The supernatant (0.5 mL) was used for assay. Xylazyme AX (dyed xylan substrate tablet) was added to each supernatant, and each tube was immediately placed in a water bath at 40 °C and incubated for exactly 30 min. After 30 min, to the solution was added 5 mL of Tris-HCl buffer solution (pH 9.0), and the mixture was stirred vigorously with a vortex mixer and kept at room temperature for 5 min. Thereafter, the sample solution was filtered through a Whatman no. 1 (9 cm) filter paper. The absorbance of each filtrate was measured at 590 nm.

Measurement of Glucose Content. To the rice flour sample (0.1 g) was added 1 mL of 60% ethyl alcohol, and the mixture was subjected to the extraction of glucose by rotation at 20 °C for 1 h. In the case of boiled rice sample, sample flours were prepared by pulverization after lyophilization.

The solution was centrifuged (1500*g*, 15 min), and the supernatant was used as a sample solution for the measurement. The glucose content in the sample solution was measured by the enzyme assay method of NADPH using the glucose assay kit (Roche, Darmstadt, Germany).

Measurement of Resistant Starch. The resistant starch in the sample was measured according to the AOAC method by a resistant starch assay kit (Megazyme, Wicklow, Ireland). In the case of boiled rice sample, sample flours were prepared by pulverization after lyophilization. Each sample (100 mg) was digested by pancreatin and amyloglucosidase, and glucose was measured by spectrophotometer at 510 nm.

Degree of Gelatinization. Degree of gelatinization was determined using the BAP method.[17] Pregelatinized starch was decomposed by β-amylase and pullulanase in this method. Dehydrated sample (80 mg) was added to distilled water (8 mL) and then dispersed with a glass homogenizer, reciprocally 20 times. Dispersed sample (2 mL) was filled to 25 mL with 0.8 M acetate buffer solution, pH 6.0 (standard solution). The other dehydrated sample (30 mg) was added to distilled water (3 mL), then mixed thoroughly, and then boiled for 10 min, followed by autoclaving at 120 °C for 15 min. This pregelatinized solution (2 mL) was added to 0.2 mL of 10 N NaOH, then kept at 50 °C for 3 min, and added to 1 mL of 2 N acetic acid. This pregelatinized sample (2 mL) was filled to 25 mL with 0.8 M acetate buffer solution, pH 6.0 (complete pregelatinized solution). Standard solution (4 mL) and complete pregelatinized solution were added to 1 mL of enzyme solution containing β-amylase (0.8 IU) and pullulanase (3.4 IU) and thereafter incubated at 40 °C for 30 min, respectively. The other 4 mL of standard solution and complete pregelatinized solution was added to 1 mL of inactivated enzyme solution (10 min boiling) (blank test sample), respectively. These samples (1 mL) were inactivated by boiling for 5 min, respectively. These samples (1 mL) were diluted five times, respectively. These samples (1 mL) were used for measurement of reducing sugars according to the Somogyi−Nelson method.[18,19]

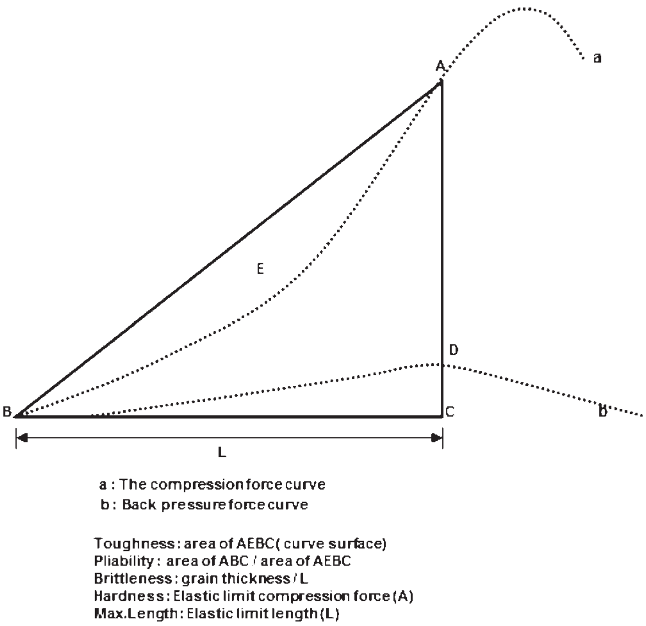

Figure 1. Continuous progressive compression test (CPC test). The milled rice samples (10 g) were added with 10 g (× 1 time, w/w), 16 g (× 1.6 times, w/w), and 20 g (× 2.0 times, w/w) of distilled water in an aluminumu cup. After soaking for 1 h, the samples were boiled in an electric rice cooker (National SR-SW182). After 2 h of boiling, the physical properties of the samples were measured by the continuous progressive compression method (CPC) using a Tensipresser (My Boy System, Taketomo Electric, Co., Tokyo, Japan) at the following conditions: distance, 30.0 mm; clearance, 0.2 mm; thickness 1, 5.0 mm; thickness 2, 5.0 mm; bite speed, 2.0 mm/s; second speed, 2.0 mm/s' multiply, 1; magnificant, 2; repeat time, 200; multirepeat, 1; add value, 0.05; baseline, 2; load cell, 10 (according to our previous paper[16]).

Measurement of Pasting Properties of Rice Flours. Pasting properties of white rice flours were measured using a Rapid-Visco-Analyzer (RVA, model Super 4, Newport Scientific Pty, Ltd., Warriewood, Australia). A programmed heating and cooling cycle was followed as outlined in the procedure of Toyoshima et al.[20] In the case of boiled rice sample, sample flours were prepared by pulverization using a coffee mill (Millser, Iwatani, Tokyo) after lyophilization with a freeze-dryer (FD 50, Eyla).

Measurement of Physical Properties of Boiled Rice Grains. After the boiled rice grains had stood for 2 h at 20 °C since the automatic finish of boiling, the physical properties of the samples were measured by the continuous progressive compression method (CPC) using a Tensipresser (My Boy System, Taketomo Electric, Co., Tokyo, Japan) under the following conditions: distance, 30.0 mm; clearance, 0.2 mm; thickness 1, 5.0 mm; thickness 2, 5.0 mm; bite speed, 2.0 mm/s; second speed, 2.0 mm/s; multiply 1; magnificant, 2; repeat time, 200; multi-repeat, 1; add value, 0.05; baseline, 2; load cell, 10, according to our previous paper.[21] The average of each parameter was calculated by measuring 20 individual grains.

Measurement of the physical properties of the boiled rice grains by CPC with the Tensipresser showed the hardness, toughness, pliability, brittleness, and maximum length of boiled rice grains. The original method of CPC test with a Tensipresser was developed for the evaluation of meat tenderness.[22] We arranged the measuring condition of CPC test for boiled rice texture.[16] Toughness is shown as area of AEBC (curve surface), pliability is shown as the ratio (area of ABC)/(area of AEBC), brittleness is shown as the ratio (grain thickness)/(maximum length), hardness is shown as elastic limit compression force (apex A), and maxmum length means elastic limit length as shown in Figure 1.

Statistical Analyses. All of the results, including the significance of regression coefficients, were subjected to a statistical analysis by the *t* test and one-way ANOVA, method of Tukey, provided in Excel Statistics (ver. 2006, Microsoft Corp., Tokyo, Japan).

RESULTS

Apparent Amylose Content. As shown in Table 1, apparent amylose contents of EM10 (36.4%), EM145 (34.2%), EM72 (31.3%), and EM174 (33.7%) were very high and EM189 (24.2%) showed an intermediate value, whereas Koshihikari (16.2%) showed a low value and Hoshiyutaka (31.0%) a high value.

Protein Content. As shown in Table 1, protein contents of EM145 (7.37%) and EM72 (8.43%) were high. EM10 (6.57%), EM189 (6.23%), EM174 (6.43%), and Hosiyutaka (6.10%) were of intermediate values, whereas Koshihikari (5.57%) showed low protein content.

***α*-Amylase Activity.** Among milled rice of *ae* mutants, the *α*-amylase activities were 0.080−0.19 (CU/g), which were 13−32 times that in Hoshiyutaka (0.006 CU/g) and 8−19 times that in Koshihikari (0.010 CU/g). *Ae* mutant cultivars had very high *α*-amylase activities; particularly EM10 showed the highest activity.

Among *ae* mutants, 65.8−86.2% of *α*-amylase activities of milled rice were decreased by roasting; Koshihikari (80%) and Hoshiyutaka (83.3%) showed a similar tendency as shown in Figure 2.

***α*-Glucosidase Activity.** Iwata reported that *α*-glucosidase activity showed a positive correlation with fresh weight, GBSS activity, and amylose content.[23] The *α*-glucosidase activity of *ae* mutants milled rice grains were 0.045−0.058 U/mL. The rice samples were classified into two groups, which were a higher *α*-glucosidase group, such as Koshihikari, EM145, and EM72, and a lower group, such as Hoshiyutaka, EM10, EM189, and EM174.

Xylanase Activity. As shown in Table 1, the activities of xylanase of *ae* mutants were 222.11−244.72 U/kg; that of Koshihikari was 230.47 U/kg and that of Hoshiyutaka, 266.17 U/kg. Particularly, high-amylose rice, Hoshiyutaka, showed the highest xylanase activity. EM174 showed the highest activity of xylanase among the *ae* mutants.

Glucose Content. As shown in Figure 3, milled rice of EM10 (0.08 g/L), EM72 (0.06 g/L), EM189 (0.05 g/L), and EM174 (0.07 g/L) showed high glucose contents; especially EM145 (0.16 g/L) was highest. Koshihikari (0.04 g/L) and Hoshiyutaka (0.02 g/L) showed very low glucose contents. As boiled rice after preroasting, EM174 showed the lowest glucose content among *ae* mutants. Glucose contents slightly decreased by roasting and increased remarkably 3.9−7.2 times by boiling, and Koshihikari (2.2 times) and Hoshiyutaka (3.0 times) showed a similar tendency.

Resistant Starch. *Ae* mutant rice grains had markedly higher amounts of resistant starches than the other rice grains as shown in Figure 4. Among the various kinds of milled rice, *ae* mutants had an average resistant starch content of 9.9% (6.7−12.4%), which was higher than that of ordinary low-amylose rice, Koshihikari (0.5%), and ordinary high-amylose rice, Hoshiyutaka (0.7%). Among the boiled rices, the amount of resistant starch of *ae* mutants was 4.8% (4.0−5.6%), which was about 4 times that in high-amylose rice, Hoshiyutaka (1.2%), and 8 times that in

Table 1. Main Chemical Components and α-Amylase, α-Glucosidase, and Xylanase Activities of Samples

	moisture		amylose		protein		α-amylase		α-glucosidase		xylanase	
sample	%	SD	%	SD	%	SD	activity (CU/g)	SD	activity (U/mL)	SD	activity (U/kg)	SD
Koshihikari	14.13 a	0.06	13.70 a	0.21	5.57 a	0.06	0.01 a	0.00	0.058 a	0.00	230.5 a	6.98
Hoshiyutaka	12.90 b	0.10	26.30 b	0.15	6.10 ab	0.10	0.01 a	0.00	0.047 b	0.00	266.2 b	17.08
EM10	14.27 a	0.06	36.40 c	0.10	6.57 bc	0.51	0.19 b	0.01	0.045 b	0.00	226.9 a	13.29
EM145	15.20 c	0.10	34.20 d	0.15	7.37 d	0.23	0.16 c	0.01	0.057 a	0.00	238.6 ac	11.93
EM72	15.77 d	0.06	31.30 e	0.20	8.43 e	0.23	0.09 d	0.00	0.056 a	0.00	222.6 a	2.49
EM189	15.20 c	0.10	24.20 f	0.10	6.23 bc	0.06	0.08 d	0.00	0.047 b	0.00	222.1 a	2.36
EM174	15.67 d	0.12	33.70 d	0.10	6.43 bc	0.06	0.10 d	0.00	0.045 b	0.00	244.7 bc	25.38

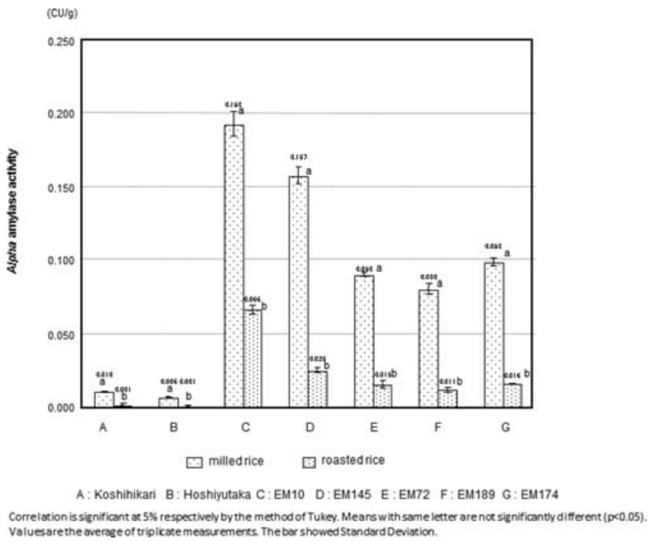

Figure 2. Influence of roasting rice on its α-amylase activity. Correlation is significant at 5% by the method of Tukey. Means with the same letter are not significantly different ($p < 0.05$). Values are the average of triplicate measurements. The bars show standard deviation. Various kinds of milled rice (100 g), such as Koshihikari, Hoshiyutaka, EM10, EM145, EM72, EM189, and EM174, were roasted for 4 min at 740 W by an electric induction heater (Panasonic K2-PH 31, IH) respectively. α-Amylase activity of raw milled rice and roasted rice was determined by the enzyme kit (Megazyme International Ireland, Ltd.). For α-amylase activity measurement, rice flour (1.5 g) was extracted with 1 mL of extraction buffer, pH 5.4, at 40 °C for 20 min and then centrifuged for 10 min at 1000g. Extraction solution (0.1 mL) and substrate (0.1 mL) were preincubated at 40 °C for 5 min. Thereafter, each sample solution was incubated at 40 °C for exactly 20 min, followed by the addition of stopping reagent (1.5 mL). The absorbance was measured at 400 nm.

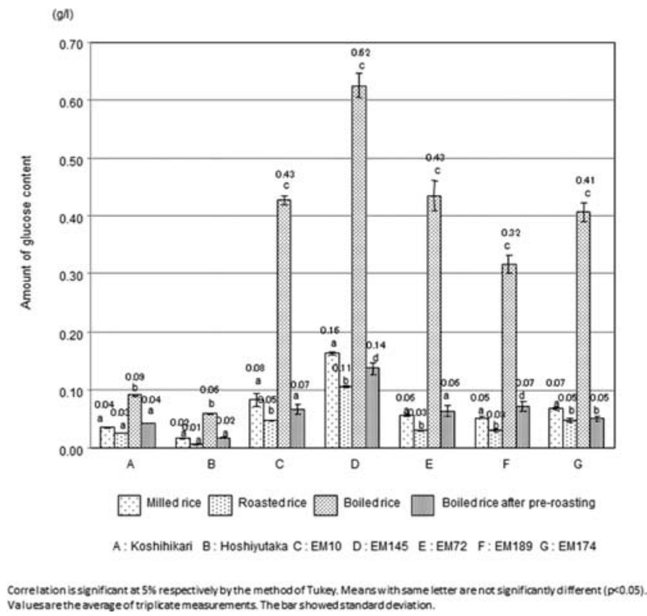

Figure 3. Glucose content of various processed rice flours prepared according to different heat treatments. Correlation is significant at 5% by the method of Tukey. Means with the same letter are not significantly different ($p < 0.05$). Values are the average of triplicate measurements. The bars show standard deviation. Rice flour sample (0.1 g) was added with 1 mL of 60% ethyl alcohol and subjected to the extraction of glucose by rotation at 20 °C for 1 h. The solution was centrifuged (1500g, 15 min), and the supernatant was used as sample solution for measurement. The glucose content in the sample solution was measured according to the enzyme assay method of NADPH using the glucose assay kit (Roche, Darmstadt, Germany).

premium Japonica rice, Koshihikari (0.6%). *Ae* mutants showed a very high amount of resistant starch even after boiling. In addition, among boiled rices after preroasting, *ae* mutants had an average resistant starch content of 7.5% (6.4−8.8%), which was higher than that of Koshihikari (0.8%) or Hoshiyutaka (1.3%).

Degree of Gelatinization. As shown in Figure 5, among the various rice samples, when the degree of gelatinization of Koshihikari showed 100%, *ae* mutants showed 44.6−55.4% and Hoshiyutaka showed 80.2%, respectively. Preroasting before boiling decreased the degree of gelatinization of *ae* mutant rice (3−12.5%), Koshihikari (23.8%), and Hoshiyutaka (2.0%) compared with those of boiled rice grains without preroasting.

Pasting Properties of Rice Flours. Among the various kinds of milled rice, *ae* mutants showed higher pasting temperatures, as high as 10 °C, than ordinary rice cultivars (Koshihikari and Hoshiyutaka), as shown in Figure 6A. *Ae* mutants showed very low maximum viscosities, minimum viscosities, and breakdown values compared with ordinary rice cultivars (Koshihikari and Hoshiyutaka); EM145 showed the lowest maximum viscosity, minimum viscosity, breakdown, final viscosity, setback, and consistency value, and EM189 showed the highest maximum viscosity, minimum viscosity, breakdown, final viscosity, setback, and consistency value among the *ae* mutants. As shown in Figure 6B, the viscosity of EM10 decreased markedly by roasting, boiling, and boiling after preroasting.

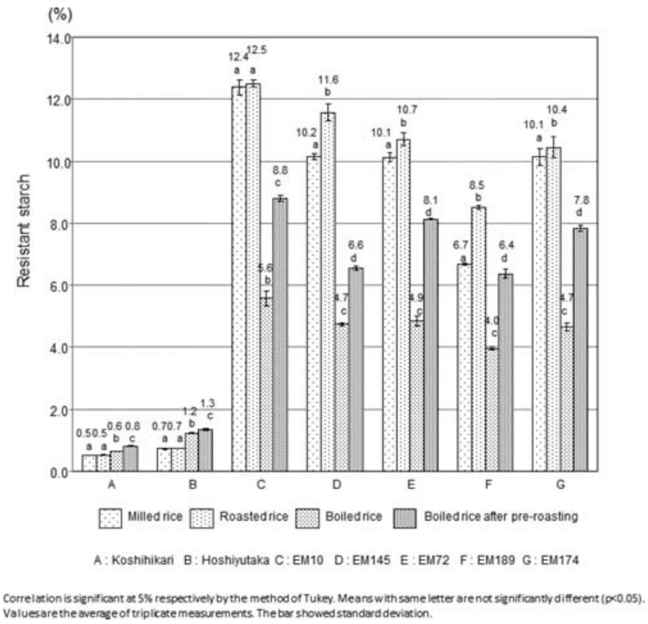

Figure 4. Resistant starch of various starches prepared with different heat treatments. Correlation is significant at 5% by the method of Tukey. Means with the same letter are not significantly different ($p < 0.05$). The resistant starch in the sample was measured according to the AOAC method by a resistant starch assay kit (Megazyme, Wicklow, Ireland). Each sample (100 mg) was digested by pancreatin and amyloglucosidase, and glucose was measured by spectrophotometer at 510 nm.

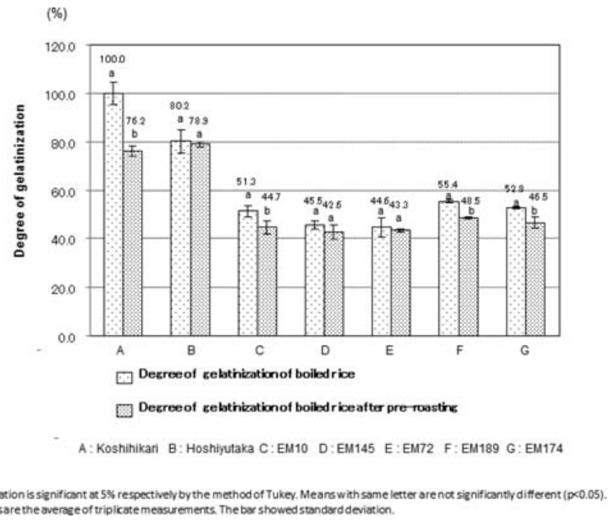

Figure 5. Degree of gelatinization of starch (BAP method). Correlation is significant at 5% by the method of Tukey. Means with the same letter are not significantly different ($p < 0.05$). The BAP method clearly differentiates the velocity of hydrolysis between gelatinized starch and retrograded starch compared with the conventional glucoamylase method and diastase method. Among the various rice samples, when the degree of gelatinization of Koshihikari showed 100%, *ae* mutants showed 44.6−55.4% and Hoshiyutaka showed 80.2%, respectively. Preroasting before boiling decreased the degree of gelatinization of *ae* mutant rice (3−12.5%), Koshihikari (23.8%), and Hoshiyutaka (2.0%) compared with boiled rice grains without preroasting.

As shown in Figure 7, among the *ae* mutants, roasted rice flours showed lower maximum viscosity (56−91%) compared with raw milled rice, and Hoshiyutaka (26%) showed a similar tendency. *Ae* mutant rice flours prepared after boiling showed lower maximum viscosities (87−94%) compared with raw milled rice flours, and Hoshiyutaka (81%) and Koshihikari (64%) revealed a similar tendency. Furthermore, *ae* mutant rice flours prepared by boiling after roasting showed lower maximum viscosities (23−73%) compared with raw milled rice flours, and Hoshiyutaka (59%) and Koshihikari (34%) showed a similar tendency.

Physical Properties of Boiled Rice Grains. Physical properties of boiled rice were changed by adjustment of amount of added water on boiling as shown in Figure 8 and Table 2.

As shown in Figure 8, Hoshiyutaka (high-amylose rice) revealed highest maximum length, an index of hard-layer thickness from surface layer toward center-core-layer of boiled rice grain, and Koshihikari (low amylase rice) showed higher maximum length than *ae* mutant rice in the case of ×1.6 and ×2.0. Among *ae* mutants, EM10 showed the lowest maximum length (×2.0) and EM189 showed the highest maximum length (×1.0).

As shown in Table 2, Hoshiyutaka (high amylose) showed the highest hardness, pliability, and toughness (except ×1) among all rice samples. Boiled rice grains of *ae* mutant showed harder texture than Koshihikari (low amylose) except EM145 (×1.0) in the case of low or ordinary amount of water addition on boiling (×1.0, ×1.6); on the contrary, they showed similar low hardness in the case of a high amount of water on boiling. On the contrary, boiled rice grains of *ae* mutant showed higher brittleness compared with high-amylose and low-amylose rice grains except EM189 (×1.0). In Table 2, results of statistical analyses on water addition and cultivar characteristics are shown.

Correlation among the Physicochemical Properties. Significant correlations among the physicochemical properties are shown in Table 3. Apparent amylose contents showed positive correlations with resistant starch ($r = 0.86$), α-amylase activity ($r = 0.81$), and pasting temperature ($r = 0.87$) and negative correlations with maximum viscosity ($r = -0.94$) and breakdown ($r = -0.95$).

Glucose content revealed a high positive correlation with α-amylase activity ($r = 0.77$) and negative correlations with maximum viscosity ($r = -0.80$), minimum viscosity ($r = -0.87$), and final viscosity ($r = -0.88$).

α-Amylase activity showed positive significant correlations with resistant starch ($r = 0.92$), consistency/breakdown ratio ($r = 0.96$), pasting temperature ($r = 0.83$), and brittleness ($r = 0.83$) and negative significant correlations with maximum viscosity ($r = -0.91$), breakdown ($r = -0.84$), maximum length ($r = -0.78$), pliability ($r = -0.76$), and degree of gelatinization ($r = 0.86$).

Xylanase activity showed a high correlation with the physical properties of boiled rice grains. Xylanase activity showed significant correlation with maximum length (×2) ($r = 0.80$) and pliability (×2) ($r = 0.76$) at the level of 5%.

The degree of gelatinization had a high correlation with α-amylase activity, brittleness, maximum length, pliability, maximum viscosity, breakdown, pasting temperature, and resistant starch. Degree of gelatinization showed significant correlation with resistant starch ($r = -0.96$) at the level of 1%.

Physical properties of *ae* mutants and ordinary rice cultivars, such as brittleness, maximum length, or pliability, had a high positive or negative correlation with α-amylase activity and xylanase activity, resistant starch, degree of gelatinization, and pasting temperature.

Effect of Boiling Rice Grains with Frozen Fruits after Roasting. It became possible to produce high-quality and biofunctional pregelatinized rice flour by boiling with frozen

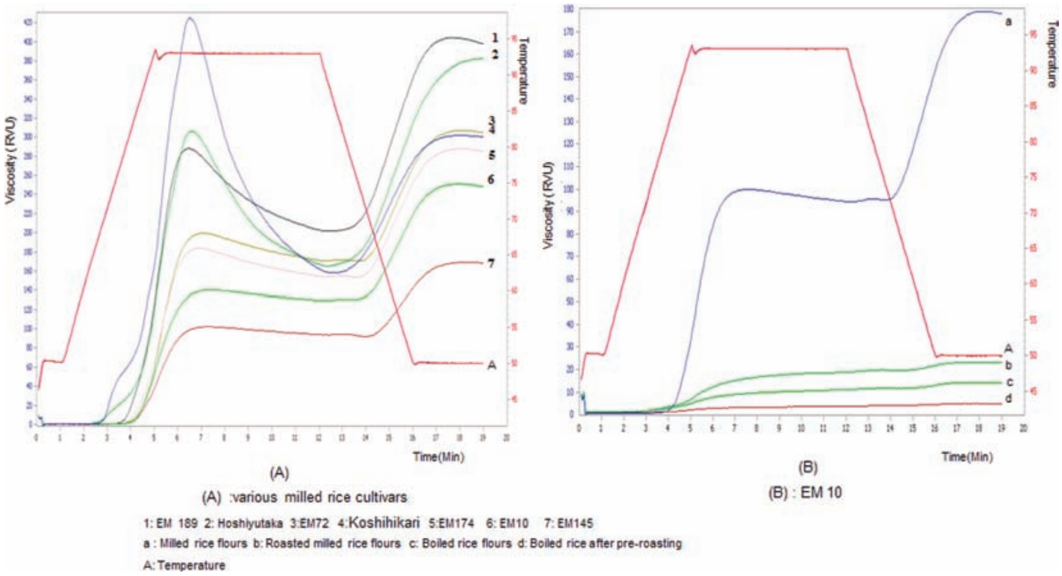

Figure 6. Pasting properties of various milled rice and effect of heat treatment on those of EM10. Pasting properties of rice flours were measured using a Rapid-Visco-Analyzer (RVA, model Super 4, Newport Scientific Pty, Ltd., Warriewood, Australia). A programmed heating and cooling cycle was followed as outlined in the procedure of Toyoshima et al.[15].

fruits (6% w/w dry base), such as blueberry, tomato, strawberry, or sweet summer orange, after rice grains had been preroasted as shown in Figure 9 . It was shown that these rice products contained high amounts of resistant starch (6.14−6.85%). Their colors were very beautiful and vivid as shown in Figure 9, and their tastes were very favorable. The resistant starch was increased about 1.2 times that in rice prepared by ordinary boiling.

■ DISCUSSION

Apparent Amylose Content. Generally, cereal starches were reported to have higher apparent amylose contents than root and tuber starches, which tend to retrograde more rapidly after boiling.[25] Among the easy-hardening cereal grains, high amylose *ae* mutant rice grains are not sticky characteristically; therefore, boiled rice grains do not adhere to each other during drying. It was found to be suitable as a material for pregelatinized rice grains or flours due to easy-handling based on very high apparent amylose content.

Protein Content. Protein is the second most abundant constituent of milled rice next to starch. The amount of protein affects the physical properties of the rice; the higher the protein content, the harder and less sticky the rice becomes upon boiling.[26] Although Okadome et al. reported that protein content affected the surface hardness in the case of high-pressure/low-pressure measurement using a Tensipresser,[27] protein content did not show significant correlation with hardness and other physicochemical properties. It seems that our sample differs from their Japonica samples cultivated with a different application of nitrogen fertilizer.

***α*-Amylase Activity.** α-Amylases are enzymes that cleave the α-1,4-D-glucosidic linkages in starch molecules. *Ae* mutant cultivars had very high α-amylase activities; particularly EM10 showed the highest activity. It is plausible because EM10 is apt to pregerminate on panicle during the consecutive rainy days just before harvest. Low dormancy of *ae* mutant rice cultivars would be the reason for such high α-amylase activities. As α-amylase is localized in only the outer layer of the rice grain,[28] it does not affect the molecular structure of starch and physical properties of the cooked rice grain markedly.

***α*-Glucosidase Activity.** Although Iwata reported that α-glucosidase activity showed a positive correlation with fresh weight, GBSS activity, and amylose content,[23] α-glucosidase activity did not show significant correlations with other physical or chemical properties in the present investigation. This would be because the activities of α-glucosidase were not so diversified as α-amylase activities among the *ae* mutant rice cultivars.

Xylanase Activity. Strength and adhesion of cell wall affect the physical properties of rice endosperm. Boiled rice is softened by the decomposition of cell wall, which suggests the important role of hemicellulosic polysaccharides (endoxylanase) for the physical properties of endosperm cell walls.[29,30] Particularly, the high-amylose rice, Hoshiyutaka, showed the highest xylanase activity. EM174 showed the highest activity of xylanase among the *ae* mutants. As shown in Table 3, xylanase activity had significant correlation with maximum length and pliability of the cooked rice grains, which reveals that xylanase activity would lead to the increase of softness and tenderness of the boiled rice grains.

Glucose Content. During the early stage of boiling, α-amylase and α-glucosidase decompose starch and generate glucose.

As shown in Figure 3, *ae* mutant rice cultivars showed high glucose contents after boiling, whereas Koshihikari and Hoshiyutaka showed very low glucose contents. Glucose contents were slightly decreased by roasting and increased remarkably by boiling. Most interestingly, boiled rice after preroasting did not show the increase of glucose during boiling among all rice samples.

We think that α-amylase and α-glucosidase were denatured by the roasting; therefore, the glucose content of boiled rice after preroasting was lower than that of the ordinary boiled rice.

Saito reported that the addition of sucrose to rice cake prevented its retrogradation and hardening.[15] However, an excess amount of sucrose makes rice cake too sticky, which causes problems for handling. It was found that the *ae* mutant rice is suitable as a material for pregelatinized flours. When we prepare rice flours as a material of various processed rice

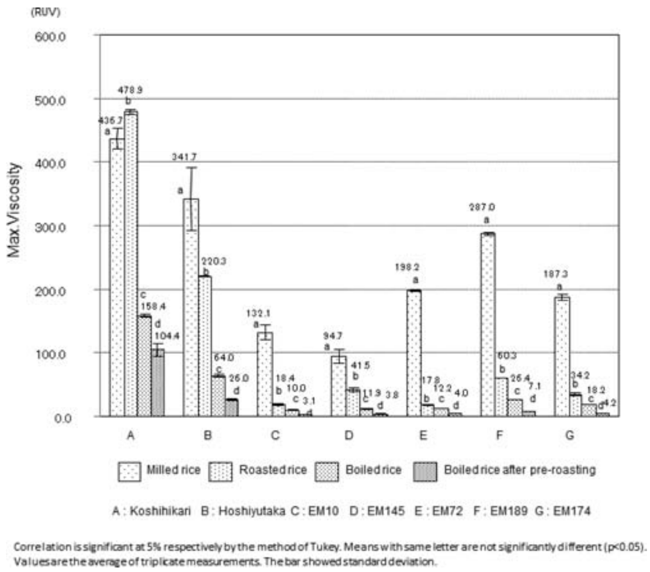

Figure 7. Max viscosities of various processed starches prepared by different heat treatments. Correlation is significant at 5% by the method of Tukey. Means with the same letter are not significantly different ($p < 0.05$). Values are the average of triplicate measurements. The bars show standard deviation.

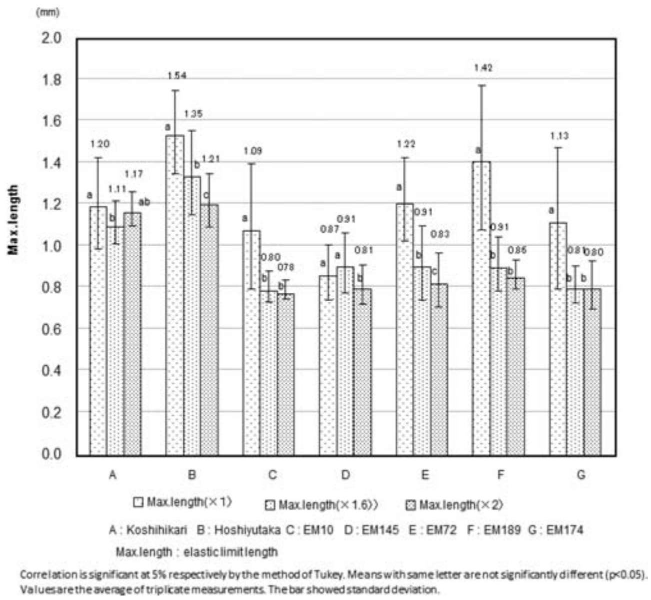

Figure 8. Effects of cultivar and cooking water on maximum length of boiled rice grains. Correlation is significant at 5% by the method of Tukey. Means with the same letter are not significantly different ($p < 0.05$). Values are the average of triplicate measurements. The bars show standard deviation. Measurement of the physical properties of the boiled rice grains was carried out by the continuous progressive compression method (CPC) with the Tensipresser according to our previous paper.[16] Maximum length means elastic limit length as shown in Figure 1.

products, sticky boiled rice grains or sticky rice dough is not suitable at the points of handling (drying and pulverizing) and diabetes prevention. *Ae* mutant rice is suitable for processing because its boiled rice grains or rice dough is nonsticky. We adopt preroasting to prevent starch digestion by amylase because an excess amount of glucose makes the boiled rice or dough too sticky. It seems that denaturing amylase by roasting prevents the increase of glucose on boiling.

Furthermore, a low glucose content after processing is necessary in terms of low-GI foods[9,10] developed from *ae* mutant rice.

Resistant Starch. Yang et al. reported the starch properties of the mutant rice, which is rich in resistant starch.[1]

Ae mutant rice grains had markedly higher amounts of resistant starches than the other rice grains, as shown in Figure 4. Among the same *ae* mutant rice cultivars, boiled rice grains after preroasting contained more resistant starches (about 1.6 times) than those only boiled without preroasting. The roasted rice grains without boiling of *ae* mutants had more resistant starch (about 1.1 times) than that of raw milled rice, which was due to the denaturing of the starch degrading enzymes. Particularly, it was caused by the decrease of α-amylase activities during roasting. Milled rice grains of *ae* mutant and ordinary rice cultivars revealed the decrease of α-amylase activities and maximum viscosities (>80%) by roasting. Branching enzyme introduces 1,6-α-glycosidic bonds between the chains of amylopectin, creating the branched amylopectin.[31] As *ae* mutants lack starch branching enzyme IIb, their amylopectin molecules have more long-chain glucans. During the roasting of grains, α-amylase activity was decreased and Maillard reaction and caramelizing reaction proceeded,[32] which led to a substantial amount of resistant starch in the preroasted rice grains or the boiled rice grains after preroasting.

Degree of Gelatinization. The gelatinization property is one of the most important rheological indicators for the cooking quality or processing characteristics of rice starch. Many investigations have shown that the rheological properties of starch, such as gelatinization, retrogradation, and pasting properties, are affected by amylopectin structure.[33−35]

The diastase,[36] glucoamylase (GA), and β-amylase−pullulanase (BAP) methods[17] were reported to determine the degree of gelatinization of starch. The BAP method clearly differentiates the velocity of hydrolysis between gelatinized starch and retrograded starch compared with the conventional glucoamylase method and diastase method. Preroasting before boiling decreased the degree of gelatinization of rice samples compared with those of boiled rice grains without preroasting.

Pasting Properties of Rice Flours. *Ae* mutants showed higher pasting temperature as much as 10 °C higher than those of ordinary rice cultivars (Koshihikari and Hoshiyutaka) as shown in Figure 6A. The starch from the mature endosperm of the wild type started to gelatinize in urea solution at 3 M, in contrast the *ae* mutants hardly gelatinized in urea up to 6 M, but they began to gelatinize at 7 M.[6] The chain length and distribution of amylopectin branches determine the gelatinization temperature of starch, enthalpy changes, and pasting properties, and the gelatinization temperature of starch increases with increasing chain length.[37−39] The results of the present studies support the view of the above-mentioned papers.

The gelatinized starch granule was resistant to swelling and breakdown by stirring. Swelling power and solubility of buckwheat starch granules were decreased by roasting as reported by Christa et al.[40]

High-amylose rice showed a higher final viscosity than low-amylose rice, and the final viscosity was related to the degree of starch retrogradation upon cooling.[31,41] The ratio of consistency/breakdown was related to expansion of rice crackers.[42] Due to the highest consistency/breakdown ratio, EM10 tends to

Table 2. Physical Properties of Cooked Milled Rice Using Tensipresser[a]

	hardness (×1)		hardness (×1.6)		hardness (×2)		pliability (×1)		pliability (×1.6)		pliability (×2)	
	gw/cm^2	SD	gw/cm^2	SD	gw/cm^2	SD		SD		SD		SD
Koshihikari	686.5 a	504.6	325.6 bx	111.6	296.6 b	79.1	1.5 a	0.1	1.3 bx	0.1	1.3 b	0.1
Hoshiyutaka	2015.0 a	373.8	766.5 by	74.8	548.8 b	155.6	1.6 a	0.1	1.6 aby	0.2	1.4 b	0.1
EM10	1171.0 a	634.5	427.9 bx	73.2	287.4 b	48.8	1.2 a	0.1	1.1 bx	0.0	1.1 b	0.0
EM145	666.4 a	278.9	565.9 abx	151.2	290.3 b	63.7	1.1 a	0.1	1.1 ax	0.1	1.1 a	0.1
EM72	1094.0 a	493.8	567.0 bx	109.5	345.4 b	80.4	1.2 a	0.1	1.2 ax	0.1	1.1 a	0.1
EM189	1741.0 a	786.4	635.9 bx	209.1	362.3 b	86.7	1.2 ad	0.1	1.1 bx	0.0	1.1 a	0.0
EM174	1233.0 a	719.3	440.8 bx	82.4	344.3 b	77.9	1.1 a	0.1	1.2 ax	0.1	1.1 a	0.1

	toughness (×1)		toughness (×1.6)		toughness (×2)		brittleness (×1)		brittleness (×1.6)		brittleness (×2)	
	gw/cm^2	SD	gw/cm^2	SD	gw/cm^2	SD		SD		SD		SD
Koshihikari	30.3 a	27.9	14.2 ax	5.4	13.7 a	3.3	1.7 a	0.3	1.9 ax	0.2	1.9 a	0.1
Hoshiyutaka	95.6 a	21.5	33.0 by	6.6	23.9 b	7.8	1.2 a	0.1	1.5 by	0.1	1.7 b	0.2
EM10	55.7 a	37.2	19.8 bz	5.4	12.5 b	3.3	1.8 a	0.4	2.2 bz	0.2	2.5 b	0.0
EM145	28.5 a	20.4	23.3 az	9.8	10.7 b	3.5	2.3 a	0.3	2.3 az	0.4	2.6 a	0.3
EM72	73.9 a	56.5	17.7 bz	5.5	13.1 b	4.1	1.7 a	0.5	2.4 bz	0.4	2.6 b	0.3
EM189	108.4 a	58.1	26.3 by	12.3	14.0 b	4.2	1.5 ad	0.5	2.2 bz	0.3	2.3 b	0.2
EM174	61.3 a	46.7	16.0 bx	3.6	10.9 b	2.9	1.8 a	0.5	2.5 bz	0.2	2.6 b	0.3

[a] Correlation is significant at 5% by the method of Tukey. Means with the same letter within a row (a−c) or column (x−z) are not significantly different ($p < 0.05$). Values are the average of triplicate measurements.

expand least as rice crackers among *ae* mutant cultivars. As shown in Figure 6B, the viscosity of EM10 decreased markedly by roasting, boiling, and boiling after preroasting.

As shown in Figure 7, *ae* mutant rice flours prepared by boiling after roasting showed lower maximum viscosities compared with raw milled rice flours, which would be due to starch gelatinization and starch digestion by α-amylase as reported by Collado and Corke for sweetpotato starch.[43] As shown in Table 1 and Figure 2, amylase activities of *ae* mutant rice cultivars are markedly higher than those of ordinary rice. Therefore, the degree of lowering of maximum viscosity was higher in the case of EM10 as shown in Figure 7.

Generally, rice endosperm starches give the A-type of X-ray diffraction pattern; on the contrary, *ae* rice starches showed the B-type patterns.[8] Many starches from roots or tubers, such as a potato starch, show B-type diffraction pattern.[44] Cereal starches show higher apparent amylose contents than root and tuber starches; therefore, cereal starches tend to retrograde more easily than root and tuber starches.[44] Compared with wild type rice, *ae* mutant rice showed lower peak viscosity, lower break-down, and higher consistency as shown in Figure 6A.

It was found that the *ae* mutant rice cultivars would be suitable as materials for processed rice products because their starches are hard to gelatinize and maintain well the shape and volume of the processed products due to hard texture and low expansion during heating.

Physical Properties of Boiled Rice Grains. We reported that the cooked rice grains (×1.6) of *ae* mutant rice EM10 were harder and less sticky than those of Koshihikari by measurement with a Tensipresser using an ordinary compression test.[14]

The physical properties of boiled rice were changed by adjustment of the amount of added water on boiling. Maximum length (×2.0) was suggested as a useful index for the characterization of *ae* mutants because all of the *ae* mutant rice grains showed lower values compared with ordinary high-amylose and low-amylose rice.

As shown in Table 2, a high amount of water is necessary to get the soft texture in the case of *ae* mutant rice. Toughness showed a similar tendency as hardness. Boiled rice grains of *ae* mutant showed lower pliability than high-amylose rice (Hoshiyutaka) and low-amylose rice (Koshihikari) grains. On the contrary, boiled rice grains of *ae* mutant showed higher brittleness compared with high-amylose and low-amylose rice grains except EM189 (×1.0).

As the boiled grains of *ae* mutant rice show nonsticky and brittle properties, they were not accepted as table rice by Japanese consumers.[14] Nevertheless, they would be suitable for processing of bread or cooking with fruits/vegetable as shown in the present paper (Figure 9) because nonsticky and tough grains are suitable for wheat/rice bread[45] or novel characteristic processed food-stuff, such as low-GI foods.

Perhaps the characteristics of boiled mutant rice grains were caused by the change in the fine structure of amylopectin with enriched long chains within a cluster, and *ae* mutants lack the starch branching enzyme IIb, which lowers the amount of short-chain glucans.[6] BE IIb plays a specific role in the transfer of short chains, which might then be extended by starch synthase (SS) to form the A and B_1 chains of amylopectin cluster in rice endosperm.[6] EM10 showed the lowest pliability (×2.0); more-over, low- or high-amylose rice showed higher pliability than *ae* mutants. As *ae* mutants have fewer branched short glucans and more long chains in the amylopectin of the starches, their starch granules are resistant to gelatinization and their boiled rice grains were nonsticky and brittle.

As *ae* mutants have been developed only 10 years ago,[6] the architecture of their starch, such as the relationship between amylase and amylopectin, has not been clarified yet. The chalki-ness of rice grains is associated with loosely packed starch

Table 3. Correlation between Chemical Components and Physical Parameters of Milled Rice Samples[a]

	amylose	glucose	α-amylase	xylanase	toughness	brittleness	max length	hardness	pliability	max visco	min visco	break-down	final visco	setback	pasting temp	consistency/ breakdown	R8	degree of gelatinization
amylose	1.00																	
glucose	0.58	1.00																
α-amylase	0.81*	0.77*	1.00															
xylanase	0.03	−0.20	−0.41	1.00														
toughness	0.01	−0.18	−0.33	0.59	1.00													
brittleness	0.57	0.59	0.88*	−0.70	−0.65	1.00												
max length	−0.54	−0.52	−0.78*	0.80*	0.56	−0.88**	1.00											
hardness	0.16	−0.14	−0.22	0.47	0.97**	−0.52	0.39	1.00										
pliability	−0.41	−0.57	−0.78*	0.82*	0.63	−0.97**	0.88**	0.52	1.00									
max visco	0.84**	−0.80*	−0.81**	0.18	0.12	−0.72	0.69	−0.02	0.61	1.00								
min visco	−0.52	−0.87*	−0.69	0.01	0.33	−0.42	0.31	0.33	0.35	0.67	1.00							
breakdown	−0.96**	−0.63	−0.84*	0.21	0.02	−0.71	0.72	−0.15	0.61	0.98**	0.43	1.00						
final visco	−0.51	−0.88**	−0.74	0.25	0.54	−0.63	0.54	0.50	0.57	0.70	0.86**	0.48	1.00					
setback	0.80*	0.21	0.52	0.01	0.38	0.36	−0.42	0.54	−0.27	−0.68	0.04	−0.84*	0.04	1.00				
pasting temp	0.87*	0.56	0.88	−0.36	−0.25	0.86	−0.84*	−0.08	−0.75	−0.80**	−0.36	−0.88*	−0.49	0.78*	1.00			
consistency/breakdown	0.88*	0.72	0.88**	−0.26	−0.30	0.70	−0.65	−0.19	−0.60	−0.89**	−0.77**	−0.78*	−0.78*	0.45	0.73	1.00		
R8	0.88*	0.65	0.82**	−0.45	−0.36	0.80**	−0.88**	−0.19	−0.81*	−0.83**	−0.50	−0.84**	−0.62	0.66	0.97**	0.86*	1.00	
degree of gelatinization	−0.75	−0.67	0.86*	0.56	0.28	0.80**	0.82**	0.11	0.88*	−0.87**	−0.39	−0.81**	0.55	−0.66	−0.86**	−0.71	0.88**	1.00

[a] Correlation is significant at 5% (*) or 1% (**) by the method of t test. Physical properties measured by adjustment of amount of added water to 1.6 times (w/w).

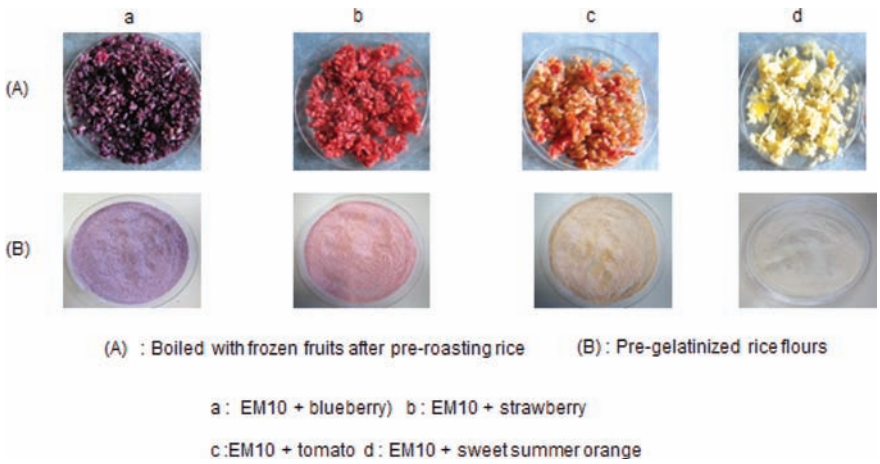

Figure 9. Development of tasty biofunctional pregerminated rice flours: (A) rice boiled with frozen fruits after preroasting; (B) pregelatinized rice flours; (a) EM10 + blueberry; (b) EM10 + strawberry; (c) EM10 + tomato; (d) EM10 + sweet summer orange. *Ae* mutants are not suitable for table rice because of their low palatability.[12] It became possible to produce high-quality and biofunctional pregelatinized rice flour by boiling with frozen fruits (6% w/w dry base), such as blueberry, tomato, strawberry, or sweet summer orange, after rice grains had been preroasted. It was shown that these rice products contained high amounts of resistant starch (6.14−6.85%). Their colors were very beautiful and vivid, and their tastes were very favorable.

granules,[46] and the presence of air spaces between granules might contribute to the chalkiness of raw rice endosperm, brittleness, and low-hardness of the rice grains after boiling.

In the present paper, we adopted the CPC test because it represents the strength of boiled rice grain structure, from the outer layer to the center core portion, against continuous stepwise compression. CPC was suggested as a useful method for the evaluation of physical properties of boiled rice of hard and nonsticky rice.[21,27,47,48] Particularly, physical properties measured by adjustment of the amount of added water to 1.6 times (w/w) led to significant high correlation with the other physical properties.

Correlation among Physicochemical Properties. Significant correlations among the physicochemical properties are shown in Table 3. Apparent amylose contents showed positive correlations with resistant starch, α-amylase activity, and pasting temperature and negative correlations with maximum viscosity and breakdown. Quevas et al.[24] reported that the gelatinization temperature by DSC has no correlation with amylose content in a large and diverse sample set. In the present investigation, amylopectin structure may have affected both apparent amylose content and pasting temperature among the limited rice samples.

Among the components, increasing the amylose content of the starch increased resistant starch.[8]

Pasting properties of *ae* mutants and ordinary rice flours (milled rice flour), such as maximum viscosity, breakdown, or pasting temperature, had a high correlation with resistant starch, degree of gelatinization, amylose content, α-amylase activity, and glucose content. As Benmoussa et al. reported that the RVA method potentially could be used as a screening tool for starch digestion properties,[49] our results also showed that RVA analysis was very useful to characterize the starch digestion properties.

As Nishi et al. pointed out, the pasting temperature of *ae* mutant rice is higher than that of ordinary rice.[6] It seems that a high pasting temperature leads to a high amount of resistant starch.

We performed this investigation to elucidate the condition of palatable and biofunctional rice and rice products. Particularly, tasty and high-resistant starch rice products are necessary. Among the rice samples we used in the present paper, those with high amylose, high α-amylase, high brittle, high pasting temperature, and high consistency/breakdown and low maximum length, low pliability, and low maximum viscosity tend to be typical high-resistant rice and promising for low-GI rice products.

Effect of Boiling Rice Grains with Frozen Fruits after Roasting. *Ae* mutants are not suitable for table rice because of their low palatability.[14] Boiled rice grains with frozen fruits after roasting showed very beautiful and vivid colors as shown in Figure 9, and their tastes were very favorable as described in the Introduction. The resistant starch was increased about 1.2 times that prepared by ordinary boiling. Yagishita et al. reported that the amino acids inhibited the gelatinization of tapioca starch due to ionic bonding between water and amino acid.[50] The present paper is the first report of the effect of co-cooking of fruits/vegetables with rice flours.

As the amylopectin of *ae* mutants has more long-chain glucans, it tends to take a helical structure in solution. It is known that the cavities in a helix easily make an inclusion complex with low molecular weight substances,[51] such as pigments, aromatic, or volatile compounds. The examples of "pre-roasted and cooked *ae* rice/fruits or rice/vegetable products" are promising as "tasty and biofunctional foods" because they are tasty, beautiful, and low-glucose foods because of high resistant starch and replacement of glucose with fructose.

Conclusions. (1) The novel *ae* mutant rice grains were characterized by having high apparent amylose contents, high glucose contents, high pasting temperature, high α-amylase activities, high resistant starch content, and low degree of gelatinization during the boiling.

(2) These *ae* mutant rice grains contained high amounts of resistant starch even after preroasting and boiling.

(3) Preroasting before boiling was shown to be effective to maintain the resistant starch contents high and to lower the degree of gelatinization. By this method, resistant starch of EM10 was markedly increased to 1.6 times that of ordinary boiled rice.

(4) Preroasting showed another effect to decrease the glucose content of boiled rice (about 84% in the case of EM10).

(5) It became possible to produce high-quality and biofunctional pregelatinized rice flour by boiling with frozen fruits, such as tomatoes, after preroasting of *ae* rice grains.

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ABBREVIATIONS USED

ae mutants, amylose extender mutants of rice; SHR, super-hard rice; GI, glycemic index; MNU, methyl nitroso urea; PNPG, *p*-nitrophenyl α-D-glucopyranoside.

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